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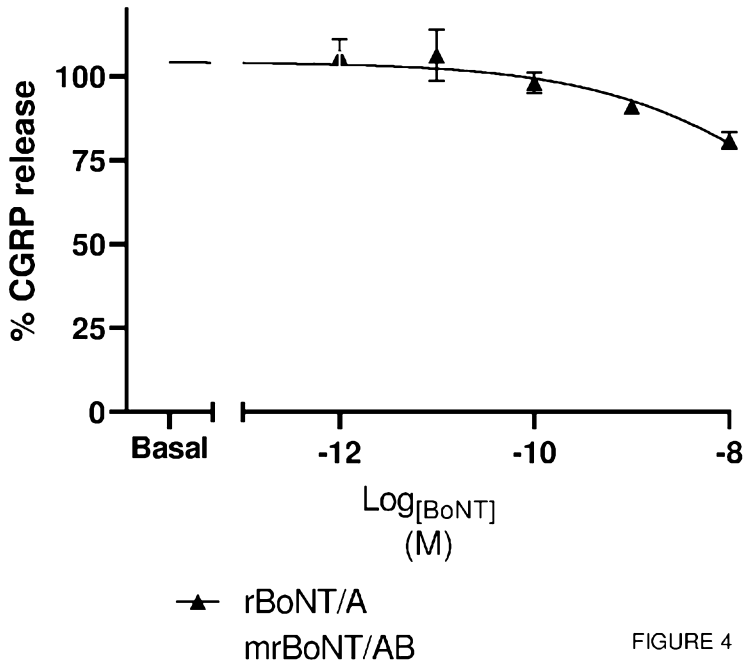

FIGURE 4

(57) **Abstract:** The present invention is directed inter alia to the treatment of pain. For example, there is provided a chimeric clostridial neurotoxin for use in treating pain by inhibiting release of a pain mediator from a neuron comprising an Aδ nerve fiber or a C nerve fiber, wherein the chimeric clostridial neurotoxin binds to the neuron comprising the Aδ nerve fiber or the C nerve fiber, respectively, and wherein the chimeric clostridial neurotoxin comprises a botulinum neurotoxin A (BoNT/A) light-chain and translocation domain (H_N domain), and a BoNT/B receptor binding domain (He domain). Also provided are methods, uses, kits, and unit dosage forms.

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TREATMENT OF PAIN

FIELD OF THE INVENTION

The present invention relates to the treatment of disorders, such as pain.

BACKGROUND

Pain is an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage. Pain is also described as a neurologic condition characterised by pathologic changes in the nervous system or, more precisely, a dysfunction of the endogenous nociceptive system (Raffaeli & Arnaudo (2017), J Pain Res, 10, 2003-2008).

Nociception is the process by which information about actual tissue damage (or the potential for such damage, should the noxious stimulus continue to be applied) is relayed to the brain. The sensory neurons involved in nociception are classified into three main groups: Group A; Group B; and Group C (Yam *et al* (2018), Int J Mol Sci, 19, 8, 2164).

Group A nerve fibers are classified as myelinated fibers and can be further subdivided into Aα, Aβ, Aγ and Aδ, each with different sets of characteristics. These fibers generally terminate in laminae I, III, IV and V of the dorsal horn of the spinal cord with some lamina II inner projection. Both Type Ia and Ib sensory fibers from muscle spindle endings and Golgi tendons are type Aα. Type Aβ fibers are typically low-threshold, cutaneous, slow or fast adapting mechanoreceptors, and include Type II afferent fibers from the stretch receptor. The Aβ-fibers typically belong to laminae III and IV. Type Aγ fibers may include Type II afferent fibers from the stretch receptors. Type Aδ fibers may include the thermal and mechanical nociceptors that terminate in the rexed laminae I and V, as well as Type III afferent fibers. Aδ-fibers are also typically the smallest myelinated nerves and may have a relatively fast conduction velocity of ~ 30 m/s. The diameter of Aδ-fibers is typically about 2–5 μm, and is typically responsive towards short-lasting and pricking pain.

Group B nerve fibers are moderately myelinated usually with conduction velocities of 3–14 m/s. The preganglionic nerve fibers of the autonomous nervous system (ANS) and general visceral afferent fibers belong to this group.

Group C nerve fibers are unmyelinated and are typically less than 2 μm in diameter and have a relatively slow conduction velocity typically of up to approximately 2 m/s. The nerve fibers at the dorsal roots (Type IV afferent fibers) and postganglionic fibers in the ANS may be categorized in this group. All these fibers are mainly nociceptive in function, carrying the sensory information and assembling around 70% of the afferent nociceptive information, which then enters the spinal cord. C-fibers may terminate in laminae I and II in the grey matter of the spinal cord. In terms of nociception, C-fiber nociceptors may be polymodal, as they are activated by thermal, mechanical, and/or chemical stimuli. For example, C-fibers may be activated via poorly localized stimuli. In terms of neurochemistry, C-fibers can be classified as either peptidergic or non-peptidergic, and about 50% of these fibers express neuropeptides including calcitonin gene-related peptide (CGRP), neurokinins and substance P (SP).

There are a variety of neurotransmitters involved in pain, including all the major types of neurotransmitters, such as inflammatory mediators: prostaglandin E2 (PGE2), prostacyclin (PGI2), leukotriene B4 (LTB4), nerve growth factor (NGF), protons, bradykinin (BK), ATP, adenosine, SP, neurokinin A (NKA), neurokinin B (NKB), 5-hydroxytryptamine (5-HT), histamine, glutamate, norepinephrine (NE) and nitric oxide (NO); and non-inflammatory mediators: CGRP, γ-aminobutyric acid (GABA), opioid peptides, glycine and cannabinoids (Yam *et al* (2018), Int J Mol Sci, 19, 8, 2164).

Of particular therapeutic interest is CGRP, which is widely produced in both the central and peripheral nervous systems; however, it is primarily located in the primary afferent nerves. As a direct derivative of the dorsal root ganglia (DRG), CGRP may be found in the dorsal horn of the spinal cord and associated with the conduction of noxious stimulation. CGRP is related to the excitatory effects of SP, which results in Ca^{2+} release. The receptors of CGRP (calcitonin receptor-like receptor (CALCRL)) are typically located in the nucleus accumbens, indicating that the CNS may control CGRP-mediated pain transmission. CGRP is widely distributed in the peripheral and central nervous system and its receptors are expressed in pain pathways. CGRP-like immunoreactivity (CGRP-LI) is typically found in 40–50% of DRG neurons. Moreover, CGRP is usually co-localized with other neuropeptides, including substance P and neurokinins in DRG neurons. Peripheral CGRP-LI fibers may terminate in lamina I, III and V of spinal cord and CGRP-containing DRG neurons innervate joints. Thus, CGRP and its receptors may be widely distributed in peripheral and central pain pathways (Schou *et al* (2017), The Journal of Headache and Pain, 18, 34, 1-17). In animals, CGRP may be released from peripheral and central nerve endings upon noxious pain and/or mechanical

stimulation of the skin. In rats, the major part of circulating CGRP may be released from perivascular nerve terminals. Acute and chronic nociception may lead to altered release of CGRP from sensory nerve endings and central terminals into the dorsal horn of the spinal cord. CGRP is known as one of the most potent vasodilators. Two isoforms have been characterized: α-CGRP and β-CGRP (Russell *et al* (2014), Physiol Rev, 94, 4, 1099-1142). The isoform α is principally expressed in primary sensory neurons, whereas the isoform β is mainly found in intrinsic enteric neurons. The mature form of this neuropeptide is composed of 37 amino acids, and its expression has been particularly noticed in sensory neurons of the DRG and trigeminal ganglion. The mature form is stored in vesicles localized in the terminal region of central and peripheral nerve endings from where it may be secreted in the dorsal spinal cord or in various peripheral tissues, especially surrounding blood vessels which may modulate vascular tone. In addition, the presence of networks of nociceptors positive to CGRP in rodent and human meningeal vessels has been observed, and about 40–50% of trigeminal ganglion neurons have been found to be positive to CGRP. Moreover, CGRP expression has been observed in areas of the CNS, such as the hypothalamus, thalamus, periaqueductal grey, superior and inferior colliculi, amygdala, trigeminocervical complex, and the cerebellum. These mentioned brain areas may be associated with migraine pathophysiology, considering the capability of CGRP to change synaptic and neuronal activity at the trigeminocervical complex, and transmission of nociceptive signals to the thalamus and cortical areas (Tardiolo *et al* (2019), Int J Mol Sci, 20(12), 2932).

Conventional treatments for pain (e.g. CGRP-associated pain) include monoclonal antibodies and small-molecule antagonists that target pain mediators (e.g. CGRP) once said mediators have already been released by the pre-synaptic neurons. As an alternative approach, certain conventional therapeutics target receptors of the pain mediators. These approaches are associated with a number of disadvantages, including: effects on chemical mediators (e.g. CGRP) systemically; nausea; vomiting; dyspepsia; diarrhoea; bradycardia; hypotension; bronchospasm; dyspnoea; fatigue; insomnia; dizziness; dry mouth; flushing; hot or cold sensations; chest pain; constipation; itchiness; drowsiness; ringing in the ears; restlessness; muscle spasms; injection site pain; upper respiratory infection; fatigue; nasopharyngitis; injection site erythema; injection site induration; anxiety; depression; injection site pruritus; influenza; urinary tract infection; somnolence; paraesthesia; increased heart rate; stroke; and/or heart attack (Woo (2020), Nature, 586, S4-S6 and Tardiolo *et al* (2019), Int J Mol Sci, 20(12), 2932). There is thus a need for improved pain therapeutics that are associated with fewer side-effects and/or which block pain mediators at the point of release.

The present invention overcomes one or more of the above-mentioned problems.

SUMMARY OF THE INVENTION

The present inventors have found that, unlike BoNT/A, a chimeric clostridial neurotoxin comprising a botulinum neurotoxin A (BoNT/A) light-chain and translocation domain (H_N domain), and a BoNT/B receptor binding domain (H_C domain) may bind to neurons comprising Aδ or C nerve fibers that secrete pain mediators and may be efficacious at inhibiting release of said mediators from said neurons. Thus, by way of such inhibition, the chimeric clostridial neurotoxins of the invention may function as analgesics that are capable of treating pain. In particular, the present inventors have shown that a chimeric clostridial neurotoxin as claimed may be efficacious at inhibiting CGRP release from said neurons. Thus, by way of said CGRP release inhibition, the chimeric clostridial neurotoxins of the invention may function as analgesics that are capable of treating CGRP-associated pain.

Without wishing to be bound by theory, it is believed that by blocking the chemical mediators at the point of secretion, the chimeric clostridial neurotoxins of the invention may prevent pain mediators (e.g. CGRP) reaching neighbouring and distal cells. Advantageously, this may provide: selective blockade of pain-related abnormal mediator release, thus preserving the mediator release elsewhere; a therapeutic with a longer duration of action (with fewer side-effects and/or an increased safety window than non-chimeric clostridial neurotoxins); and/or fewer side effects when compared to conventional therapeutics. In particular, blockade of CGRP action once released and/or CGRP receptors by conventional therapeutics can result in nausea, fatigue and increased heart rate, stroke, and/or heart attack. Said side effects may be minimised/avoided by the present invention.

The inventors have additionally found that a chimeric clostridial neurotoxin may be able to cleave SNAP25 in central nervous system structures relevant to migraine pathophysiology. Advantageously, the chimeric clostridial neurotoxin may be particularly efficacious in the treatment of migraine (e.g. migraine pain).

DETAILED DESCRIPTION

In one aspect, the invention provides a chimeric clostridial neurotoxin for use in treating pain, wherein the chimeric clostridial neurotoxin comprises a botulinum neurotoxin A (BoNT/A) light-chain and translocation domain (H_N domain), and a BoNT/B receptor binding domain (H_C domain).

In one aspect, the invention provides a method for treating pain, the method comprising administering to a subject a chimeric clostridial neurotoxin, wherein the chimeric clostridial neurotoxin comprises a botulinum neurotoxin A (BoNT/A) light-chain and translocation domain (H_N domain), and a BoNT/B receptor binding domain (H_C domain).

In one aspect, the invention provides the use of a chimeric clostridial neurotoxin in the manufacture of a medicament for treating pain, wherein the chimeric clostridial neurotoxin comprises a botulinum neurotoxin A (BoNT/A) light-chain and translocation domain (H_N domain), and a BoNT/B receptor binding domain (H_C domain).

In one aspect, the invention provides a chimeric clostridial neurotoxin for use in treating migraine (preferably migraine pain), wherein the chimeric clostridial neurotoxin comprises a botulinum neurotoxin A (BoNT/A) light-chain and translocation domain (H_N domain), and a BoNT/B receptor binding domain (H_C domain).

In one aspect, the invention provides a method for treating migraine (preferably migraine pain), the method comprising administering to a subject a chimeric clostridial neurotoxin, wherein the chimeric clostridial neurotoxin comprises a botulinum neurotoxin A (BoNT/A) light-chain and translocation domain (H_N domain), and a BoNT/B receptor binding domain (H_C domain).

In one aspect, the invention provides the use of a chimeric clostridial neurotoxin in the manufacture of a medicament for treating migraine (preferably migraine pain), wherein the chimeric clostridial neurotoxin comprises a botulinum neurotoxin A (BoNT/A) light-chain and translocation domain (H_N domain), and a BoNT/B receptor binding domain (H_C domain).

The migraine may be episodic migraine or chronic migraine (preferably chronic migraine). A subject may have episodic migraine if the subject experiences headaches (e.g. migraine) on fewer than 15 days per month (e.g. at least 1 but less than 15 days per month), preferably if the subject experiences headaches (e.g. migraine) on at least 4 but less than 15 days per month. In other words, episodic migraine may be defined as headache (e.g. migraine) on fewer than 15 days per month (e.g. at least 1 but less than 15 days per month), preferably as headache (e.g. migraine) on at least 4 but less than 15 days per month. A subject may have chronic migraine if the subject experiences headaches (e.g. migraine) on at least 15 days per month. A subject may have chronic migraine if the subject experiences headaches (e.g. migraine) on at least 15 days per month for at least 3 months, with the features of migraine on at least 8 days per month. In other words, chronic migraine may be defined as headache

(e.g. migraine) on at least 15 days per month. In other words, chronic migraine may be defined as headache (e.g. migraine) on at least 15 days per month for at least 3 months, with the features of migraine on at least 8 days per month. In one embodiment, a chronic migraine may last 4 hours a day or longer. In addition to headache pain, a migraine may be associated with one or more additional symptom(s), including increased light sensitivity, nausea, and/or vomiting.

Preferably when treating migraine, the chimeric clostridial neurotoxin treats migraine pain.

In one aspect, the invention provides a chimeric clostridial neurotoxin for use in treating pain by inhibiting release of a pain mediator from a neuron comprising an Aδ nerve fiber or a C nerve fiber, wherein the chimeric clostridial neurotoxin binds to the neuron comprising the Aδ nerve fiber or the C nerve fiber, respectively, and wherein the chimeric clostridial neurotoxin comprises a botulinum neurotoxin A (BoNT/A) light-chain and translocation domain (H_N domain), and a BoNT/B receptor binding domain (H_C domain).

In a related aspect, the invention provides a method for treating pain by inhibiting release of a pain mediator from a neuron comprising an Aδ nerve fiber or a C nerve fiber, the method comprising administering to a subject a chimeric clostridial neurotoxin, wherein the chimeric clostridial neurotoxin binds to the neuron comprising the Aδ nerve fiber or the C nerve fiber, respectively, and wherein the chimeric clostridial neurotoxin comprises a botulinum neurotoxin A (BoNT/A) light-chain and translocation domain (H_N domain), and a BoNT/B receptor binding domain (H_C domain).

In another related aspect, the invention provides the use of a chimeric clostridial neurotoxin in the manufacture of a medicament for treating pain by inhibiting release of a pain mediator from a neuron comprising an Aδ nerve fiber or a C nerve fiber, wherein the chimeric clostridial neurotoxin binds to the neuron comprising the Aδ nerve fiber or the C nerve fiber, respectively, and wherein the chimeric clostridial neurotoxin comprises a botulinum neurotoxin A (BoNT/A) light-chain and translocation domain (H_N domain), and a BoNT/B receptor binding domain (H_C domain).

In one embodiment, the invention provides a chimeric clostridial neurotoxin for use in treating CGRP-associated pain by inhibiting release of CGRP from a neuron comprising an Aδ nerve fiber or a C nerve fiber, wherein the chimeric clostridial neurotoxin binds to the neuron comprising the Aδ nerve fiber or the C nerve fiber, respectively, and wherein the chimeric

clostridial neurotoxin comprises a botulinum neurotoxin A (BoNT/A) light-chain and translocation domain (H_N domain), and a BoNT/B receptor binding domain (H_C domain). Corresponding methods of treatment and uses are also provided.

A mediator may be any molecule released from a neuron that has a role in a disorder (such as pain). Inhibition of release of said mediator by a chimeric clostridial neurotoxin in accordance with the invention may treat said disorder (e.g. may treat pain).

A mediator may be a neurotransmitter.

The inhibition of release of a mediator from a neuron may be partial or complete inhibition, preferably complete inhibition. For example, the chimeric clostridial neurotoxin may inhibit at least 40%, 50%, 60%, 70%, 80%, 90%, 95% or 99% of the mediator being released from a neuron. Preferably, the chimeric clostridial neurotoxin inhibits 100% of the mediator being released from the neuron.

A pain mediator may be a neurotransmitter.

The inhibition of release of the pain mediator from the neuron may be partial or complete inhibition, preferably complete inhibition. For example, the chimeric clostridial neurotoxin may inhibit at least 40%, 50%, 60%, 70%, 80%, 90%, 95% or 99% of the pain mediator being released from the neuron. Preferably, the chimeric clostridial neurotoxin inhibits 100% of the pain mediator being released from the neuron.

The inhibition is preferably inhibition of SNARE-associated (e.g. SNAP25-associated) release.

The chimeric clostridial neurotoxin of the invention preferably inhibits release of the mediator from the neuron by a greater amount than BoNT/A (preferably native BoNT/A shown as SEQ ID NO: 6 [such as a di-chain form of SEQ ID NO: 6]) inhibits release of the mediator from the neuron. At a given dose (e.g. 1 nM), the chimeric clostridial neurotoxin of the invention may inhibit at least 10% or 20% (preferably at least 30%) more mediator from the neuron than BoNT/A at the same dose (e.g. 1 nM). At a given dose (e.g. 1 nM), the chimeric clostridial neurotoxin of the invention may inhibit 10-90%, or 20-90% (preferably 30-85%) more mediator from the neuron than BoNT/A at the same dose (e.g. 1 nM). Thus, a much lower dose of the chimeric clostridial neurotoxin when compared to BoNT/A may be required to

inhibit the same amount of release of the mediator from the neuron. For example, the dose of chimeric clostridial neurotoxin may be at least 100 times lower, 200 times lower, or 500 times lower, preferably 1000 times lower than the dose of BoNT/A required to inhibit the same amount of release of the mediator from the neuron. The dose of chimeric clostridial neurotoxin may be at least 500-2000 times lower, or 750-1750 times, preferably 1000-1500 times lower than the dose of BoNT/A required to inhibit the same amount of release of the mediator from the neuron.

The chimeric clostridial neurotoxin of the invention preferably inhibits release of the pain mediator from the neuron by a greater amount than BoNT/A (preferably native BoNT/A shown as SEQ ID NO: 6 [such as a di-chain form of SEQ ID NO: 6]) inhibits release of the pain mediator from the neuron. At a given dose (e.g. 1 nM), the chimeric clostridial neurotoxin of the invention may inhibit at least 10% or 20% (preferably at least 30%) more pain mediator from the neuron than BoNT/A at the same dose (e.g. 1 nM). At a given dose (e.g. 1 nM), the chimeric clostridial neurotoxin of the invention may inhibit 10-90%, or 20-90% (preferably 30-85%) more pain mediator from the neuron than BoNT/A at the same dose (e.g. 1 nM). Thus, a much lower dose of the chimeric clostridial neurotoxin when compared to BoNT/A may be required to inhibit the same amount of release of the pain mediator from the neuron. For example, the dose of chimeric clostridial neurotoxin may be at least 100 times lower, 200 times lower, or 500 times lower, preferably 1000 times lower than the dose of BoNT/A required to inhibit the same amount of release of the pain mediator from the neuron. The dose of chimeric clostridial neurotoxin may be at least 500-2000 times lower, or 750-1750 times, preferably 1000-1500 times lower than the dose of BoNT/A required to inhibit the same amount of release of the pain mediator from the neuron.

The chimeric clostridial neurotoxin may inhibit the release of a plurality of mediators from a neuron.

The chimeric clostridial neurotoxin may inhibit the release of a plurality of pain mediators from a neuron.

The chimeric clostridial neurotoxin of the invention preferably has analgesic properties. In other words, a chimeric clostridial neurotoxin of the invention is preferably an analgesic chimeric clostridial neurotoxin.

Preferably, a chimeric clostridial neurotoxin of the invention neither promotes neuronal growth nor neuronal repair to treat pain. In other words, preferably, the chimeric clostridial neurotoxin does not treat pain by any of the following means: by promoting neuronal growth, by promoting neuronal repair, or by promoting neuronal growth and repair.

Preferably, a chimeric clostridial neurotoxin of the invention neither promotes neuronal growth nor neuronal repair to treat a disorder described herein. In other words, preferably, the chimeric clostridial neurotoxin does not treat a disorder described herein by any of the following means: by promoting neuronal growth, by promoting neuronal repair, or by promoting neuronal growth and repair.

The term "promotes neuronal growth and/or neuronal repair" encompasses an increase in the rate of neuronal growth and/or neuronal repair. The term "neuronal growth and/or neuronal repair" encompasses the rebuilding of damaged neuronal circuits, thereby restoring activity and/or neuronal communication in a network or population of neurons. Thus, the term "neuronal repair" as used herein encompasses repair of a specific neuron as well as repair of a neuronal circuit. The term also encompasses neuronal plasticity. The term "neuronal plasticity" as used herein encompasses axonal sprouting, dendritic sprouting, neurogenesis (e.g. the production of new neurons), maturation, differentiation, and/or synaptic plasticity (e.g. including changes to synaptic strength, activity, anatomy, and/or connectivity). The term "promotes neuronal growth and/or neuronal repair" also encompasses promoting the establishment of functional synapses (e.g. at or near to a site of injury). The term "neuronal growth" as used herein encompasses growth of any part of a neuron, including growth of axons and/or dendrites. Said term encompasses an increase in neurite length, neurite number (e.g. number of neurites per cell), and/or an increase in the length and/or numbers of projections from a cell body or cell membrane of a neuron, e.g. axonal growth of a neuron and/or axonal sprouting, e.g. a neuron in a subject. Said axonal growth may promote connections and/or chemical communication between neurons.

Preferably, a chimeric clostridial neurotoxin of the invention does not promote a neuroimmune response to treat pain. Preferably, a chimeric clostridial neurotoxin of the invention does not promote a neuroimmune response to treat a disorder described herein. A neuroimmune response in this context encompasses a microglial response. Thus, in one embodiment a chimeric clostridial neurotoxin of the invention does not promote a microglial response to treat pain. Thus, in one embodiment a chimeric clostridial neurotoxin of the invention does not promote a microglial response to treat a disorder described herein.

In a preferred embodiment, the pain is not pain associated with, or caused by, a brain disorder. In a preferred embodiment, the disorder described herein is not a disorder associated with, or caused by, a brain disorder. The term "brain disorder" used in this context is interchangeable with "brain disease". A "brain disorder" as used in this context encompasses a disorder that originates from within or outside the brain, and includes disorders associated with bodily insults that cause brain tissue damage. Examples of brain disorders encompassed in this context include any one (or more) of traumatic brain injury, cancer (e.g. a brain tumour), infectious disease (e.g. encephalitis, meningitis, a brain abscess, and encephalitis), stroke, a neurodegenerative disorder (e.g. Alzheimer's disease, Parkinson's disease, Parkinson's disease related disorders, motor neuron disease (e.g. amyotrophic lateral sclerosis), prion disease, Huntington's disease, spinocerebellar ataxia, ataxia, Hallervorden-Spatz disease, and frontotemporal lobar degeneration), brain aneurysm, multiple sclerosis, anoxic injury, toxic injury and metabolic injury. A brain disorder may be caused by traumatic brain injury, cancer, infectious disease (e.g. encephalitis, meningitis, a brain abscess, and encephalitis), stroke, a neurodegenerative disorder (e.g. Alzheimer's disease, Parkinson's disease, Parkinson's disease related disorders, motor neuron disease (e.g. amyotrophic lateral sclerosis), prion disease, Huntington's disease, spinocerebellar ataxia, ataxia, Hallervorden-Spatz disease, and frontotemporal lobar degeneration), brain aneurysm, multiple sclerosis, anoxic injury, toxic injury and/or metabolic injury.

The chimeric clostridial neurotoxin preferably binds to a neuron comprising an Aδ fiber or a C fiber. Said binding may be mediated by the BoNT/B H_C domain of the chimeric clostridial neurotoxin (e.g. the H_{CC} portion thereof). Following binding to the neuron, the chimeric clostridial neurotoxin may be internalised via an endosome and the BoNT/A light-chain may be translocated from the endosome into the cytosol of the neuron by the BoNT/A translocation domain. Once in the cytosol, the light-chain may cleave a SNARE protein (e.g. SNAP25), thereby inhibiting release/secretion from said neuron (including release/secretion of a pain mediator from said neuron).

Neurons comprising an Aδ fiber or a C fiber are described in Pichon & Chesler (2014), Frontiers in Neuroanatomy (https://doi.org/10.3389/fnana.2014.00021) and Yam *et al* (2018), Int J Mol Sci, 19, 8, 2164. The term "fiber" (e.g. in the context of an Aδ fiber or a C fiber) preferably refers to an axon of a neuron. Typically, a plurality of fibers (e.g. a plurality of Aδ fibers or a plurality of C fibers, respectively) together may define a greater neural/neuronal structure in a subject, e.g. as a bundle of fibers. For example, a bundle of Aδ fibers or a

bundle of C fibers. Said plurality of fibers may, in some embodiments, include fibers additional to Aδ fibers or C fibers. For example, a nerve may comprise a plurality of neurons, including a neuron comprising an Aδ fiber and/or a neuron comprising a C fiber.

The chimeric clostridial neurotoxin may bind to a neuron comprising an Aδ fiber. Aδ fibers (or neurons comprising the same) may be characterised as being peptidergic, fast conducting, lightly myelinated, involved in sharp/fast pain, involved in nociception and/or involved in temperature sensation. Preferably, an Aδ fiber (or neuron comprising the same) may have a conduction velocity of 5-75 m/s (e.g. 5-35 m/s) and/or a diameter of about 1-5 μm (e.g. 2-5 μm). Neurons comprising an Aδ fiber bound by a chimeric clostridial neurotoxin of the invention are those that are capable of releasing pain mediators. In particular, said neurons may be capable of releasing CGRP and thus have a role in CGRP-associated pain. By binding to a neuron comprising an Aδ fiber, the chimeric clostridial neurotoxin inhibits release of a pain mediator from said neuron by cleaving a SNARE protein (e.g. SNAP25) thereof, thereby inhibiting release/secretion of the pain mediator from said neuron.

The chimeric clostridial neurotoxin may bind to a neuron comprising a C fiber. C fibers (or neurons comprising the same) may be characterised as being peptidergic, low (e.g. slow) conducting, unmyelinated, involved in dull/slow pain, involved in neuropathic pain, involved in thermal sensation, and/or involved in the itch sensation. The neurons comprising a C fiber may be polymodal. Preferably, a C fiber (or neuron comprising the same) may have a conduction velocity of 0.5-2 m/s and/or a diameter of about 0.2-1.5 μm (e.g. 0.2-0.5 μm). Neurons comprising a C fiber bound by a chimeric clostridial neurotoxin of the invention are those that are capable of releasing pain mediators. In particular, said neurons may be capable of releasing CGRP and thus have a role in CGRP-associated pain. By binding to a neuron comprising a C fiber, the chimeric clostridial neurotoxin may inhibit release of a pain mediator from said neuron by cleaving a SNARE protein (e.g. SNAP25) thereof, thereby inhibiting release/secretion of the pain mediator from said neuron.

Preferably a neuron to which a chimeric clostridial neurotoxin binds is a neuron comprising a C fiber.

Expression of tropomyosin receptor kinase A (TrkA) may be a marker for distinguishing a neuron comprising an Aδ nerve fiber or a C nerve fiber (e.g. from a neuron comprising an Aβ fiber). In other words, a neuron comprising an Aδ nerve fiber or a C nerve fiber of the invention may be one that expresses TrkA.

In use, the chimeric clostridial neurotoxin may bind to a plurality of neurons comprising at least a neuron that comprises an Aδ fiber and a neuron that comprises a C fiber. The plurality of neurons may be part of a greater neural/neuronal structure in a subject, e.g. comprising a bundle of fibers.

A neuron comprising an Aδ nerve fiber or a C nerve fiber may be a neuron of the central nervous system (e.g. the hypothalamus, thalamus, periaqueductal grey, superior colliculi, inferior colliculi, amygdala, trigeminocervical complex, and/or the cerebellum) or peripheral nervous system. A chimeric clostridial neurotoxin may inhibit release of a mediator from a neuron of the central nervous system when treating certain conditions, such as headache pain, preferably migraine pain. A chimeric clostridial neurotoxin may inhibit release of a pain mediator from a neuron of the central nervous system when treating certain pain conditions, such as headache pain, preferably migraine pain.

A neuron comprising an Aδ nerve fiber or a C nerve fiber according to the invention is preferably a sensory neuron. The sensory neuron may be a primary sensory neuron, such as a primary afferent neuron. For example, a neuron to which the chimeric clostridial neurotoxin binds may be a sensory neuron of the dorsal route ganglia and/or trigeminal ganglia. Additionally or alternatively, the neuron may be an intrinsic enteric neuron.

The chimeric clostridial neurotoxin of the invention may bind to a neuron comprising an Aδ fiber or C fiber with an affinity that is greater than the affinity with which BoNT/A (preferably native BoNT/A shown as SEQ ID NO: 6 [such as a di-chain form of SEQ ID NO: 6]) binds to the neuron. In particular, the chimeric clostridial neurotoxin of the invention may bind to a neuron comprising an Aδ fiber or C fiber with an affinity that is at least 2x, 5x, 10x, 50x, 100x, 1,000x or 10,000x greater than the affinity with which BoNT/A binds to the neuron.

The chimeric clostridial neurotoxin of the invention may bind to a neuron comprising an Aδ fiber or C fiber with an affinity that is greater than the affinity with which the chimeric clostridial neurotoxin binds to a neuron (preferably sensory neuron) that does not comprise an Aδ fiber or C fiber (e.g. a neuron that comprises an Aβ fiber). For example, the chimeric clostridial neurotoxin of the invention may bind to a neuron comprising an Aδ fiber or C fiber with an affinity that is at least 2x, 5x, 10x, 50x, 100x, 1,000x or 10,000x greater than the affinity with which the chimeric clostridial neurotoxin binds to a neuron (preferably sensory

neuron) that does not comprise an Aδ fiber or C fiber (e.g. a neuron that comprises an Aβ fiber).

Aβ fibers (or neurons comprising the same) may be characterised as being myelinated, fast conducting, involved in touch, and/or responsive to other non-noxious stimuli generally. Preferably, an Aβ fiber (or neuron comprising the same) may have a conduction velocity of 80-120 m/s and/or a diameter of about 6-20 μm. Expression of neurofilament 200 (NF200) may be a marker for distinguishing a neuron comprising an Aβ nerve fiber (e.g. from a neuron comprising an Aδ fiber or a C fiber). In other words, a neuron comprising an Aβ fiber may be one that expresses NF200.

In other embodiments, the chimeric clostridial neurotoxin may be able to exert an effect at a site distal to the site of administration (e.g. injection). For example, following administration of the chimeric clostridial neurotoxin SNARE protein cleavage (e.g. SNAP25 cleavage) may occur at a site distal to the site of administration (e.g. injection). Preferably, such an effect occurs via neuronal transport of the chimeric clostridial neurotoxin from its site of administration to the distal site. When treating pain (preferably headache pain, most preferably migraine pain) or migraine, the chimeric clostridial neurotoxin preferably exerts an effect at a site distal to the site of administration (e.g. injection). In one embodiment, this effect may be additional to a peripheral effect. Accordingly, preferably, the chimeric clostridial neurotoxin may be transported via neuronal transport when treating pain (preferably headache pain, most preferably migraine pain) or migraine.

Neuronal transport may be retrograde transport or anterograde transport, preferably retrograde transport. The transport may be axonal transport.

“Retrograde transport” may be a form of axonal transport (aka. axoplasmic transport or axoplasmic flow); a cellular process normally responsible for movement of mitochondria, lipids, synaptic vesicles, proteins, and other organelles to and from a neuron's cell body, through the cytoplasm of its axon called the axoplasm. Axons are on the order of meters long, such that neurons cannot rely on diffusion to carry products of the nucleus and organelles to the end of their axons, hence the use of axonal transport. Axonal transport may also be responsible for moving molecules destined for degradation from the axon back to the cell body, where they are broken down by lysosomes.

"Retrograde transport" may refer to movement toward the cell body of a neuron and "anterograde transport" may refer to movement toward the synapse of a neuron.

In one embodiment, neuronal (e.g. retrograde) transport to a neuron of the central nervous system may refer to transport (e.g. axonal transport) of the chimeric clostridial neurotoxin toward a neuron cell body that is positioned in the proximity of the central nervous system.

Neuronal (e.g. retrograde) transport is now described in more detail. In one embodiment, the chimeric clostridial neurotoxin may bind to a first neuron (such as a primary sensory afferent) at a site of administration. The chimeric clostridial neurotoxin may be internalised by the first neuron, transported within the first neuron, and then released from the first neuron. Preferably, the clostridial neurotoxin binds to a first neuron at a site of intramuscular or intradermal administration (e.g. intramuscular or intradermal injection). Such a neuron may be a peripheral neuron, preferably a neuron comprising an Aδ fiber or a C fiber. Once released, the chimeric clostridial neurotoxin may bind to a second neuron, be internalised, and cleave a SNARE protein (e.g. SNAP25) within said second neuron. Alternatively, the chimeric clostridial neurotoxin may bind to the second neuron, be internalised by the second neuron, transported within the second neuron, and then released from the second neuron. This process may be repeated until the chimeric clostridial neurotoxin binds to a neuron (e.g. a third neuron), is internalised, and cleaves a SNARE protein (e.g. SNAP25) within said neuron. A second neuron may be a secondary sensory afferent. Preferably, a second neuron is a neuron of the central nervous system, such as a neuron present in the brain, brainstem, or spinal cord. The second neuron may be a neuron present in the trigeminal ganglia (e.g. and SNARE cleavage may occur in an axon thereof).

In some embodiments, when administered intramuscularly, the chimeric clostridial neurotoxin may be neuronally (e.g. retrogradely) transported via a motor neuron, released from the motor neuron, and enter a second neuron, preferably a neuron of the central nervous system. Said neuron may be a sensory neuron.

In one embodiment, when administered intramuscularly, the chimeric clostridial neurotoxin may diffuse to and bind to a sensory neuron present in the periosteum or skin (e.g. terminating in the periosteum or skin).

Without wishing to be bound by theory, it is believed that, by the neuronal (e.g. retrograde) transport mechanism referred to above, the chimeric clostridial neurotoxin of the invention

may inhibit secretion from one or more neurons of the central nervous system. Accordingly, the chimeric clostridial neurotoxin may travel by neuronal (e.g. retrograde) transport to a neuron of the central nervous system and cleaves a SNARE protein (e.g. SNAP25) of said neuron.

In a preferred embodiment, by inhibiting secretion (e.g. inhibiting release of a mediator (e.g. pain mediator) from one or more neuron(s) of the central nervous system, the chimeric clostridial neurotoxin may treat pain or a disorder described herein. This may be particularly relevant in the treatment of pain or migraine, preferably treating migraine pain. The chimeric clostridial neurotoxin may travel by neuronal (e.g. retrograde) transport to the neuron of the central nervous system and cleave a SNARE protein (e.g. SNAP25) of said neuron. Accordingly, the chimeric clostridial neurotoxin may treat pain (e.g. headache pain or migraine pain) or migraine by inhibiting secretion from a neuron of the central nervous system, preferably by inhibiting secretion of a mediator, more preferably a pain mediator from a neuron of the central nervous system.

A neuron of the central nervous system may be a neuron of the brainstem, spinal cord, and/or brain. For example, a neuron of the central nervous system may be a neuron of the: trigeminal nuclei (e.g. the spinal trigeminal nucleus, such as the spinal trigeminal sensory nucleus), spinal cord (preferably a neuron of the dorsal horn of the spinal cord), hypothalamus, thalamus, periaqueductal grey, superior colliculi, inferior colliculi, amygdala, trigeminocervical complex, cortex, and/or the cerebellum. A neuron of the trigeminal nuclei may be a neuron of the trigeminal nucleus caudalis (e.g. pars caudalis).

In a preferred embodiment, the chimeric clostridial neurotoxin cleaves a SNARE protein (e.g. SNAP25) in a neuron of the brainstem, more preferably a neuron of the trigeminal nuclei (even more preferably the spinal trigeminal (sensory) nucleus). The chimeric clostridial neurotoxin may inhibit secretion (e.g. of a mediator, preferably a pain mediator) from said neuron. Cleavage of said SNARE protein may occur via neuronal (e.g. retrograde) transport of the chimeric clostridial neurotoxin from the site of administration. Such a neuron may be targeted by administering the chimeric clostridial neurotoxin to muscles, the periosteum and/or skin innervated by sensory trigeminal neurons (e.g. muscles, the periosteum, and/or skin located in the face and/or scalp of a subject). Alternatively, the neuron may comprise an Aδ nerve fiber or a C nerve fiber, the chimeric clostridial neurotoxin may bind thereto, and then cleave a SNARE protein thereof (e.g. following transport/diffusion through the cytoplasm of the neuron). Said cleavage may be at a neuronal terminal present in the spinal trigeminal

sensory nuclei. Most preferably, said SNARE cleavage and inhibition of secretion results in the treatment of migraine or migraine pain.

In one embodiment, the chimeric clostridial neurotoxin cleaves a SNARE protein (e.g. SNAP25) a neuron of the trigeminal motor nuclei. The chimeric clostridial neurotoxin may inhibit secretion from said neuron. Cleavage of said SNARE protein may occur via neuronal (e.g. retrograde) transport of the chimeric clostridial neurotoxin from the site of administration.

In another preferred embodiment, the chimeric clostridial neurotoxin cleaves a SNARE protein (e.g. SNAP25) in a neuron of the spinal cord, such as the cervical spinal cord. More preferably, said neuron is a neuron present in the dorsal horn (e.g. associated with sensory neurons) of the spinal cord. The chimeric clostridial neurotoxin may inhibit secretion (e.g. of a mediator, preferably a pain mediator) from said neuron. Cleavage of said SNARE protein may occur via neuronal (e.g. retrograde) transport of the chimeric clostridial neurotoxin from the site of administration. Such a neuron may be targeted by administering the chimeric clostridial neurotoxin to muscles, the periosteum and/or skin innervated by sensory spinal neurons (e.g. muscles, the periosteum, and/or skin located at the back of head and/or neck of a subject). Alternatively, the neuron may comprise an Aδ nerve fiber or a C nerve fiber, the chimeric clostridial neurotoxin may bind thereto, and then cleave a SNARE protein thereof (e.g. following transport/diffusion through the cytoplasm of the neuron). Said cleavage may be at a neuronal terminal present in the spinal cord. Most preferably, said SNARE cleavage and inhibition of secretion results in the treatment of migraine or migraine pain.

In one embodiment, the chimeric clostridial neurotoxin cleaves a SNARE protein (e.g. SNAP25) in a neuron of the ventral horn (e.g. associated with motor neurons) of the spinal cord. The chimeric clostridial neurotoxin may inhibit secretion (e.g. of a mediator, preferably a pain mediator) from said neuron. Cleavage of said SNARE protein may occur via neuronal (e.g. retrograde) transport of the chimeric clostridial neurotoxin from the site of administration.

In another preferred embodiment, the chimeric clostridial neurotoxin cleaves a SNARE protein (e.g. SNAP25) in a neuron of the trigeminal ganglia, such as in the axon thereof. The chimeric clostridial neurotoxin may inhibit secretion (e.g. of a mediator, preferably a pain mediator) from said neuron. Cleavage of said SNARE protein may occur via neuronal (e.g. retrograde) transport of the chimeric clostridial neurotoxin from the site of administration.

Alternatively, the neuron may comprise an Aδ nerve fiber or a C nerve fiber, the chimeric clostridial neurotoxin may bind thereto, and then cleave a SNARE protein thereof (e.g. following transport/diffusion through the cytoplasm of the neuron). Most preferably, said SNARE cleavage and inhibition of secretion results in the treatment of migraine or migraine pain.

The neuronal (e.g. retrograde) transport of clostridial neurotoxins has been described (*see* Bomba-Warczak *et al* (2016), Cell Rep., 16(7), 1974-1987) and, without wishing to be bound by theory, is believed to occur by binding of a clostridial neurotoxin to a non-canonical receptor (e.g. in the present case via binding to a receptor other than SYTI or SYTII), incorporation into a non-acidified organelle, neuronal (e.g. retrograde) transport (e.g. away from the periphery of the body towards the central nervous system), and release from the neuron into the extracellular space. In such instances, it has been described that the clostridial neurotoxin may remain intact (i.e. the di-chain comprising an L-chain and H-chain joined together by a di-sulphide bond remains intact), allowing for binding via a canonical intoxication route to a second neuron (e.g. via SYTI or SYTII in the context of a chimeric clostridial neurotoxin of the invention).

A portion of the chimeric clostridial neurotoxin administered to a subject may bind to a neuron comprising the Aδ nerve fiber or the C nerve fiber and inhibit release of a mediator (e.g. pain mediator) from said neuron, and a portion of the chimeric clostridial may exert an effect at a site distal to the site of administration. The portion that exerts its effect at a site distal to the site of administration may inhibit secretion from a neuron of the central nervous system, preferably inhibit secretion of a mediator (e.g. a neurotransmitter), more preferably a pain mediator from a neuron of the central nervous system. The chimeric clostridial neurotoxin may travel by neuronal (e.g. retrograde) transport to the neuron of the central nervous system and cleave a SNARE protein (e.g. SNAP25) of said neuron.

In some embodiments the neuronal (e.g. retrograde) transport of the chimeric clostridial neurotoxin may comprise transynaptic movement (e.g. transcytosis) of the chimeric clostridial neurotoxin from one neuron to another.

Inhibition of secretion from a neuron may be partial or complete inhibition, preferably complete inhibition. For example, the chimeric clostridial neurotoxin may inhibit at least 40%, 50%, 60%, 70%, 80%, 90%, 95% or 99% of secretion from the neuron. Preferably, the

chimeric clostridial neurotoxin inhibits 100% of secretion from the neuron. The secretion in this context is preferably SNARE-associated (e.g. SNAP25-associated) secretion.

In a preferred embodiment, a chimeric clostridial neurotoxin of the invention may treat migraine or a disorder described herein (preferably pain) by inhibiting release of a mediator (e.g. pain mediator) from a neuron comprising an Aδ nerve fiber or a C nerve fiber, wherein the chimeric clostridial neurotoxin binds to the neuron comprising the Aδ nerve fiber or the C nerve fiber, respectively and by inhibiting secretion (e.g. of a mediator, preferably a pain mediator) from a neuron of the central nervous system.

Bacteria in the genus *Clostridia* produce highly potent and specific protein toxins, which can poison neurons and other cells to which they are delivered. Examples of such clostridial toxins include the neurotoxins produced by *C. tetani* (TeNT) and by *C. botulinum* (BoNT) serotypes A-G, and X (*see* WO 2018/009903 A2), as well as those produced by *C. baratii* and *C. butyricum*. Both tetanus and botulinum toxins act by inhibiting the function of affected neurons, specifically the release of neurotransmitters. While botulinum toxin typically acts at the neuromuscular junction and inhibits cholinergic transmission in the peripheral nervous system, tetanus toxin acts in the central nervous system.

In nature, clostridial neurotoxins are synthesised as a single-chain polypeptide that is modified post-translationally by a proteolytic cleavage event to form two polypeptide chains joined together by a disulphide bond. Cleavage occurs at a specific cleavage site, often referred to as the activation site (e.g. activation loop) that is located between the cysteine residues that provide the inter-chain disulphide bond. It is this di-chain form that is the active form of the toxin. The two chains are termed the heavy-chain (H-chain), which has a molecular mass of approximately 100 kDa, and the light-chain (L-chain), which has a molecular mass of approximately 50 kDa. The H-chain comprises an N-terminal translocation component (H_N domain) and a C-terminal targeting component (H_C domain). The cleavage site is located between the L-chain and the translocation domain components. Following binding of the H_C domain to its target neuron and internalisation of the bound toxin into the cell via an endosome, the H_N domain translocates the L-chain across the endosomal membrane and into the cytosol, and the L-chain provides a protease function (also known as a non-cytotoxic protease).

Non-cytotoxic proteases act by proteolytically cleaving intracellular transport proteins known as SNARE proteins (e.g. SNAP25, VAMP, or Syntaxin, preferably SNAP25). The acronym

SNARE derives from the term Soluble NSF Attachment Receptor, where NSF means N-ethylmaleimide-Sensitive Factor. SNARE proteins are integral to intracellular vesicle fusion, and thus to secretion of molecules via vesicle transport from a cell. The protease function is a zinc-dependent endopeptidase activity and exhibits a high substrate specificity for SNARE proteins. Accordingly, once delivered to a desired target cell, the non-cytotoxic protease is capable of inhibiting cellular secretion from the target cell. The L-chain proteases of clostridial neurotoxins are non-cytotoxic proteases that cleave SNARE proteins.

In view of the ubiquitous nature of SNARE proteins, clostridial neurotoxins such as botulinum toxin have been successfully employed in a wide range of therapies.

For further details on the genetic basis of toxin production in *Clostridium botulinum* and *C. tetani*, *see* Henderson *et al* (1997) in *The Clostridia: Molecular Biology and Pathogenesis, Academic press*.

Clostridial neurotoxin domains are described in more detail below.

Examples of L-chain reference sequences include:

Botulinum type A neurotoxin: amino acid residues 1-448

Botulinum type B neurotoxin: amino acid residues 1-440

The above-identified reference sequences should be considered a guide, as slight variations may occur according to sub-serotypes. By way of example, US 2007/0166332 (hereby incorporated by reference in its entirety) cites slightly different clostridial sequences:

Botulinum type A neurotoxin: amino acid residues M1-K448

Botulinum type B neurotoxin: amino acid residues M1-K441

The translocation domain is a fragment of the H-chain of a clostridial neurotoxin approximately equivalent to the amino-terminal half of the H-chain, or the domain corresponding to that fragment in the intact H-chain.

Examples of reference translocation domains include:

Botulinum type A neurotoxin - amino acid residues (449-871)

Botulinum type B neurotoxin - amino acid residues (441-858)

The above-identified reference sequence should be considered a guide as slight variations may occur according to sub-serotypes. By way of example, US 2007/0166332 (hereby incorporated by reference thereto) cites slightly different clostridial sequences:

Botulinum type A neurotoxin - amino acid residues (A449-K871)
Botulinum type B neurotoxin - amino acid residues (A442-S858)

In the context of the present invention, a variety of BoNT/A H_N regions comprising a translocation domain can be useful in aspects of the present invention. The H_N regions from the heavy-chain of BoNT/A are approximately 410-430 amino acids in length and comprise a translocation domain. Research has shown that the entire length of a H_N region from a clostridial neurotoxin heavy-chain is not necessary for the translocating activity of the translocation domain. Thus, aspects of this embodiment can include BoNT/A H_N regions comprising a translocation domain having a length of, for example, at least 350 amino acids, at least 375 amino acids, at least 400 amino acids or at least 425 amino acids. Other aspects of this embodiment can include BoNT/A H_N regions comprising a translocation domain having a length of, for example, at most 350 amino acids, at most 375 amino acids, at most 400 amino acids or at most 425 amino acids.

The term H_N embraces naturally-occurring BoNT/A H_N portions, and modified BoNT/A H_N portions having amino acid sequences that do not occur in nature and/or synthetic amino acid residues. Preferably, said modified BoNT/A H_N portions still demonstrate the above-mentioned translocation function.

Examples of clostridial neurotoxin receptor binding domain (H_C) reference sequences include:

BoNT/A - N872-L1296
BoNT/B - E859-E1291

The ~50 kDa H_C domain of a clostridial neurotoxin (such as a BoNT) comprises two distinct structural features that are referred to as the H_{CC} and H_{CN} domains, each typically of ~25 kDa. Amino acid residues involved in receptor binding are believed to be primarily located in the H_{CC} domain. The H_C domain of a native clostridial neurotoxin may comprise approximately 400-440 amino acid residues. This fact is confirmed by the following publications, each of which is herein incorporated in its entirety by reference thereto: Umland TC (1997) Nat. Struct. Biol. 4: 788-792; Herreros J (2000) Biochem. J. 347: 199-204; Halpern

J (1993) J. Biol. Chem. 268: 15, pp. 11188-11192; Rummel A (2007) PNAS 104: 359-364; Lacey DB (1998) Nat. Struct. Biol. 5: 898-902; Knapp (1998) Am. Cryst. Assoc. Abstract Papers 25: 90; Swaminathan and Eswaramoorthy (2000) Nat. Struct. Biol. 7: 1751-1759; and Rummel A (2004) Mol. Microbiol. 51(3), 631-643.

Examples of (reference) H_{CN} domains include:

Botulinum type A neurotoxin - amino acid residues (872-1110)

Botulinum type B neurotoxin - amino acid residues (859-1097)

The above sequence positions may vary a little according to serotype/ sub-type, and further examples of (reference) H_{CN} domains include:

Botulinum type A neurotoxin - amino acid residues (874-1110)

Botulinum type B neurotoxin - amino acid residues (861-1097)

Examples of (reference) H_{CC} domains include:

Botulinum type A neurotoxin - amino acid residues (Y1111-L1296)

Botulinum type B neurotoxin - amino acid residues (Y1098-E1291)

WO 2017/191315 A1 (which is incorporated herein by reference) teaches chimeric clostridial neurotoxins and methods for preparing and manufacturing the same. Thus, a chimeric clostridial neurotoxin comprising a botulinum neurotoxin A (BoNT/A) light-chain and translocation domain (BoNT/A H_N), and a BoNT/B receptor binding domain (H_C domain) for use in the present invention may be one taught in WO 2017/191315 A1.

The term “chimeric clostridial neurotoxin” or “chimeric neurotoxin” as used herein means a neurotoxin comprising (preferably consisting of) a clostridial neurotoxin light-chain and translocation domain (H_N domain) from a first clostridial neurotoxin serotype and a receptor binding domain (H_C domain) originating from a second different clostridial neurotoxin serotype. Specifically, a chimeric clostridial neurotoxin for use in the invention comprises a botulinum neurotoxin A (BoNT/A) light-chain and translocation domain (H_N domain), and a BoNT/B receptor binding domain (H_C domain). The BoNT/A LH_N domain of the chimeric clostridial neurotoxin is covalently linked to the BoNT/B H_C domain. The chimeric clostridial neurotoxin of the invention may be referred to as a chimeric botulinum neurotoxin. Said chimeric clostridial neurotoxin is also referred to herein as “BoNT/AB”, “mrBoNT/AB” or a “BoNT/AB chimera”.

The L-chain and H_N domain (optionally including a complete or partial activation loop, e.g. a complete activation loop when the chimeric clostridial neurotoxin is in a single-chain form and a cleaved/partial activation loop when in a di-chain form) may be collectively referred to as an LH_N domain. The LH_N domain thus does not further comprise an H_C domain.

The chimeric clostridial neurotoxin may consist essentially of a botulinum neurotoxin A (BoNT/A) light-chain and translocation domain (H_N domain), and a BoNT/B receptor binding domain (H_C domain).

The term "consist(s) essentially of" as used in this context means that the chimeric clostridial neurotoxin does not further comprise one or more amino acid residues that confer additional functionality to the polypeptide, e.g. when administered to a subject. In other words, a polypeptide that "consists essentially of" a botulinum neurotoxin A (BoNT/A) light-chain and translocation domain (H_N domain), and a BoNT/B receptor binding domain (H_C domain) may further comprise one or more amino acid residues (to those of the botulinum neurotoxin A (BoNT/A) light-chain and translocation domain (H_N domain), and BoNT/B receptor binding domain (H_C domain)) but said one or more further amino acid residues do not confer additional functionality to the polypeptide, e.g. when administered to a subject. Additional functionality may include enzymatic activity, binding activity and/or any physiological activity whatsoever.

The chimeric clostridial neurotoxin may comprise non-clostridial neurotoxin sequences in addition to any clostridial neurotoxin sequences so long as the non-clostridial neurotoxin sequences do not disrupt the ability of the chimeric clostridial neurotoxin to achieve its therapeutic effect (preferably to treat pain). Preferably, the non-clostridial neurotoxin sequence is not one having catalytic activity, e.g. enzymatic activity. In one embodiment the chimeric clostridial neurotoxin of the invention does not comprise a non-clostridial catalytically active domain. In one embodiment, a chimeric clostridial neurotoxin does not comprise a further catalytically active domain. In one embodiment, the non-clostridial sequence is not one that binds to a cellular receptor. In other words, in one embodiment, the non-clostridial sequence is not a ligand for a cellular receptor. A cellular receptor may be a proteinaceous cellular receptor, such as an integral membrane protein. Examples of cellular receptors can be found in the IUPHAR Guide to Pharmacology Database, version 2019.4, available at https://www.guidetopharmacology.org/download.jsp#db_reports. Non-clostridial neurotoxin sequences may include tags to aid in purification, such as His-tags. In one

embodiment, a chimeric clostridial neurotoxin of the invention does not comprise a label or a site for adding a label, such as a sortase acceptor or donor site.

Preferably, a chimeric clostridial neurotoxin may consist of a botulinum neurotoxin A (BoNT/A) light-chain and translocation domain (H_N domain), and a BoNT/B receptor binding domain (H_C domain).

The chimeric clostridial neurotoxin comprises a light-chain that is capable of exhibiting non-cytotoxic protease activity and of cleaving a SNARE protein in the cytosol of a target neuron. As explained above, the di-chain form is the active form of a clostridial neurotoxin. Thus, the invention excludes the use of a chimeric clostridial neurotoxin comprising a light-chain that has been catalytically inactivated (a "catalytically inactive light-chain"), e.g. by way of one or more mutations. Such catalytically inactive light-chains (and clostridial neurotoxins comprising the same) are known in the art. A catalytically inactive L-chain may have one or more mutations that inactivate said catalytic activity. For example, a catalytically inactive L-chain may comprise a mutation of an active site residue. A mutation may be a substitution or a deletion, in particular a substitution with a chemically-similar amino acid. Glutamic acid may be substituted with glutamine, histidine may be substituted with tyrosine, arginine may be substituted with glutamine, and/or tyrosine may be substituted with phenylalanine. Alternatively, any residue may be substituted with alanine. A catalytically inactive BoNT/A L-chain may comprise a mutation at H223, E224, H227, E262, R363, and/or Y366, e.g. a mutation of at least E224 and H227. A catalytically inactive BoNT/A L-chain may comprise a substitution at E224 with glutamine (E224Q) and substitution at H227 with tyrosine (H227Y).

The term "catalytically inactive" as used herein in respect of a clostridial neurotoxin L-chain means that said L-chain exhibits substantially no non-cytotoxic protease activity, e.g. no non-cytotoxic protease activity. A catalytically inactive clostridial neurotoxin L-chain may be one that does not cleave a protein of the exocytic fusion apparatus in a target cell. The term "substantially no non-cytotoxic protease activity" means that the clostridial neurotoxin L-chain has less than 5% of the non-cytotoxic protease activity of a catalytically active clostridial neurotoxin L-chain (preferably an L-chain of native BoNT/A shown as SEQ ID NO: 6), for example less than 2%, 1% or less than 0.1% of the non-cytotoxic protease activity of a catalytically active clostridial neurotoxin L-chain. Non-cytotoxic protease activity can be determined *in vitro* by incubating a test clostridial neurotoxin L-chain with a SNARE protein and comparing the amount of SNARE protein cleaved by the test clostridial neurotoxin L-chain when compared to the amount of SNARE protein cleaved by a catalytically active

clostridial neurotoxin L-chain (preferably an L-chain of native BoNT/A shown as SEQ ID NO: 6) under the same conditions. Routine techniques, such as SDS-PAGE and Western blotting can be used to quantify the amount of SNARE protein cleaved. Suitable *in vitro* assays are described in WO 2019/145577 A1, which is incorporated herein by reference.

Cell-based and *in vivo* assays may also be used to determine if a clostridial neurotoxin comprising an L-chain and a functional cell binding and translocation domain has non-cytotoxic protease activity. Assays such as the Digit Abduction Score (DAS) assay, the dorsal root ganglia (DRG) assay, spinal cord neuron (SCN) assay, and mouse phrenic nerve hemidiaphragm (PNHD) assay are routine in the art. A suitable assay for determining non-cytotoxic protease activity may be one described in Aoki KR, Toxicon 39: 1815-1820; 2001 or Donald *et al* (2018), Pharmacol Res Perspect, e00446, 1-14, which are incorporated herein by reference.

When administered to a subject, a chimeric clostridial neurotoxin is preferably in its active di-chain form where the light-chain and heavy-chain are joined together by a disulphide bond. Where a clostridial neurotoxin (e.g. chimeric clostridial neurotoxin) is defined herein by way of a polypeptide sequence (SEQ ID NO), an L-chain portion of the sequence (SEQ ID NO) may constitute a first chain of the di-chain clostridial neurotoxin (e.g. di-chain chimeric clostridial neurotoxin) and the H_N and H_C domains together may constitute a second chain of the di-chain clostridial neurotoxin (e.g. di-chain chimeric clostridial neurotoxin), wherein the first and second chains are joined together by a di-sulphide bond. The skilled person will appreciate that a protease may cleave at one or more positions within the activation loop of the clostridial neurotoxin (e.g. chimeric clostridial neurotoxin), preferably at two positions within the activation loop. Where cleavage occurs at more than one position (preferably at two positions) within the activation loop, a small fragment of the C-terminal L-chain portion of the sequence may be absent from the di-chain clostridial neurotoxin sequence (e.g. di-chain chimeric clostridial neurotoxin). In view of this, the sequence of the di-chain clostridial neurotoxin (e.g. di-chain chimeric clostridial neurotoxin) may be slightly different to that of the corresponding single-chain clostridial neurotoxin (e.g. single-chain chimeric clostridial neurotoxin). The small fragment may be 1-15 amino acids. In particular, in one embodiment, when Lys-C is used to covert a single-chain chimeric clostridial neurotoxin into a di-chain clostridial neurotoxin, the small fragment of the C-terminal L-chain portion of the sequence that is absent may be SEQ ID NO: 15 or 16.

The C-terminal amino acid residue of the LH_N domain may correspond to the first amino acid residue of the 3_{10} helix separating the LH_N and H_C domains of BoNT/A, and the N-terminal amino acid residue of the H_C domain may correspond to the second amino acid residue of the 3_{10} helix separating the LH_N and H_C domains in BoNT/B.

An example of a BoNT/A polypeptide sequence is provided as SEQ ID NO: 6.

An example of a BoNT/B polypeptide sequence is provided as SEQ ID NO: 7 (UniProt accession number B1INP5).

Reference herein to the "first amino acid residue of the 3_{10} helix separating the LH_N and H_C domains of BoNT/A" means the N-terminal residue of the 3_{10} helix separating the LH_N and H_C domains.

Reference herein to the "second amino acid residue of the 3_{10} helix separating the LH_N and H_C domains of BoNT/B" means the amino acid residue following the N-terminal residue of the 3_{10} helix separating the LH_N and H_C domains.

A "3_{10} helix" is a type of secondary structure found in proteins and polypeptides, along with α-helices, β-sheets and reverse turns. The amino acids in a 3_{10} helix are arranged in a right-handed helical structure where each full turn is completed by three residues and ten atoms that separate the intramolecular hydrogen bond between them. Each amino acid corresponds to a 120° turn in the helix (i.e., the helix has three residues per turn), and a translation of 2.0 Å (= 0.2 nm) along the helical axis, and has 10 atoms in the ring formed by making the hydrogen bond. Most importantly, the N-H group of an amino acid forms a hydrogen bond with the C = O group of the amino acid three residues earlier; this repeated i + 3 → i hydrogen bonding defines a 3_{10} helix. A 3_{10} helix is a standard concept in structural biology with which the skilled person is familiar.

This 3_{10} helix corresponds to four residues which form the actual helix and two cap (or transitional) residues, one at each end of these four residues. The term "3_{10} helix separating the LH_N and H_C domains" as used herein consists of those 6 residues.

Through carrying out structural analyses and sequence alignments, a 3_{10} helix separating the LH_N and H_C domains was identified. This 3_{10} helix is surrounded by an α-helix at its N-terminus (i.e. at the C-terminal part of the LH_N domain) and by a β-strand at its C-terminus

(i.e. at the N-terminal part of the H_C domain). The first (N-terminal) residue (cap or transitional residue) of the 3_{10} helix also corresponds to the C-terminal residue of this α-helix.

The 3_{10} helix separating the LH_N and H_C domains can be for example determined from publicly available crystal structures of botulinum neurotoxins, for example 3BTA (http://www.rcsb.org/pdb/explore/explore.do?structureId=3BTA) and 1EPW (http://www.rcsb.org/pdb/explore/explore.do?structureId=1EPW) for botulinum neurotoxins A1 and B1 respectively.

In silico modelling and alignment tools which are publicly available can also be used to determine the location of the 3_{10} helix separating the LH_N and H_C domains in other neurotoxins, for example the homology modelling servers LOOPP (Learning, Observing and Outputting Protein Patterns, http://loopp.org), PHYRE (Protein Homology/analogY Recognition Engine, http://www.sbg.bio.ic.ac.uk/phyre2/) and Rosetta (https://www.rosettacommons.org/), the protein superposition server SuperPose (http://wishart.biology.ualberta.ca/superpose/), the alignment program Clustal Omega (http://www.clustal.org/omega/), and a number of other tools/services listed at the Internet Resources for Molecular and Cell Biologists (http://molbiol-tools.ca/). In particular, the region around the "H_N/H_{CN}" junction may be structurally highly conserved which renders it an ideal region to superimpose different serotypes.

For example, the following methodology may be used to determine the sequence of this 3_{10} helix in other neurotoxins:

1. The structural homology modelling tool LOOP (http://loopp.org) may be used to obtain a predicted structure of other BoNT serotypes based on the BoNT/A1 crystal structure (3BTA.pdb);
2. The structural (pdb) files thus obtained may be edited to include only the N-terminal end of the H_{CN} domain and about 80 residues before it (which are part of the H_N domain), thereby retaining the "H_N/H_{CN}" region which is structurally highly conserved;
3. The protein superposition server SuperPose (http://wishart.biology.ualberta.ca/superpose/) may be used to superpose each serotype onto the 3BTA.pdb structure;
4. The superposed pdb files may be inspected to locate the 3_{10} helix at the start of the H_C domain of BoNT/A1, and corresponding residues in the other serotype may then be identified.

5. The other BoNT serotype sequences may be aligned with Clustal Omega in order to check that corresponding residues are correct.

Examples of LH_N, H_C and 3_{10} helix domains determined by this method are presented below:

Neurotoxin	**Accession Number (Plus Sequence Version after Decimal)**	**LH_N**	**H_C**	**3_{10} helix**
BoNT/A1 (SEQ ID NO: 6)	A5HZZ9.1	1-872	873-1296	^{872}NIINTS^{877}
BoNT/A2	X73423.3	1-872	873-1296	^{872}NIVNTS^{877}
BoNT/A3	DQ185900.1 (aka Q3LRX9.1)	1-872	873-1292	^{872}NIVNTS^{877}
BoNT/A4	EU341307.1 (aka Q3LRX8.1)	1-872	873-1296	^{872}NITNAS^{877}
BoNT/A5	EU679004.1 (aka C1IPK2.1)	1-872	873-1296	^{872}NIINTS^{877}
BoNT/A6	FJ981696.1	1-872	873-1296	^{872}NIINTS^{877}
BoNT/A7	JQ954969.1 (aka K4LN57.1)	1-872	873-1296	^{872}NIINTS^{877}
BoNT/A8	KM233166.1	1-872	873-1297	^{872}NITNTS^{877}
BoNT/B1 (SEQ ID NO: 7)	B1INP5.1	1-859	860-1291	^{859}EILNNI^{864}
BoNT/B2	AB084152.1 (aka Q8GR96.1)	1-859	860-1291	^{859}EILNNI^{864}
BoNT/B3	EF028400.1 (aka A2I2S2.1)	1-859	860-1291	^{859}EILNNI^{864}
BoNT/B4	EF051570.1 (aka A2I2W0.1)	1-859	860-1291	^{859}EILNNI^{864}
BoNT/B5	EF033130.1 (aka A2I2U6.1)	1-859	860-1291	^{859}DILNNI^{864}
BoNT/B6	AB302852.1 (aka A8R089.1)	1-859	860-1291	^{859}EILNNI^{864}

Neurotoxin	Accession Number (Plus Sequence Version after Decimal)	LH_N	H_C	3_{10} helix
BoNT/B7	JQ354985.1 (aka H9CNK9.1)	1-859	860-1291	^{859}EILNNI^{864}
BoNT/B8	JQ964806.1 (aka I6Z8G9.1)	1-859	860-1292	^{859}EILNNI^{864}

Using structural analysis and sequence alignments, it was found that the β-strand following the 3_{10} helix separating the LH_N and H_C domains is a conserved structure in all botulinum and tetanus neurotoxins and starts at the 8th residue when starting from the first residue of the 3_{10} helix separating the LH_N and H_C domains (e.g., at residue 879 for BoNT/A1).

A BoNT/AB chimera may comprise an LH_N domain from BoNT/A covalently linked to a H_C domain from BoNT/B, wherein the C-terminal amino acid residue of the LH_N domain corresponds to the eighth amino acid residue N-terminally to the β-strand located at the beginning (N-term) of the H_C domain of BoNT/A, and wherein the N-terminal amino acid residue of the H_C domain corresponds to the seventh amino acid residue N-terminally to the β-strand located at the beginning (N-term) of the H_C domain of BoNT/B.

A BoNT/AB chimera may comprise an LH_N domain from BoNT/A covalently linked to a H_C domain from BoNT/B, wherein the C-terminal amino acid residue of the LH_N domain corresponds to the C-terminal amino acid residue of the α-helix located at the end (C-terminus) of the LH_N domain of BoNT/A, and wherein the N-terminal amino acid residue of the H_C domain corresponds to the amino acid residue immediately C-terminal to the C-terminal amino acid residue of the α-helix located at the end (C-terminus) of the LH_N domain of BoNT/B.

The rationale of the design process of the BoNT/AB chimera was to try to ensure that the secondary structure was not compromised and thereby minimise any changes to the tertiary structure and to the function of each domain. Without wishing to be bound by theory, it is hypothesized that by not disrupting the four central amino acid residues of the 3_{10} helix in the BoNT/AB chimera ensures an optimal conformation for the chimeric neurotoxin, thereby allowing for the chimeric neurotoxin to exert its functions to their full capacity. In fact, surprisingly, retaining solely the first amino acid residue of the 3_{10} helix of the BoNT/A and

the second amino acid residue of the 3_{10} helix onwards of BoNT/B not only allows the production of soluble and functional BoNT/AB chimera, but further leads to improved properties over other BoNT/AB chimeras, in particular an increased potency, an increased Safety Ratio and/or a longer duration of action (as well as an increased Safety Ratio and/or duration of action when compared to native BoNT/A [e.g. SEQ ID NO: 6]).

Undesired effects of a neurotoxin (caused by diffusion of the neurotoxin away from the site of administration) can be assessed experimentally by measuring percentage bodyweight loss in a relevant animal model (e.g. a mouse, where loss of bodyweight is detected within seven days of administration). Conversely, desired on-target effects of a neurotoxin can be assessed experimentally by the Digital Abduction Score (DAS) assay, a measurement of muscle paralysis. The DAS assay may be performed by injection of 20 µL of neurotoxin, formulated in Gelatin Phosphate Buffer, into the mouse gastrocnemius/soleus complex, followed by assessment of Digital Abduction Score using the method of Aoki (Aoki KR, Toxicon 39: 1815-1820; 2001). In the DAS assay, mice are suspended briefly by the tail in order to elicit a characteristic startle response in which the mouse extends its hind limbs and abducts its hind digits. Following neurotoxin injection, the varying degrees of digit abduction are scored on a five point scale (0=normal to 4=maximal reduction in digit abduction and leg extension).

The Safety Ratio of a neurotoxin may then be expressed as the ratio between the amount of neurotoxin required for a 10% drop in a bodyweight of a mouse (measured at peak effect within the first seven days after dosing in a mouse) and the amount of neurotoxin required for a DAS score of 2. High Safety Ratio scores are therefore desired, and indicate a neurotoxin that is able to effectively paralyse a target muscle with little undesired off-target effects.

A high Safety Ratio is particularly advantageous in therapy because it represents an increase in the therapeutic index. In other words, this means that reduced dosages can be used compared to alternative clostridial neurotoxin therapeutics and/or that increased dosages can be used without any additional (e.g. deleterious) effects. Deleterious effects may include systemic toxicity and/or undesired spread to adjacent muscles. The possibility to use higher doses of neurotoxin without additional effects is particularly advantageous as higher doses usually lead to a longer duration of action of the neurotoxin.

The potency of a chimeric clostridial neurotoxin may be expressed as the minimal dose of neurotoxin which leads to a given DAS score when administered to a mouse

gastrocnemius/soleus complex, for example a DAS score of 2 (ED_{50} dose) or a DAS score of 4. The Potency of a chimeric clostridial neurotoxin may be also expressed as the EC_{50} dose in a cellular assay measuring SNARE cleavage by the neurotoxin, for example the EC_{50} dose in a cellular assay measuring SNAP25 cleavage by a chimeric clostridial neurotoxin.

The duration of action of a chimeric clostridial neurotoxin may be expressed as the time required for retrieving a DAS score of 0 after administration of a given dose of neurotoxin, for example the minimal dose of neurotoxin leading to a DAS score of 4, to a mouse gastrocnemius/soleus complex.

The chimeric clostridial neurotoxin may have a Safety Ratio of greater than 7, wherein the Safety Ratio is calculated as: dose of toxin required for -10% bodyweight change measured as pg/mouse divided by DAS ED_{50} measured as pg/mouse, wherein ED_{50} = dose required to produce a DAS score of 2. For example, a chimeric clostridial neurotoxin may have a Safety Ratio of at least 8, 9, 10, 15, 20, 25, 30, 35, 40, 45 or 50.

Preferably, the chimeric clostridial neurotoxin has a Safety Ratio of at least 10 (e.g. a Safety Ratio of 10), more preferably at least 12 or 13 (e.g. 14-15). The chimeric clostridial neurotoxin may have a Safety Ratio of greater than 7 up to 50 e.g. 8-45, 10-20 or 12-15.

The chimeric clostridial neurotoxin of the invention preferably has a longer duration of action (e.g. an improvement in one or more symptoms of at least 5%, 10%, 25%, or 50%) when compared to BoNT/A (e.g. SEQ ID NO: 6, such as SEQ ID NO: 6 in a di-chain form). Said duration of action may be at least 1.25x, 1.5x, 1.75x, 2.0x, or 2.25x greater. The duration of action of said chimeric clostridial neurotoxin may be between 4.5 and 9 months or between 6 and 9 months. For example, a duration of action may be at least 4.5 months (from onset), 5.0 months, 5.5 months, 6 months, 6.5 months, 7.0 months, 7.5 months, 8.0 months, 8.5 months, or 9.0 months. In particular embodiments, a duration of action may be greater than 9.0 months.

Thus, in one embodiment, a chimeric clostridial neurotoxin may treat a disorder (e.g. pain or a sensory disorder, preferably pain) of a subject for a longer duration (e.g. from administration) than that of a subject treated with BoNT/A (e.g. SEQ ID NO: 6, such as SEQ ID NO: 6 in a di-chain form). Said duration may be a duration from administration that is consistent with the duration of action of a chimeric clostridial neurotoxin of the invention. Thus, a chimeric clostridial neurotoxin may treat a disorder of a subject for a duration from

administration that is at least 1.25x, 1.5x, 1.75x, 2.0x, or 2.25x greater than the duration of treatment from administration with BoNT/A (e.g. SEQ ID NO: 6, such as SEQ ID NO: 6 in a di-chain form). A chimeric clostridial neurotoxin may treat a disorder of a subject for a duration from administration of between 4.5 and 9 months or between 6 and 9 months, for example, at least 4.5 months, 5.0 months, 5.5 months, 6 months, 6.5 months, 7.0 months, 7.5 months, 8.0 months, 8.5 months, or 9.0 months from administration. In particular embodiments, a chimeric clostridial neurotoxin may treat a disorder of a subject for a duration from administration of greater than 9.0 months.

Thus, in one aspect, the invention provides a method for treating a disorder (e.g. pain or a sensory disorder, preferably pain) of a subject for a longer duration (e.g. from administration) than that of a subject treated with BoNT/A (e.g. SEQ ID NO: 6, such as SEQ ID NO: 6 in a di-chain form), the method comprising administering a chimeric clostridial neurotoxin to the subject, wherein the chimeric clostridial neurotoxin inhibits release of a mediator (e.g. a pain mediator) from a neuron comprising an Aδ nerve fiber or a C nerve fiber, wherein the chimeric clostridial neurotoxin binds to the neuron comprising the Aδ nerve fiber or the C nerve fiber, respectively, and wherein the chimeric clostridial neurotoxin comprises a botulinum neurotoxin A (BoNT/A) light-chain and translocation domain (H_N domain), and a BoNT/B receptor binding domain (H_C domain).

In a related aspect, the invention provides a chimeric clostridial neurotoxin for use in a method for treating a disorder (e.g. pain or a sensory disorder, preferably pain) of a subject for a longer duration (e.g. from administration) than that of a subject treated with BoNT/A (e.g. SEQ ID NO: 6, such as SEQ ID NO: 6 in a di-chain form), the method comprising administering a chimeric clostridial neurotoxin to the subject, wherein the chimeric clostridial neurotoxin inhibits release of a mediator (e.g. a pain mediator) from a neuron comprising an Aδ nerve fiber or a C nerve fiber, wherein the chimeric clostridial neurotoxin binds to the neuron comprising the Aδ nerve fiber or the C nerve fiber, respectively, and wherein the chimeric clostridial neurotoxin comprises a botulinum neurotoxin A (BoNT/A) light-chain and translocation domain (H_N domain), and a BoNT/B receptor binding domain (H_C domain).

In another related aspect, the invention provides the use of a chimeric clostridial neurotoxin in the manufacture of a medicament for treating a disorder (e.g. pain or a sensory disorder, preferably pain) of a subject for a longer duration (e.g. from administration) than that of a subject treated with BoNT/A (e.g. SEQ ID NO: 6, such as SEQ ID NO: 6 in a di-chain form), wherein the chimeric clostridial neurotoxin inhibits release of a mediator (e.g. a pain

mediator) from a neuron comprising an Aδ nerve fiber or a C nerve fiber, wherein the chimeric clostridial neurotoxin binds to the neuron comprising the Aδ nerve fiber or the C nerve fiber, respectively, and wherein the chimeric clostridial neurotoxin comprises a botulinum neurotoxin A (BoNT/A) light-chain and translocation domain (H_N domain), and a BoNT/B receptor binding domain (H_C domain).

Thus, in one aspect, the invention provides a method for treating a disorder (e.g. pain or a sensory disorder, preferably pain) of a subject for a longer duration (e.g. from administration) than that of a subject treated with BoNT/A (e.g. SEQ ID NO: 6, such as SEQ ID NO: 6 in a di-chain form), the method comprising administering a chimeric clostridial neurotoxin to the subject, wherein the chimeric clostridial neurotoxin comprises a botulinum neurotoxin A (BoNT/A) light-chain and translocation domain (H_N domain), and a BoNT/B receptor binding domain (H_C domain).

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In another related aspect, the invention provides the use of a chimeric clostridial neurotoxin in the manufacture of a medicament for treating a disorder (e.g. pain or a sensory disorder, preferably pain) of a subject for a longer duration (e.g. from administration) than that of a subject treated with BoNT/A (e.g. SEQ ID NO: 6, such as SEQ ID NO: 6 in a di-chain form), wherein the chimeric clostridial neurotoxin comprises a botulinum neurotoxin A (BoNT/A) light-chain and translocation domain (H_N domain), and a BoNT/B receptor binding domain (H_C domain).

The disorder is preferably migraine or migraine pain.

The term “treat a disorder of a subject for a longer duration (e.g. from administration) than that of a subject treated with BoNT/A” or “treating a disorder of a subject for a longer duration (e.g. from administration) than that of a subject treated with BoNT/A” may mean that one or more symptoms of the disorder of the subject are reduced for a longer time period following

administration of the chimeric clostridial neurotoxin of the invention, when compared to administration of BoNT/A. Said duration of action may be at least 1.25x, 1.5x, 1.75x, 2.0x, or 2.25x greater. The duration of action of chimeric clostridial neurotoxin may be between 6 and 9 months. For example, a duration of action may be at least: 4.5 months (from onset), 5.0 months, 5.5 months, 6 months, 6.5 months, 7.0 months, 7.5 months, 8.0 months, 8.5 months or 9.0 months. In particular embodiments, a duration of action may be greater than 9.0 months. Said reduction may be determined by comparison to an equivalent control subject exhibiting equivalent symptoms that has been treated with BoNT/A. At a time period where the severity of one or more symptoms of the control subject are substantially the same (e.g. the same) as before BoNT/A treatment, a subject treated with the chimeric clostridial neurotoxin according to the invention may exhibit an improvement in the equivalent one or more symptoms of at least 5%, 10%, 25%, or 50% when compared to the severity of the one or more symptoms before treatment with the chimeric clostridial neurotoxin.

In one embodiment, a chimeric clostridial neurotoxin may treat a disorder (e.g. pain or a sensory disorder, preferably pain) of a subject with greater efficacy than that of a subject treated with BoNT/A (e.g. SEQ ID NO: 6, such as SEQ ID NO: 6 in a di-chain form).

Thus, in one aspect, the invention provides a method for treating a disorder (e.g. pain or a sensory disorder, preferably pain) of a subject with greater efficacy than that of a subject treated with BoNT/A (e.g. SEQ ID NO: 6, such as SEQ ID NO: 6 in a di-chain form), the method comprising administering a chimeric clostridial neurotoxin to the subject, wherein the chimeric clostridial neurotoxin inhibits release of a mediator (e.g. a pain mediator) from a neuron comprising an Aδ nerve fiber or a C nerve fiber, wherein the chimeric clostridial neurotoxin binds to the neuron comprising the Aδ nerve fiber or the C nerve fiber, respectively, and wherein the chimeric clostridial neurotoxin comprises a botulinum neurotoxin A (BoNT/A) light-chain and translocation domain (H_N domain), and a BoNT/B receptor binding domain (H_C domain).

In a related aspect, the invention provides a chimeric clostridial neurotoxin for use in a method for treating a disorder (e.g. pain or a sensory disorder, preferably pain) of a subject with greater efficacy than that of a subject treated with BoNT/A (e.g. SEQ ID NO: 6, such as SEQ ID NO: 6 in a di-chain form), the method comprising administering a chimeric clostridial neurotoxin to the subject, wherein the chimeric clostridial neurotoxin inhibits release of a mediator (e.g. a pain mediator) from a neuron comprising an Aδ nerve fiber or a C nerve fiber, wherein the chimeric clostridial neurotoxin binds to the neuron comprising the Aδ nerve

fiber or the C nerve fiber, respectively, and wherein the chimeric clostridial neurotoxin comprises a botulinum neurotoxin A (BoNT/A) light-chain and translocation domain (H_N domain), and a BoNT/B receptor binding domain (H_C domain).

In another related aspect, the invention provides the use of a chimeric clostridial neurotoxin in the manufacture of a medicament for treating a disorder (e.g. pain or a sensory disorder, preferably pain) of a subject with greater efficacy than that of a subject treated with BoNT/A (e.g. SEQ ID NO: 6, such as SEQ ID NO: 6 in a di-chain form), wherein the chimeric clostridial neurotoxin inhibits release of a mediator (e.g. a pain mediator) from a neuron comprising an Aδ nerve fiber or a C nerve fiber, wherein the chimeric clostridial neurotoxin binds to the neuron comprising the Aδ nerve fiber or the C nerve fiber, respectively, and wherein the chimeric clostridial neurotoxin comprises a botulinum neurotoxin A (BoNT/A) light-chain and translocation domain (H_N domain), and a BoNT/B receptor binding domain (H_C domain).

Thus, in one aspect, the invention provides a method for treating a disorder (e.g. pain or a sensory disorder, preferably pain) of a subject with greater efficacy than that of a subject treated with BoNT/A (e.g. SEQ ID NO: 6, such as SEQ ID NO: 6 in a di-chain form), the method comprising administering a chimeric clostridial neurotoxin to the subject, wherein the chimeric clostridial neurotoxin comprises a botulinum neurotoxin A (BoNT/A) light-chain and translocation domain (H_N domain), and a BoNT/B receptor binding domain (H_C domain).

In a related aspect, the invention provides a chimeric clostridial neurotoxin for use in a method for treating a disorder (e.g. pain or a sensory disorder, preferably pain) of a subject with greater efficacy than that of a subject treated with BoNT/A (e.g. SEQ ID NO: 6, such as SEQ ID NO: 6 in a di-chain form), the method comprising administering a chimeric clostridial neurotoxin to the subject, wherein the chimeric clostridial neurotoxin comprises a botulinum neurotoxin A (BoNT/A) light-chain and translocation domain (H_N domain), and a BoNT/B receptor binding domain (H_C domain).

In another related aspect, the invention provides the use of a chimeric clostridial neurotoxin in the manufacture of a medicament for treating a disorder (e.g. pain or a sensory disorder, preferably pain) of a subject with greater efficacy than that of a subject treated with BoNT/A (e.g. SEQ ID NO: 6, such as SEQ ID NO: 6 in a di-chain form), wherein the chimeric clostridial neurotoxin comprises a botulinum neurotoxin A (BoNT/A) light-chain and translocation domain (H_N domain), and a BoNT/B receptor binding domain (H_C domain).

The disorder is preferably migraine or migraine pain.

The term “treat a disorder of a subject with greater efficacy than that of a subject treated with BoNT/A” or “treating a disorder of a subject with greater efficacy than that of a subject treated with BoNT/A” may mean that one or more symptoms of the disorder of the subject are reduced by a greater amount following administration of the chimeric clostridial neurotoxin of the invention, when compared to administration of BoNT/A. Said reduction may be determined by comparison to an equivalent control subject exhibiting equivalent symptoms that has been treated with BoNT/A. At a given time period following administration, a subject treated with the chimeric clostridial neurotoxin according to the invention may exhibit a reduction in severity of one or more symptoms of at least 5%, 10%, 25%, or 50% when compared to the severity of the equivalent one or more symptoms of a control subject at the same time period following administration of BoNT/A (e.g. SEQ ID NO: 6, such as SEQ ID NO: 6 in a di-chain form). In another embodiment, greater efficacy may mean that a maximal reduction in severity of one or more symptoms of a subject treated with the chimeric clostridial neurotoxin is greater than the maximal reduction in severity of the equivalent one or more symptoms of a control subject treated with BoNT/A (e.g. SEQ ID NO: 6, such as SEQ ID NO: 6 in a di-chain form).

In one embodiment, a chimeric clostridial neurotoxin may reduce pain (e.g. migraine pain) of a subject by a greater amount than that of a subject treated with BoNT/A (e.g. SEQ ID NO: 6, such as SEQ ID NO: 6 in a di-chain form).

Thus, in one aspect, the invention provides a method for reducing pain of a subject by a greater amount than that of a subject treated with BoNT/A (e.g. SEQ ID NO: 6, such as SEQ ID NO: 6 in a di-chain form), the method comprising administering a chimeric clostridial neurotoxin to the subject, wherein the chimeric clostridial neurotoxin inhibits release of a pain mediator from a neuron comprising an Aδ nerve fiber or a C nerve fiber, wherein the chimeric clostridial neurotoxin binds to the neuron comprising the Aδ nerve fiber or the C nerve fiber, respectively, and wherein the chimeric clostridial neurotoxin comprises a botulinum neurotoxin A (BoNT/A) light-chain and translocation domain (H_N domain), and a BoNT/B receptor binding domain (H_C domain).

In a related aspect, the invention provides a chimeric clostridial neurotoxin for use in a method for reducing pain of a subject by a greater amount than that of a subject treated with

BoNT/A (e.g. SEQ ID NO: 6, such as SEQ ID NO: 6 in a di-chain form), the method comprising administering a chimeric clostridial neurotoxin to the subject, wherein the chimeric clostridial neurotoxin inhibits release of a pain mediator from a neuron comprising an Aδ nerve fiber or a C nerve fiber, wherein the chimeric clostridial neurotoxin binds to the neuron comprising the Aδ nerve fiber or the C nerve fiber, respectively, and wherein the chimeric clostridial neurotoxin comprises a botulinum neurotoxin A (BoNT/A) light-chain and translocation domain (H_N domain), and a BoNT/B receptor binding domain (H_C domain).

In another related aspect, the invention provides use of a chimeric clostridial neurotoxin in the manufacture of a medicament for reducing pain of a subject by a greater amount than that of a subject treated with BoNT/A (e.g. SEQ ID NO: 6, such as SEQ ID NO: 6 in a di-chain form), wherein the chimeric clostridial neurotoxin inhibits release of a pain mediator from a neuron comprising an Aδ nerve fiber or a C nerve fiber, wherein the chimeric clostridial neurotoxin binds to the neuron comprising the Aδ nerve fiber or the C nerve fiber, respectively, and wherein the chimeric clostridial neurotoxin comprises a botulinum neurotoxin A (BoNT/A) light-chain and translocation domain (H_N domain), and a BoNT/B receptor binding domain (H_C domain).

Thus, in one aspect, the invention provides a method for reducing pain of a subject by a greater amount than that of a subject treated with BoNT/A (e.g. SEQ ID NO: 6, such as SEQ ID NO: 6 in a di-chain form), the method comprising administering a chimeric clostridial neurotoxin to the subject, wherein the chimeric clostridial neurotoxin comprises a botulinum neurotoxin A (BoNT/A) light-chain and translocation domain (H_N domain), and a BoNT/B receptor binding domain (H_C domain).

In a related aspect, the invention provides a chimeric clostridial neurotoxin for use in a method for reducing pain of a subject by a greater amount than that of a subject treated with BoNT/A (e.g. SEQ ID NO: 6, such as SEQ ID NO: 6 in a di-chain form), the method comprising administering a chimeric clostridial neurotoxin to the subject, wherein the chimeric clostridial neurotoxin comprises a botulinum neurotoxin A (BoNT/A) light-chain and translocation domain (H_N domain), and a BoNT/B receptor binding domain (H_C domain).

In another related aspect, the invention provides use of a chimeric clostridial neurotoxin in the manufacture of a medicament for reducing pain of a subject by a greater amount than that of a subject treated with BoNT/A (e.g. SEQ ID NO: 6, such as SEQ ID NO: 6 in a di-chain form), wherein the chimeric clostridial neurotoxin comprises a botulinum neurotoxin A

(BoNT/A) light-chain and translocation domain (H_N domain), and a BoNT/B receptor binding domain (H_C domain).

The pain is preferably migraine pain.

The term “reduce pain of a subject by a greater amount than that of a subject treated with BoNT/A” or “reducing pain of a subject by a greater amount than that of a subject treated with BoNT/A” may mean that the pain of the subject is reduced by a greater amount following administration of the chimeric clostridial neurotoxin of the invention, when compared to administration of BoNT/A. Said reduction may be determined by comparison to an equivalent control subject exhibiting equivalent pain that has been treated with BoNT/A. At a given time period following administration, a subject treated with the chimeric clostridial neurotoxin according to the invention may exhibit a reduction in pain of at least 5%, 10%, 25%, or 50% when compared to the severity of the equivalent pain of a control subject at the same time period following administration of BoNT/A (e.g. SEQ ID NO: 6, such as SEQ ID NO: 6 in a di-chain form). In another embodiment, a maximal reduction in pain of a subject treated with the chimeric clostridial neurotoxin is greater than the maximal reduction in equivalent pain of a control subject treated with BoNT/A (e.g. SEQ ID NO: 6, such as SEQ ID NO: 6 in a di-chain form).

In one embodiment, a chimeric clostridial neurotoxin may reduce an amount of a pain mediator (e.g. a migraine pain mediator) in a biofluid and/or brain of a subject by a greater amount than the amount of the same pain mediator in the same biofluid and/or brain of a subject treated with BoNT/A (e.g. SEQ ID NO: 6, such as SEQ ID NO: 6 in a di-chain form).

Thus, in one aspect, the invention provides a method for reducing an amount of a pain mediator in a biofluid and/or brain of a subject by a greater amount than the amount of the same pain mediator in the same biofluid and/or brain of a subject treated with BoNT/A (e.g. SEQ ID NO: 6, such as SEQ ID NO: 6 in a di-chain form), the method comprising administering a chimeric clostridial neurotoxin to the subject, wherein the chimeric clostridial neurotoxin inhibits release of a pain mediator from a neuron comprising an Aδ nerve fiber or a C nerve fiber, wherein the chimeric clostridial neurotoxin binds to the neuron comprising the Aδ nerve fiber or the C nerve fiber, respectively, and wherein the chimeric clostridial neurotoxin comprises a botulinum neurotoxin A (BoNT/A) light-chain and translocation domain (H_N domain), and a BoNT/B receptor binding domain (H_C domain).

In a related aspect, the invention provides a chimeric clostridial neurotoxin for use in a method for reducing an amount of a pain mediator in a biofluid and/or brain of a subject by a greater amount than the amount of the same pain mediator in the same biofluid and/or brain of a subject treated with BoNT/A (e.g. SEQ ID NO: 6, such as SEQ ID NO: 6 in a di-chain form), the method comprising administering a chimeric clostridial neurotoxin to the subject, wherein the chimeric clostridial neurotoxin inhibits release of a pain mediator from a neuron comprising an Aδ nerve fiber or a C nerve fiber, wherein the chimeric clostridial neurotoxin binds to the neuron comprising the Aδ nerve fiber or the C nerve fiber, respectively, and wherein the chimeric clostridial neurotoxin comprises a botulinum neurotoxin A (BoNT/A) light-chain and translocation domain (H_N domain), and a BoNT/B receptor binding domain (H_C domain).

In another related aspect, the invention provides use of a chimeric clostridial neurotoxin in the manufacture of a medicament for reducing an amount of a pain mediator in a biofluid and/or brain of a subject by a greater amount than the amount of the same pain mediator in the same biofluid and/or brain of a subject treated with BoNT/A (e.g. SEQ ID NO: 6, such as SEQ ID NO: 6 in a di-chain form), wherein the chimeric clostridial neurotoxin inhibits release of a pain mediator from a neuron comprising an Aδ nerve fiber or a C nerve fiber, wherein the chimeric clostridial neurotoxin binds to the neuron comprising the Aδ nerve fiber or the C nerve fiber, respectively, and wherein the chimeric clostridial neurotoxin comprises a botulinum neurotoxin A (BoNT/A) light-chain and translocation domain (H_N domain), and a BoNT/B receptor binding domain (H_C domain).

Thus, in one aspect, the invention provides a method for reducing an amount of a pain mediator in a biofluid and/or brain of a subject by a greater amount than the amount of the same pain mediator in the same biofluid and/or brain of a subject treated with BoNT/A (e.g. SEQ ID NO: 6, such as SEQ ID NO: 6 in a di-chain form), the method comprising administering a chimeric clostridial neurotoxin to the subject, wherein the chimeric clostridial neurotoxin comprises a botulinum neurotoxin A (BoNT/A) light-chain and translocation domain (H_N domain), and a BoNT/B receptor binding domain (H_C domain).

In a related aspect, the invention provides a chimeric clostridial neurotoxin for use in a method for reducing an amount of a pain mediator in a biofluid and/or brain of a subject by a greater amount than the amount of the same pain mediator in the same biofluid and/or brain of a subject treated with BoNT/A (e.g. SEQ ID NO: 6, such as SEQ ID NO: 6 in a di-chain form), the method comprising administering a chimeric clostridial neurotoxin to the subject,

wherein the chimeric clostridial neurotoxin comprises a botulinum neurotoxin A (BoNT/A) light-chain and translocation domain (H_N domain), and a BoNT/B receptor binding domain (H_C domain).

In another related aspect, the invention provides use of a chimeric clostridial neurotoxin in the manufacture of a medicament for reducing an amount of a pain mediator in a biofluid and/or brain of a subject by a greater amount than the amount of the same pain mediator in the same biofluid and/or brain of a subject treated with BoNT/A (e.g. SEQ ID NO: 6, such as SEQ ID NO: 6 in a di-chain form), wherein the chimeric clostridial neurotoxin comprises a botulinum neurotoxin A (BoNT/A) light-chain and translocation domain (H_N domain), and a BoNT/B receptor binding domain (H_C domain).

The term “reduce an amount of a pain mediator in a biofluid and/or brain of a subject by a greater amount than the amount of the same pain mediator in the same biofluid and/or brain of a subject treated with BoNT/A” or “reducing an amount of a pain mediator in a biofluid and/or brain of a subject by a greater amount than the amount of the same pain mediator in the same biofluid and/or brain of a subject treated with BoNT/A” may mean that the amount of the pain mediator of the subject is reduced by a greater amount following administration of the chimeric clostridial neurotoxin of the invention, when compared to administration of BoNT/A. Said reduction may be determined by comparison to an amount of the same pain mediator in the same biofluid and/or brain of an equivalent control subject that has been treated with BoNT/A. At a given time period following administration, a subject treated with the chimeric clostridial neurotoxin according to the invention may exhibit a reduction in the amount of the pain mediator in its biofluid and/or brain of at least 5%, 10%, 25%, or 50% when compared to the amount of the same pain mediator in the same biofluid and/or brain of a control subject at the same time period following administration of BoNT/A (e.g. SEQ ID NO: 6, such as SEQ ID NO: 6 in a di-chain form). In another embodiment, a maximal reduction in the amount of the pain mediator in the biofluid and/or brain of a subject treated with the chimeric clostridial neurotoxin is greater than the maximal reduction of the same pain mediator in the same biofluid and/or brain of a control subject treated with BoNT/A (e.g. SEQ ID NO: 6, such as SEQ ID NO: 6 in a di-chain form). Said pain mediator may be a migraine pain mediator. Said pain mediator is preferably CGRP. Preferably, the biofluid is blood (including a fraction thereof).

CGRP may be used as a relevant marker to assess the efficacy of analgesics, such as painkillers. CGRP may thus be used as a biomarker for determining the suitability of a

clostridial neurotoxin for treating pain (e.g. migraine pain). Thus, in one aspect, the invention provides a method for determining whether or not a clostridial neurotoxin is suitable for treating pain, the method comprising:

(a) comparing a level of CGRP comprised in a first sample with the level of CGRP comprised in a second sample, wherein the first sample has been obtained from a subject prior to administration of the clostridial neurotoxin, and wherein the second sample has been obtained from the same subject after administration of the clostridial neurotoxin; and

(b) determining that the clostridial neurotoxin is suitable for treating pain when the level of CGRP in the second sample is lower than the level of CGRP in the first sample; or

(c) determining that the clostridial neurotoxin is unsuitable for treating pain when the level of CGRP in the second sample is not lower (e.g. is higher or the same) than the level of CGRP in the first sample. The term "lower" as used in this context preferably means statistically-significantly lower and "is not lower" preferably means is not statistically-significantly different (e.g. is the same) or is statistically significantly higher.

The clostridial neurotoxin may be any suitable clostridial neurotoxin known in the art, for example, a chimeric clostridial neurotoxin as described herein. Said clostridial neurotoxin may be a BoNT/A, BoNT/B, BoNT/C, BoNT/D, BoNT/E, BoNT/F, BoNT/G, BoNT/X, or tetanus neurotoxin (TeNT).

A BoNT/A may comprise a polypeptide sequence having at least 70% sequence identity to SEQ ID NO: 6. For example, a BoNT/A may comprise a polypeptide sequence having at least 80%, 90%, 95% or 99.9% sequence identity to SEQ ID NO: 6. Preferably, a BoNT/A may comprise (more preferably consist of) SEQ ID NO: 6.

A BoNT/B may comprise a polypeptide sequence having at least 70% sequence identity to SEQ ID NO: 7. For example, a BoNT/B may comprise a polypeptide sequence having at least 80%, 90%, 95% or 99.9% sequence identity to SEQ ID NO: 7. Preferably, a BoNT/B may comprise (more preferably consist of) SEQ ID NO: 7.

A BoNT/C may comprise a polypeptide sequence having at least 70% sequence identity to SEQ ID NO: 8. For example, a BoNT/C may comprise a polypeptide sequence having at least 80%, 90%, 95% or 99.9% sequence identity to SEQ ID NO: 8. Preferably, a BoNT/C may comprise (more preferably consist of) SEQ ID NO: 8.

A BoNT/D may comprise a polypeptide sequence having at least 70% sequence identity to SEQ ID NO: 9. For example, a BoNT/D may comprise a polypeptide sequence having at least 80%, 90%, 95% or 99.9% sequence identity to SEQ ID NO: 9. Preferably, a BoNT/D may comprise (more preferably consist of) SEQ ID NO: 9.

A BoNT/E may comprise a polypeptide sequence having at least 70% sequence identity to SEQ ID NO: 10. For example, a BoNT/E may comprise a polypeptide sequence having at least 80%, 90%, 95% or 99.9% sequence identity to SEQ ID NO: 10. Preferably, a BoNT/E may comprise (more preferably consist of) SEQ ID NO: 10.

A BoNT/F may comprise a polypeptide sequence having at least 70% sequence identity to SEQ ID NO: 11. For example, a BoNT/F may comprise a polypeptide sequence having at least 80%, 90%, 95% or 99.9% sequence identity to SEQ ID NO: 11. Preferably, a BoNT/F may comprise (more preferably consist of) SEQ ID NO: 11.

A BoNT/G may comprise a polypeptide sequence having at least 70% sequence identity to SEQ ID NO: 12. For example, a BoNT/G may comprise a polypeptide sequence having at least 80%, 90%, 95% or 99.9% sequence identity to SEQ ID NO: 12. Preferably, a BoNT/G may comprise (more preferably consist of) SEQ ID NO: 12.

A BoNT/X may comprise a polypeptide sequence having at least 70% sequence identity to SEQ ID NO: 13. For example, a BoNT/X may comprise a polypeptide sequence having at least 80%, 90%, 95% or 99.9% sequence identity to SEQ ID NO: 13. Preferably, a BoNT/X may comprise (more preferably consist of) SEQ ID NO: 13.

A TeNT may comprise a polypeptide sequence having at least 70% sequence identity to SEQ ID NO: 14. For example, a TeNT may comprise a polypeptide sequence having at least 80%, 90%, 95% or 99.9% sequence identity to SEQ ID NO: 14. Preferably, a TeNT may comprise (more preferably consist of) SEQ ID NO: 14.

In one embodiment, before using a clostridial neurotoxin in a method for determining whether or not a clostridial neurotoxin is suitable for treating pain, the clostridial neurotoxin will be converted into its di-chain form, e.g. as described herein.

The first and second samples may be blood samples, optionally subjected to one or more processing steps. The first and second samples are preferably equivalent (e.g. of the same

type and optionally have been subjected to the same processing steps). The level of CGRP may be determined using any suitable technique, including quantitative Western blotting, and/or mass spectrometry.

The BoNT/A light-chain, BoNT/A translocation domain, and/or BoNT/B H_C domain may be a modified BoNT/A light-chain, BoNT/A translocation domain, and/or BoNT/B H_C domain or a derivative thereof, including but not limited to those described below. A modified BoNT/A light-chain, BoNT/A translocation domain, and/or BoNT/B H_C domain or derivative may contain one or more amino acids that has been modified as compared to the native (unmodified) form of the BoNT/A light-chain, BoNT/A translocation domain, and/or BoNT/B H_C domain, or may contain one or more inserted amino acids that are not present in the native (unmodified) form of the BoNT/A light-chain, BoNT/A translocation domain, and/or BoNT/B H_C domain. By way of example, a modified BoNT/A light-chain, BoNT/A translocation domain, and/or BoNT/B H_C domain may have modified amino acid sequences in one or more domains relative to the native (unmodified) BoNT/A light-chain, BoNT/A translocation domain, and/or BoNT/B H_C domain sequence. Such modifications may modify functional aspects thereof, for example biological activity or persistence. Thus, in one embodiment, the BoNT/A light-chain, BoNT/A translocation domain, and/or BoNT/B H_C domain is a modified BoNT/A light-chain, BoNT/A translocation domain, and/or BoNT/B H_C domain, or modified BoNT/A light-chain, BoNT/A translocation domain, and/or BoNT/B H_C domain derivative.

A modified BoNT/B H_C domain may have one or more modifications modifying binding to target nerve cells, for example providing higher or lower affinity binding when compared to the native (unmodified) BoNT/B H_C domain. Such modifications in the BoNT/B H_C domain may include modifying residues in the ganglioside binding site of the H_C domain or in the protein (e.g. synaptotagmin) binding site that alter binding to the ganglioside receptor and/or the protein receptor of the target nerve cell. Examples of such modified neurotoxins are described in WO 2006/027207 and WO 2006/114308, both of which are hereby incorporated by reference in their entirety.

A modified light-chain may have one or more modifications in the amino acid sequence thereof, for example modifications in the substrate binding or catalytic domain which may alter or modify the SNARE protein specificity of the modified light-chain, with the proviso that said modifications do not catalytically inactivate said light-chain. Examples of such modified

neurotoxins are described in WO 2010/120766 and US 2011/0318385, both of which are hereby incorporated by reference in their entirety.

The LH_N domain from BoNT/A may correspond to amino acid residues 1 to 872 of SEQ ID NO: 6, or a polypeptide sequence having at least 70% sequence identity thereto. The LH_N domain from BoNT/A may correspond to amino acid residues 1 to 872 of SEQ ID NO: 6, or a polypeptide sequence having at least 80%, 90% or 95% sequence identity thereto. Preferably, the LH_N domain from BoNT/A corresponds to amino acid residues 1 to 872 of SEQ ID NO: 6.

The H_C domain from BoNT/B may correspond to amino acid residues 860 to 1291 of SEQ ID NO: 7, or a polypeptide sequence having at least 70% sequence identity thereto. The H_C domain from BoNT/B may correspond to amino acid residues 860 to 1291 of SEQ ID NO: 7, or a polypeptide sequence having at least 80%, 90% or 95% sequence identity thereto. Preferably, the H_C domain from BoNT/B corresponds to amino acid residues 860 to 1291 of SEQ ID NO: 7.

Preferably, the BoNT/AB chimera comprises a BoNT/A1 LH_N domain and a BoNT/B1 H_C domain. More preferably, the LH_N domain corresponds to amino acid residues 1 to 872 of BoNT/A1 (SEQ ID NO: 6) and the H_C domain corresponds to amino acid residues 860 to 1291 of BoNT/B1 (SEQ ID NO: 7).

Most preferably, a BoNT/B H_C domain further comprises at least one amino acid residue substitution, insertion, indel or deletion in the H_{CC} subdomain which has the effect of increasing the binding affinity of BoNT/B neurotoxin for human Syt II as compared to the natural BoNT/B sequence. Suitable amino acid residue substitutions, insertions, indels or deletions in the BoNT/B H_{CC} subdomain have been disclosed in WO 2013/180799 and in WO 2016/154534 (both herein incorporated by reference).

A suitable amino acid residue substitution, insertion, indel or deletion in the BoNT/B H_{CC} subdomain may include substitution mutations selected from the group consisting of: V1118M; Y1183M; E1191M; E1191I; E1191Q; E1191T; S1199Y; S1199F; S1199L; S1201V; E1191C, E1191V, E1191L, E1191Y, S1199W, S1199E, S1199H, W1178Y, W1178Q, W1178A, W1178S, Y1183C, Y1183P and combinations thereof.

A suitable amino acid residue substitution, insertion, indel or deletion in the BoNT/B H_{CC} subdomain may further include combinations of two substitution mutations selected from the group consisting of: E1191M and S1199L, E1191M and S1199Y, E1191M and S1199F, E1191Q and S1199L, E1191Q and S1199Y, E1191Q and S1199F, E1191M and S1199W, E1191M and W1178Q, E1191C and S1199W, E1191C and S1199Y, E1191C and W1178Q, E1191Q and S1199W, E1191V and S1199W, E1191V and S1199Y, or E1191V and W1178Q.

A suitable amino acid residue substitution, insertion, indel or deletion in the BoNT/B H_{CC} subdomain may also include a combination of three substitution mutations which are E1191M, S1199W and W1178Q.

Preferably, the amino acid residue substitution, insertion, indel or deletion in the BoNT/B H_{CC} subdomain includes a combination of two substitution mutations which are E1191M and S1199Y. Such modifications are present in chimeric clostridial neurotoxins SEQ ID NO: 1 and SEQ ID NO: 4. E1191M may correspond to position 1204 of SEQ ID NO: 1 and S1199Y may correspond to position 1212. Thus, SEQ ID NO: 1 may comprise 1204M and 1212Y.

The modification may be a modification when compared to unmodified BoNT/B shown as SEQ ID NO: 7, wherein the amino acid residue numbering is determined by alignment with SEQ ID NO: 7. As the presence of a methionine residue at position 1 of SEQ ID NO: 7 (as well as the SEQ ID NOs corresponding to chimeric clostridial neurotoxin polypeptides described herein) is optional, the skilled person will take the presence/absence of the methionine residue into account when determining amino acid residue numbering. For example, where SEQ ID NO: 7 includes a methionine, the position numbering will be as defined above (e.g. E1191 will be E1191 of SEQ ID NO: 7). Alternatively, where the methionine is absent from SEQ ID NO: 7 the amino acid residue numbering should be modified by -1 (e.g. E1191 will be E1190 of SEQ ID NO: 7). Accordingly, an initial methionine amino acid residue of a polypeptide sequence of the chimeric clostridial neurotoxin may be optional or absent. Similar considerations apply when the methionine at position 1 of the other polypeptide sequences described herein is present/absent, and the skilled person will readily determine the correct amino acid residue numbering using techniques routine in the art. Alignment may be carried out using any of the methods described herein for determining sequence homology and/or % sequence identity.

The term “deletion” as used herein refers to removal of one or more amino acid residues of a polypeptide without replacement of one or more amino acid residues at the site of deletion. Thus, where one amino acid residue has been deleted from a polypeptide sequence having *x* number of amino acid residues (for example), the resultant polypeptide has *x*-1 amino acid residues.

The term “indel” as used herein refers to deletion of one or more amino acid residues of a polypeptide and insertion at the deletion site of a different number of amino acid residues (either greater or fewer amino acid residues) when compared to the number of amino acid residues deleted. Thus, for an indel where two amino acid residues have been deleted from a polypeptide sequence having *x* number of amino acid residues (for example), the resultant polypeptide has *x*-1 amino acid residues or *x*+≥1 amino acid residues. The insertion and deletion can be carried out in any order, sequentially or simultaneously.

The term “substitution” as used herein refers to replacement of one or more amino acid residues with the same number of amino acid residues at the same site. Thus, for a substitution of a polypeptide sequence having *x* number of amino acid residues (for example), the resultant polypeptide also has *x* amino acid residues. Preferably a substitution is a substitution at a single amino acid position.

The term “insertion” as used herein refers to addition of one or more amino acid residues of a polypeptide without deletion of one or more amino acid residues of the polypeptide at the site of insertion. Thus, where one amino acid residue has been inserted into a polypeptide sequence having *x* number of amino acid residues (for example), the resultant polypeptide has *x*+1 amino acid residues.

Methods for modifying proteins by substitution, insertion, deletion of amino acid residues or via indels are known in the art. By way of example, amino acid modifications may be introduced by modification of a nucleic acid sequence (e.g. DNA sequence) encoding a polypeptide. This can be achieved using standard molecular cloning techniques, for example by site-directed mutagenesis where short strands of DNA (oligonucleotides) coding for the desired amino acid(s) are used to replace the original coding sequence using a polymerase enzyme, or by inserting/deleting parts of the gene with various enzymes (e.g., ligases and restriction endonucleases). Alternatively, a modified gene sequence can be chemically synthesised. Typically a modification may be carried out by either modifying a nucleic acid encoding a native clostridial neurotoxin (or part thereof) such that the modified chimeric

clostridial neurotoxin (or part thereof) encoded by the nucleic acid comprises the modification(s). Alternatively, a nucleic acid that encodes a modified clostridial neurotoxin (or part thereof) comprising the modification(s) may be synthesized.

A chimeric clostridial neurotoxin for use in the invention may comprise a polypeptide sequence having at least 70% sequence identity to a polypeptide sequence selected from SEQ ID NOs: 1-5. For example, the chimeric clostridial neurotoxin may comprise a polypeptide sequence having at least 80%, 90%, 95% or 99.9% sequence identity to a polypeptide sequence selected from SEQ ID NOs: 1-5. Preferably, a chimeric clostridial neurotoxin for use in the invention may comprise (more preferably consist of) a polypeptide sequence selected from SEQ ID NOs: 1-5. Of said chimeric clostridial neurotoxins, SEQ ID NO: 1 is preferred.

Thus, it is preferred that the chimeric clostridial neurotoxin comprises a polypeptide sequence having at least 70% sequence identity to SEQ ID NO: 1. More preferably, the chimeric clostridial neurotoxin may comprise a polypeptide sequence having at least 80%, 90%, 95% or 99.9% sequence identity to SEQ ID NO: 1. Most preferably, a chimeric clostridial neurotoxin for use in the invention may comprise (more preferably consist of) SEQ ID NO: 1.

A di-chain chimeric clostridial neurotoxin of the invention may comprise an L-chain portion of a polypeptide sequence having at least 70%, 80%, 90%, 95%, 99.9%, or 100% sequence identity to any one of SEQ ID NOs: 1-5 constituting a first chain of the di-chain chimeric clostridial neurotoxin, and may comprise the H_N and H_C domains of a polypeptide sequence having at least 70%, 80%, 90%, 95%, 99.9%, or 100% sequence identity to any one of SEQ ID NOs: 1-5 together constituting a second chain of the di-chain chimeric clostridial neurotoxin, wherein the first and second chains are joined together by a di-sulphide bond.

Where cleavage occurs at more than one position (preferably at two positions) within the activation loop of a chimeric clostridial neurotoxin comprising a polypeptide sequence having at least 70%, 80%, 90%, 95%, 99.9%, or 100% sequence identity to any one of SEQ ID NOs: 1-5, a small fragment of the C-terminal L-chain portion of the sequence having at least 70%, 80%, 90%, 95%, 99.9%, or 100% sequence identity to any one of SEQ ID NOs: 1-5 may be absent from the di-chain chimeric clostridial neurotoxin. In view of this, the sequence of the di-chain chimeric clostridial neurotoxin (e.g. comprising a polypeptide sequence having at least 70%, 80%, 90%, 95%, 99.9%, or 100% sequence identity to any one of SEQ ID NOs:

1-5) may be slightly different to that of the corresponding single-chain chimeric clostridial neurotoxin comprising a polypeptide sequence having at least 70%, 80%, 90%, 95%, 99.9%, or 100% sequence identity to any one of SEQ ID NOs: 1-5. The small fragment may be 1-15 amino acids. In particular, in one embodiment, when Lys-C is used to covert a single-chain chimeric clostridial neurotoxin into a di-chain clostridial neurotoxin, the small fragment of the C-terminal L-chain portion of the sequence that is absent may be SEQ ID NO: 15 or 16.

Preferably, a di-chain chimeric clostridial neurotoxin of the invention may comprise an L-chain portion of a polypeptide sequence having at least 70%, 80%, 90%, 95%, 99.9%, or 100% sequence identity to SEQ ID NO: 1 constituting a first chain of the di-chain chimeric clostridial neurotoxin, and may comprise the H_N and H_C domains of a polypeptide sequence having at least 70%, 80%, 90%, 95%, 99.9%, or 100% sequence identity to SEQ ID NO: 1 together constituting a second chain of the di-chain chimeric clostridial neurotoxin, wherein the first and second chains are joined together by a di-sulphide bond.

Where cleavage occurs at more than one position (preferably at two positions) within the activation loop of a chimeric clostridial neurotoxin comprising a polypeptide sequence having at least 70%, 80%, 90%, 95%, 99.9%, or 100% sequence identity to SEQ ID NO: 1, a small fragment of the C-terminal L-chain portion of the sequence having at least 70%, 80%, 90%, 95%, 99.9%, or 100% sequence identity to SEQ ID NO: 1 may be absent from the di-chain chimeric clostridial neurotoxin. In view of this, the sequence of the di-chain chimeric clostridial neurotoxin (e.g. comprising a polypeptide sequence having at least 70%, 80%, 90%, 95%, 99.9%, or 100% sequence identity to SEQ ID NO: 1) may be slightly different to that of the corresponding single-chain chimeric clostridial neurotoxin comprising a polypeptide sequence having at least 70%, 80%, 90%, 95%, 99.9%, or 100% sequence identity to SEQ ID NO: 1. The small fragment may be 1-15 amino acids. In particular, in one embodiment, when Lys-C is used to covert a single-chain chimeric clostridial neurotoxin into a di-chain clostridial neurotoxin, the small fragment of the C-terminal L-chain portion of the sequence that is absent may be SEQ ID NO: 15 or 16.

In a particularly preferred embodiment, a di-chain chimeric clostridial neurotoxin comprises (or consists of) a light-chain comprising a polypeptide sequence having at least 70%, 80%, 90%, 95%, or 99.9% sequence identity to SEQ ID NO: 17 or 18 (preferably SEQ ID NO: 17) and a heavy-chain comprising a polypeptide sequence having at least 70%, 80%, 90%, 95%, or 99.9% sequence identity to SEQ ID NO: 19, wherein the light-chain and heavy-chain are joined together by a di-sulphide bond. More preferably, a di-chain chimeric clostridial

neurotoxin comprises (or consists of) a light-chain comprising SEQ ID NO: 17 or 18 (preferably SEQ ID NO: 17) and a heavy-chain comprising SEQ ID NO: 19, wherein the light-chain and heavy-chain are joined together by a di-sulphide bond. Even more preferably, a di-chain chimeric clostridial neurotoxin comprises (or consists of) a light-chain having SEQ ID NO: 17 and a heavy-chain having SEQ ID NO: 19, wherein the light-chain and heavy-chain are joined together by a di-sulphide bond. The di-sulphide bond is preferably formed by and/or is between cysteine residue 429 of SEQ ID NO: 17 or 18 and cysteine residue 6 of SEQ ID NO: 19.

In a preferred embodiment, a chimeric clostridial neurotoxin of the invention does not comprise a therapeutic or diagnostic agent (e.g. a nucleic acid, protein, peptide or small molecule therapeutic or diagnostic agent) additional to the light-chain and heavy-chain. For example, in one embodiment, the chimeric clostridial neurotoxin may not comprise a covalently or non-covalently associated therapeutic or diagnostic agent. Thus, a chimeric clostridial neurotoxin of the invention preferably does not function as a delivery vehicle for a further therapeutic or diagnostic agent.

In embodiments where a chimeric clostridial neurotoxin described herein has a tag for purification (e.g. a His-tag) and/or a linker, said tag and/or linker are optional.

The chimeric clostridial neurotoxin of the present invention may be free from the complexing proteins that are present in a naturally occurring clostridial neurotoxin complex.

The chimeric clostridial neurotoxin of the present invention can be produced using recombinant nucleic acid technologies. Thus, in one embodiment, a chimeric clostridial neurotoxin (as described herein) is a recombinant chimeric clostridial neurotoxin.

In one embodiment a nucleic acid (for example, DNA) comprising a nucleic acid sequence encoding a chimeric clostridial neurotoxin is provided. In one embodiment, the nucleic acid sequence is prepared as part of a DNA vector comprising a promoter and a terminator. The nucleic acid sequence may be selected from any of the nucleic acid sequences described herein.

In a preferred embodiment, the vector has a promoter selected from:

Promoter	Induction Agent	Typical Induction Condition
Tac (hybrid)	IPTG	0.2 mM (0.05-2.0mM)
AraBAD	L-arabinose	0.2% (0.002-0.4%)
T7-lac operator	IPTG	0.2 mM (0.05-2.0mM)

In another preferred embodiment, the vector has a promoter selected from:

Promoter	Induction Agent	Typical Induction Condition
Tac (hybrid)	IPTG	0.2 mM (0.05-2.0mM)
AraBAD	L-arabinose	0.2% (0.002-0.4%)
T7-lac operator	IPTG	0.2 mM (0.05-2.0mM)
T5-lac operator	IPTG	0.2 mM (0.05-2.0mM)

The nucleic acid molecules may be made using any suitable process known in the art. Thus, the nucleic acid molecules may be made using chemical synthesis techniques. Alternatively, the nucleic acid molecules of the invention may be made using molecular biology techniques.

The DNA construct of the present invention is preferably designed *in silico*, and then synthesised by conventional DNA synthesis techniques.

The above-mentioned nucleic acid sequence information is optionally modified for codon-biasing according to the ultimate host cell (e.g. *E. coli*) expression system that is to be employed.

The terms “nucleotide sequence” and “nucleic acid” are used synonymously herein. Preferably the nucleotide sequence is a DNA sequence.

A chimeric clostridial neurotoxin of the invention may be present as a single-chain or as a di-chain. However, it is preferred that the chimeric clostridial neurotoxin is present as a di-chain in which the L-chain is linked to the H-chain (or component thereof, e.g. the H_N domain) via a di-sulphide bond.

Production of a single-chain chimeric clostridial neurotoxin having a light-chain and a heavy-chain may be achieved using a method comprising expressing a nucleic acid encoding a chimeric clostridial neurotoxin in an expression host, lysing the host cell to provide a host cell homogenate containing the single-chain chimeric clostridial neurotoxin, and isolating the

single-chain chimeric clostridial neurotoxin. The single-chain chimeric clostridial neurotoxin described herein may be proteolytically processed using a method comprising contacting a single-chain chimeric clostridial neurotoxin with a protease (e.g. Lys-C) that hydrolyses a peptide bond in the activation loop of the chimeric clostridial neurotoxin, thereby converting the single-chain chimeric clostridial neurotoxin into a corresponding di-chain chimeric clostridial neurotoxin (e.g. wherein the light-chain and heavy-chain are joined together by a disulphide bond). A di-chain chimeric clostridial neurotoxin is preferably obtainable by such a method.

Thus, a chimeric clostridial neurotoxin used in the invention is preferably a di-chain chimeric clostridial neurotoxin that has been produced from a single-chain BoNT/A, wherein the single-chain BoNT/A comprises or consists of a polypeptide sequence described herein. For example, it is preferred that the chimeric clostridial neurotoxin used in the invention is a di-chain chimeric clostridial neurotoxin that has been produced from a polypeptide comprising a polypeptide sequence having at least 70% (e.g. at least 80%, 90%, 95% or 99.9%) sequence identity to SEQ ID NO: 1. Most preferably, the chimeric clostridial neurotoxin used in the invention is a di-chain chimeric clostridial neurotoxin that has been produced from a polypeptide comprising (even more preferably consisting of) SEQ ID NO: 1. Accordingly, in some embodiments, the chimeric clostridial neurotoxin is a di-chain chimeric clostridial neurotoxin in which the light-chain (L-chain) is linked to the heavy-chain (H-chain) via a di-sulphide bond obtainable by a method comprising contacting a single-chain chimeric clostridial neurotoxin comprising SEQ ID NO: 1 with a protease that hydrolyses a peptide bond in the activation loop thereof, thereby converting the single-chain chimeric clostridial neurotoxin into the corresponding di-chain chimeric clostridial neurotoxin. In some embodiments, the chimeric clostridial neurotoxin is a di-chain chimeric clostridial neurotoxin in which the L-chain is linked to the H-chain via a di-sulphide bond obtainable by a method comprising contacting a single-chain chimeric clostridial neurotoxin consisting of SEQ ID NO: 1 with a protease that hydrolyses a peptide bond in the activation loop thereof, thereby converting the single-chain chimeric clostridial neurotoxin into the corresponding di-chain chimeric clostridial neurotoxin.

The term “obtainable” as used herein also encompasses the term “obtained”. In one embodiment the term “obtainable” means obtained.

The protease used to cleave the activation loop is preferably Lys-C. Suitable proteases and methods for cleaving activation loops to produce di-chain clostridial neurotoxins are taught in

WO 2014/080206, WO2014/079495, and EP2677029A2, which are incorporated herein by reference. Lys-C may cleave an activation loop C-terminal to one or more of the lysine residues present therein. Where Lys-C cleaves the activation loop more than once, the skilled person will appreciate that a small peptide of the activation loop of a di-chain modified BoNT/A may be absent when compared to a SEQ ID NO shown herein (preferably SEQ ID NO: 15 or 16 may be absent).

The term "one or more" as used herein may mean at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, or 20. In one embodiment, wherein "one or more" precedes a list, "one or more" may mean all of the members of the list. Similarly, the term "at least one" as used herein may mean at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, or 20. In one embodiment, wherein "at least one" precedes a list, "at least one" may mean all of the members of the list.

A "subject" as used herein may be a mammal, such as a human or other mammal. Preferably "subject" means a human subject.

A subject for treatment in accordance with the invention may be a subject that is unsuitable for treatment with a non-chimeric clostridial neurotoxin. Said subject may be a subject that is resistant to treatment with a non-chimeric clostridial neurotoxin. Resistance may arise due to development of an immune response to a clostridial neurotoxin, including production of anti-clostridial neurotoxin antibodies, by a subject. In one embodiment, a subject for treatment in accordance with the invention may be a subject that is unsuitable for treatment with BoNT/A. Said subject may be resistant to treatment with BoNT/A.

The term "disorder" as used herein also encompasses a "disease". In one embodiment the disorder is a disease.

The term "treat" or "treating" as used herein encompasses prophylactic treatment (e.g. to prevent onset of pain) as well as corrective treatment (e.g. treatment of a subject already suffering from pain). Preferably "treat" or "treating" as used herein means corrective treatment.

In one embodiment, a chimeric clostridial neurotoxin is administered to a subject that is not experiencing pain or a symptom of a disorder at the time of treatment. Such administration may be suitable to achieve prophylactic treatment of pain or a disorder described herein. In one embodiment, the treatment of migraine (e.g. migraine pain) may be the prophylactic

treatment of migraine. In one embodiment, a subject that is not experiencing migraine pain or a symptom of migraine at the time of treatment is administered the chimeric clostridial neurotoxin.

The term "treat" or "treating" as used herein refers to a disorder (preferably pain) and/or a symptom thereof.

Therefore, a chimeric clostridial neurotoxin of the invention may be administered to a subject in a therapeutically effective amount or a prophylactically effective amount. Preferably a chimeric clostridial neurotoxin of the invention is administered to a subject in a therapeutically effective amount.

A "therapeutically effective amount" is any amount of the chimeric clostridial neurotoxin, which when administered alone or in combination with another agent (preferably alone) to a subject for treating said disorder (preferably pain) (or a symptom thereof) is sufficient to effect such treatment of said disorder (preferably pain) or a symptom thereof.

A "prophylactically effective amount" is any amount of the chimeric clostridial neurotoxin that, when administered alone or in combination with another agent (preferably alone) to a subject, inhibits or delays the onset or reoccurrence of a disorder (preferably pain) (or a symptom thereof). In some embodiments, the prophylactically effective amount prevents the onset or reoccurrence of the disorder (preferably pain) entirely. "Inhibiting" the onset means either lessening the likelihood of onset (preferably of pain) (or symptom thereof), preventing the magnitude of the peak effect of the disorder (preferably pain), and/or preventing the onset entirely.

The chimeric clostridial neurotoxin may treat pain without treating an underlying disorder that causes said pain.

The chimeric clostridial neurotoxin may treat one or more additional symptoms of a disorder in addition to treating pain. In one embodiment, the chimeric clostridial neurotoxin may treat one or more additional symptoms associated with secretion from a neuron, e.g. release of a mediator (e.g. pain mediator), described herein. For example, CGRP may be involved in a number of symptoms associated with migraine, such as photophobia. Thus, treatment for migraine or migraine pain in accordance with the present invention may also treat one or more additional symptoms of migraine, such as photophobia.

The chimeric clostridial neurotoxin of the invention may be formulated in any suitable manner for administration to a subject, for example as part of a pharmaceutical composition. Such a pharmaceutical composition may comprise a chimeric clostridial neurotoxin of the invention and a pharmaceutically acceptable carrier, excipient, adjuvant, propellant and/or salt.

The chimeric clostridial neurotoxin of the present invention may be formulated for oral, parenteral, continuous infusion, inhalation or topical application. Compositions suitable for injection may be in the form of solutions, suspensions or emulsions, or dry powders which are dissolved or suspended in a suitable vehicle prior to use.

In one aspect, the invention provides a unit dosage form of chimeric clostridial neurotoxin for treating pain, the unit dosage form comprising:

a. 0.2 Units up to 707 Units of the chimeric clostridial neurotoxin, wherein 1 Unit is an amount of the chimeric clostridial neurotoxin that corresponds to the calculated median lethal dose (LD_{50}) in mice; or
b. 5 pg to 17,000 pg of the chimeric clostridial neurotoxin; and
c. optionally a pharmaceutically acceptable carrier, excipient, adjuvant, and/or salt.

It is preferred that the chimeric clostridial neurotoxin of the unit dosage form comprises a polypeptide sequence having at least 70% sequence identity to SEQ ID NO: 1. For example, a polypeptide sequence having at least 80%, 90%, 95% or 99.9% sequence identity to SEQ ID NO: 1. Most preferably, the chimeric clostridial neurotoxin may comprise (more preferably consist of) SEQ ID NO: 1.

A unit dosage form for treating pain may comprise 0.2 Units up to 707 Units of chimeric clostridial neurotoxin. An upper limit of said range may be 700, 650, 600, 550, 500, 450, 400, 350, 300, 250, 200, 150 or 100 Units of chimeric clostridial neurotoxin, preferably the upper limit is 666 Units. A lower limit of said range may be 40, 45, 50, 60, 65, 70, 75, 80, 85, 90, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, or 700 Units of chimeric clostridial neurotoxin, preferably the lower limit is 42 Units or 31 Units. The lower limit of said range may be greater than 125 Units. Preferably, the unit dosage form comprises 31 Units to 707 Units of chimeric clostridial neurotoxin. More preferably, the unit dosage form comprises 42 Units to 666 Units of chimeric clostridial neurotoxin, for example 200 Units to 400 Units of the chimeric clostridial neurotoxin or 41 Units to 229 Units such as 83 Units to 188 Units, 83 Units to 125 Units (e.g. 104 Units) or 145 Units to 188 Units of the chimeric clostridial

neurotoxin. Preferably, the unit dosage form comprises 166 Units of the chimeric clostridial neurotoxin. The unit dosage form may comprise 47 Units to 707 Units of chimeric clostridial neurotoxin, e.g. 187 Units to 282 Units, of the chimeric clostridial neurotoxin or 47 to 258 Units such as 94 Units to 211 Units, 94 Units to 141 Units (e.g. 117 Units) or 164 to 211 Units of the chimeric clostridial neurotoxin. The unit dosage form may comprise 188 Units of the chimeric clostridial neurotoxin.

A unit dosage form for treating pain may comprise 5 pg to 17,000 pg of chimeric clostridial neurotoxin. An upper limit of said range may be 16,500, 15,500, 14,500, 13,500, 12,500, 11,500, 10,500, 9,500, 8,500, 7,500, 6,500, 5,500, 4,500, 3,500, 2,500, 1,500 or 500 pg of chimeric clostridial neurotoxin, preferably the upper limit is 16,000 pg. A lower limit of said range may be 750, 850, 950, 1000, 1500, 2000, 2,500, 3,000, 3,500, 4,000, 4,500 or 5,000 pg of chimeric clostridial neurotoxin, preferably the lower limit is 1000 pg or 750 pg. The lower limit of said range may be greater than 3,000 pg. Preferably, the unit dosage form comprises 750 pg to 17,000 pg of chimeric clostridial neurotoxin. More preferably, the unit dosage form comprises 1000 pg to 16,000 pg of chimeric clostridial neurotoxin, e.g. 4,000 pg to 6,000 pg, of the chimeric clostridial neurotoxin or 1,000 to 5,500 pg such as 2,000 pg to 4,500 pg, 2,000 pg to 3,000 pg (e.g. 2,500 pg) or 3,500 to 4,500 pg of the chimeric clostridial neurotoxin. Preferably, the unit dosage form comprises 4,000 pg of the chimeric clostridial neurotoxin.

In some embodiments, the unit dosage form for treating pain may be for treating headache pain (e.g. migraine pain) or migraine, and may comprise:

a. 42 Units up to 258 Units (e.g. 42 to 229 Units) of chimeric clostridial neurotoxin, wherein 1 Unit is an amount of the chimeric clostridial neurotoxin that corresponds to the calculated median lethal dose (LD_{50}) in mice; or
b. 1,000 pg to 5,500 pg of chimeric clostridial neurotoxin; and
c. optionally a pharmaceutically acceptable carrier, excipient, adjuvant, and/or salt.

Potency of a chimeric clostridial neurotoxin for use according to the invention may be determined by a mouse LD_{50} assay according to standard techniques. In said assay, 1 Unit is defined as an amount of the chimeric clostridial neurotoxin that corresponds to the calculated median lethal dose (LD_{50}) in mice. Preferably, the calculated median lethal intraperitoneal dose in mice.

An amount of a chimeric clostridial neurotoxin that corresponds to 1 Unit in said assay may be 20-24.04 pg, e.g. 21.3 pg or 24.04 pg. Preferably, an amount of a chimeric clostridial neurotoxin that corresponds to 1 Unit in said assay may be 24.04 pg.

When referring to Units herein, the Units are preferably LD_{50} Units.

In another aspect, the invention provides a unit dosage form for treating headache pain (e.g. migraine pain) or migraine, the unit dosage form comprising:

a. 42 Units up to 258 Units (e.g. 42 to 229 Units) of chimeric clostridial neurotoxin, wherein 1 Unit is an amount of the chimeric clostridial neurotoxin that corresponds to the calculated median lethal dose (LD_{50}) in mice; or
b. 1,000 pg to 5,500 pg of chimeric clostridial neurotoxin; and
c. optionally a pharmaceutically acceptable carrier, excipient, adjuvant, and/or salt.

It is preferred that the chimeric clostridial neurotoxin of the unit dosage form comprises a polypeptide sequence having at least 70% sequence identity to SEQ ID NO: 1. For example, a polypeptide sequence having at least 80%, 90%, 95% or 99.9% sequence identity to SEQ ID NO: 1. Most preferably, a chimeric clostridial neurotoxin may comprise (more preferably consist of) SEQ ID NO: 1.

A unit dosage form for treating headache pain (e.g. migraine pain) or migraine may be 42 Units to 229 Units. An upper limit of the unit dosage form may be 225, 220, 215, 210, 205, 200, 190, 180, 170, 160, 150, 125, 100, or 83 Units of chimeric clostridial neurotoxin, preferably the upper limit is 212 Units, more preferably 208 Units. A lower limit of the unit dosage form may be 46, 50, 55, 60, 65, 70, 75, 80, or 90, 100, 110, 120, 130, 140, 150, 160 or 166 Units of chimeric clostridial neurotoxin, preferably the lower limit is 58 Units, more preferably 62 Units. The lower limit of said range may be greater than 125 Units. The unit dosage form may comprise 58 Units to 212 Units (e.g. 62 Units to 208 Units), 83 Units to 212 Units, 125 to 212 Units or 125 to 166 Units of chimeric clostridial neurotoxin. The unit dosage form may comprise greater than 125 Units up to 229 Units of chimeric clostridial neurotoxin. Preferably, the unit dosage form comprises 83 Units to 188 Units, 83 Units to 125 Units (e.g. 104 Units) or 145 Units to 188 Units of the chimeric clostridial neurotoxin. Preferably, the unit dosage form comprises 166 Units of the chimeric clostridial neurotoxin. The unit dosage form may comprise 47 Units to 258 Units of chimeric clostridial neurotoxin, e.g. 94 Units to 211 Units, 94 Units to 141 Units (e.g. 117 Units) or 164

to 211 Units of the chimeric clostridial neurotoxin. The unit dosage form may comprise 188 Units of the chimeric clostridial neurotoxin.

A unit dosage form for treating headache pain (e.g. migraine pain) or migraine may be 1,000 pg to 5,500 pg. An upper limit of the unit dosage form may be 5,250, 5,200, 5,100, 5,000, 4,500, 4,000, 3,500, 3,000, 2,500, or 2,000 pg of chimeric clostridial neurotoxin, preferably the upper limit is 5,100 pg, more preferably 5,000 pg. A lower limit of the unit dosage form may be 1,100, 1,200, 1,250, 1,300, 1,350, 1,400, or 1,450, 1,500, 2,000, 2,500, 3,000, 3,500, or 4,000 pg of chimeric clostridial neurotoxin, preferably the lower limit is 1,400 pg, more preferably 1,500 pg. The lower limit of said range may be greater than 3,000 pg. The unit dosage form may comprise 1,400 pg to 5,100 pg, 2,000 pg to 5,100 pg, 3,000 to 5,100 pg or 3,000 to 4,000 pg of chimeric clostridial neurotoxin. The unit dosage form may comprise greater than 3,000 pg up to 5,500 pg of chimeric clostridial neurotoxin. Preferably, the unit dosage form comprises 2,000 pg to 4,500 pg, 2,000 pg to 3,000 pg (e.g. 2,500 pg) or 3,500 to 4,500 pg of the chimeric clostridial neurotoxin. Preferably, the unit dosage form comprises 4,000 pg of the chimeric clostridial neurotoxin.

In the case of a chimeric clostridial neurotoxin that is to be delivered locally, the chimeric clostridial neurotoxin may be formulated as a cream (e.g. for topical application), or for sub-dermal injection.

Local delivery means may include an aerosol, or other spray (e.g. a nebuliser). In this regard, an aerosol formulation of a chimeric clostridial neurotoxin enables delivery to the lungs and/or other nasal and/or bronchial or airway passages.

A chimeric clostridial neurotoxin may be administered to the face, neck, and/or skull of a subject. For example, a chimeric clostridial neurotoxin may be administered to a muscular and/or dermal component thereof. A chimeric clostridial neurotoxin may be administered to two or more of the face, neck, and skull, preferably to the face, neck, and skull. In particular, the chimeric clostridial neurotoxin may be administered in the region of the face, neck, and/or skull of a subject.

A chimeric clostridial neurotoxin of the invention may be administered to a subject by intrathecal or epidural injection in the spinal column at the level of the spinal segment involved in the innervation of an affected organ.

A route of administration may be via laparoscopic and/or localised injection. In one embodiment a chimeric clostridial neurotoxin of the invention is administered at or near to a site to be treated, preferably at a site to be treated. For example, the chimeric clostridial neurotoxin may be administered intrathecally or intraspinally. In one embodiment the route of administration of a chimeric clostridial neurotoxin of the invention may be intraspinal, and/or intrathecal.

In one embodiment a chimeric clostridial neurotoxin of the invention may be administered peripherally. In one embodiment, the chimeric clostridial neurotoxin may be administered subcutaneously.

A chimeric clostridial neurotoxin of the invention may be administered via injection. The chimeric clostridial neurotoxin may be administered at at least 5, 10, 15, 20, 25 or 30 injection sites per treatment session. The chimeric clostridial neurotoxin may be administered by injection at up to 50, 45, 40, 35, 30, 25, or 20 injection sites per treatment session. The chimeric clostridial neurotoxin may be administered by injection at up to 20 or 15 injection sites per treatment session. Preferably, the chimeric clostridial neurotoxin may be administered by injection at up to 10 injection sites per treatment session, for example up to 9, 8, 7, 6, 5, 4, 3 or 2. In one embodiment a chimeric clostridial neurotoxin may be administered at 1-40, 5-40, 8-38, 30-40 (e.g. 35) or 15-25 (e.g. 20) injection sites per treatment session. In one embodiment a chimeric clostridial neurotoxin may be administered at 1-10, 3-10, 5-10 or 7-10 injection sites per treatment. In one embodiment, a chimeric clostridial neurotoxin may be administered at 25-35 (e.g. 31) injection sites per treatment session. Preferably, a chimeric clostridial neurotoxin may be administered at 25-30 (e.g. 28) injection sites per treatment session.

A chimeric clostridial neurotoxin of the invention may be administered intradermally, for example by intradermal injection. The chimeric clostridial neurotoxin may be administered by intradermal injection at at least 5, 10, 15, 20, 25 or 30 injection sites per treatment session. The chimeric clostridial neurotoxin may be administered by intradermal injection at up to 50, 45, 40, 35, 30, 25, or 20 injection sites per treatment session. The chimeric clostridial neurotoxin may be administered by intradermal injection at up to 20 or 15 injection sites per treatment session. Preferably, the chimeric clostridial neurotoxin may be administered by intradermal injection at up to 10 injection sites per treatment session, for example up to 9, 8, 7, 6, 5, 4, 3 or 2. In one embodiment a chimeric clostridial neurotoxin may be administered at 1-40, 5-40, 8-38, 30-40 (e.g. 35) or 15-25 (e.g. 20) injection sites per treatment session. In

one embodiment a chimeric clostridial neurotoxin may be administered at 1-10, 3-10, 5-10 or 7-10 injection sites per treatment. In one embodiment, a chimeric clostridial neurotoxin may be administered at 25-35 (e.g. 31) injection sites per treatment session. Preferably, a chimeric clostridial neurotoxin may be administered at 25-30 (e.g. 28) injection sites per treatment session. An intradermal injection may be made in the region of a muscle, such as a muscle described herein. In one embodiment, intradermal injection may be to the skin overlaying a muscle.

Most preferably, a chimeric clostridial neurotoxin may be administered intramuscularly, for example by intramuscular injection. The specific muscles to which the chimeric clostridial neurotoxin is administered will depend on the nature and location of the disorder (preferably pain) to be treated.

A chimeric clostridial neurotoxin may be administered to one or more muscles of a subject selected from the: frontalis, corrugator (e.g. corrugator supercilii), procerus (e.g. procerus nasalis), occipitalis, temporalis, trapezius, masseter, nasalis, orbicularis oculi, cervical paraspinal muscles, temporal fascia, auricularis superior, auricularis anterior, auricularis posterior, sternocleidomastoid, platysma, dilatator naris anterior, dilatator naris posterior, depressor septi, mentalis, orbicularis oris, zygomaticus, risorius, buccinator, occipitofrontalis, levator labii superioris, depressor labii inferioris, depressor anguli oris, thyrohyoid, omohyoid, sternohyoid, splenius cervicis, splenius capitis, semispinalis cervicis, semispinalis capitis, levator scapulae, digastric, or scalene muscle(s).

For example, the chimeric clostridial neurotoxin may be administered to one or more muscles of a subject selected from the: frontalis, corrugator, procerus (e.g. procerus nasalis), occipitalis, temporalis, trapezius, masseter, nasalis, orbicularis oculi, cervical paraspinal muscles, temporal fascia, auricularis superior, auricularis anterior, auricularis posterior, sternocleidomastoid, platysma, dilatator naris anterior, dilatator naris posterior, depressor septi, mentalis, orbicularis oris, zygomaticus, risorius, buccinator, occipitofrontalis, levator labii superioris, depressor labii inferioris, depressor anguli oris, thyrohyoid, omohyoid, sternohyoid, splenius cervicis, levator scapulae, digastric, and scalene muscle(s).

Where the disorder is headache pain (e.g. migraine pain) or migraine, the invention may comprise administering the chimeric clostridial neurotoxin to the: frontalis, corrugator (e.g. corrugator supercilia), procerus (e.g. procerus nasalis), occipitalis, temporalis, trapezius, masseter, nasalis, orbicularis oculi, cervical paraspinal muscles, temporal fascia, auricularis

superior, auricularis anterior, auricularis posterior, sternocleidomastoid, platysma, dilatator naris anterior, dilatator naris posterior, depressor septi, mentalis, orbicularis oris, zygomaticus, risorius, buccinator, occipitofrontalis, levator labii superioris, depressor labii inferioris, depressor anguli oris, thyrohyoid, omohyoid, sternohyoid, splenius cervicis, levator scapulae, digastric, and scalene muscle(s). Where the disorder is headache pain (e.g. migraine pain) or migraine, the chimeric clostridial neurotoxin may be administered to one or more muscles of a subject selected from the: frontalis, corrugator (e.g. corrugator supercilia), procerus (e.g. procerus nasalis), occipitalis, temporalis, trapezius, masseter, nasalis, orbicularis oculi, cervical paraspinal muscles, temporal fascia, auricularis superior, auricularis anterior, auricularis posterior, sternocleidomastoid, platysma, dilatator naris anterior, dilatator naris posterior, depressor septi, mentalis, orbicularis oris, zygomaticus, risorius, buccinator, occipitofrontalis, levator labii superioris, depressor labii inferioris, depressor anguli oris, thyrohyoid, omohyoid, sternohyoid, splenius cervicis, levator scapulae, digastric, and scalene muscle(s). Preferably, the chimeric clostridial neurotoxin is administered to one or more of the: procerus, corrugator supercilia, masseter, temporalis, occipitalis, and trapezius. Where there are two versions of the same muscle (e.g. two occipitalis muscles), the chimeric clostridial neurotoxin may be administered to one or both of said muscles according to the subject's need. Preferably, the chimeric clostridial neurotoxin is administered to both of said muscles.

A chimeric clostridial neurotoxin may be administered intramuscularly, for example by intramuscular injection. The specific muscles to which the chimeric clostridial neurotoxin is administered will depend on the nature and location of the disorder (preferably pain) to be treated. Where the disorder is headache pain (e.g. migraine pain) or migraine, the invention may comprise administering the chimeric clostridial neurotoxin to the: frontalis, corrugator (e.g. corrugator supercilii), procerus (e.g. procerus nasalis), occipitalis, temporalis, trapezius, masseter, nasalis, orbicularis oculi, cervical paraspinal muscles, temporal fascia, auricularis superior, auricularis anterior, auricularis posterior, sternocleidomastoid, platysma, dilatator naris anterior, dilatator naris posterior, depressor septi, mentalis, orbicularis oris, zygomaticus, risorius, buccinator, occipitofrontalis, levator labii superioris, depressor labii inferioris, depressor anguli oris, thyrohyoid, omohyoid, sternohyoid, splenius cervicis, splenius capitis, semispinalis cervicis, semispinalis capitis, levator scapulae, digastric, or scalene muscle(s). Where the disorder is headache pain (e.g. migraine pain) or migraine, the chimeric clostridial neurotoxin may be administered to one or more muscles of a subject selected from the: frontalis, corrugator (e.g. corrugator supercilii), procerus (e.g. procerus nasalis), occipitalis, temporalis, trapezius, masseter, nasalis, orbicularis oculi, cervical

paraspinal muscles, temporal fascia, auricularis superior, auricularis anterior, auricularis posterior, sternocleidomastoid, platysma, dilatator naris anterior, dilatator naris posterior, depressor septi, mentalis, orbicularis oris, zygomaticus, risorius, buccinator, occipitofrontalis, levator labii superioris, depressor labii inferioris, depressor anguli oris, thyrohyoid, omohyoid, sternohyoid, splenius cervicis, splenius capitis, semispinalis cervicis, semispinalis capitis, levator scapulae, digastric, and scalene muscle(s). Preferably, the chimeric clostridial neurotoxin is administered to one or more of the: procerus, corrugator supercilii, masseter, temporalis, occipitalis, and trapezius. Where there are two versions of the same muscle (e.g. two occipitalis muscles), the chimeric clostridial neurotoxin may be administered to one or both of said muscles according to the subject's need. Preferably, the chimeric clostridial neurotoxin is administered to both of said muscles.

The invention may comprise administering the chimeric clostridial neurotoxin to at least one of a: frontalis muscle, corrugator (e.g. corrugator supercilii) muscle, procerus (e.g. procerus nasalis), occipitalis (e.g. upper or lower occipitalis) muscle, temporalis muscle, trapezius (e.g. upper, mid or lower trapezius) muscle, masseter muscle, nasalis muscle, orbicularis oculi muscle, cervical paraspinal muscle, temporal fascia muscle, auricularis superior muscle, auricularis anterior muscle, auricularis posterior muscle, sternocleidomastoid muscle, platysma muscle, dilatator naris anterior muscle, dilatator naris posterior muscle, depressor septi muscle, mentalis muscle, orbicularis oris muscle, zygomaticus muscle, risorius muscle, buccinator muscle, occipitofrontalis muscle, levator labii superioris muscle, depressor labii inferioris muscle, depressor anguli oris muscle, thyrohyoid muscle, omohyoid muscle, sternohyoid muscle, splenius cervicis muscle, splenius capitis muscle, semispinalis cervicis muscle, semispinalis capitis muscle, levator scapulae muscle, digastric muscle, or scalene muscle. Preferably, the invention may comprise administering the chimeric clostridial neurotoxin to at least one of a: frontalis muscle, corrugator (e.g. corrugator supercilii) muscle, nasalis muscle, orbicularis oculi muscle, temporalis muscle, occipitalis muscle, or trapezius muscle. More preferably, the invention may comprise administering the chimeric clostridial neurotoxin to a: frontalis muscle, corrugator (e.g. corrugator supercilii) muscle, nasalis muscle, orbicularis oculi muscle, temporalis muscle, occipitalis muscle, and trapezius muscle. Where there are two versions of the same muscle (e.g. two occipitalis muscles), the chimeric clostridial neurotoxin may be administered to one or both of said muscles according to the subject's need. Preferably, the chimeric clostridial neurotoxin is administered to both of said muscles. Said administering may be particularly relevant in the treatment of headache pain (e.g. migraine pain) or migraine.

Where the pain is arthritic pain, the chimeric clostridial neurotoxin may be administered to one or more muscles of the hands, wrist, knees, and/or feet of a subject, e.g. depending on the location of the arthritis and/or arthritic pain. When administered to the hands, wrist, knees or feet of the subject, administration may be unilateral (e.g. where arthritis or arthritic pain is present in only one hand, wrist, knee, and/or foot) or bilateral (e.g. where arthritis or arthritic pain is present in both hands, wrists, knees, and/or feet). A chimeric clostridial neurotoxin may be administered to one or more muscles of the hand of a subject selected from the: flexor pollicis brevis, palmar interossei, abductor pollicis brevis, flexor pollicis brevis, abductor pollicis, opponens pollicis, dorsal interosseus, abductor digiti minimi, flexor digiti minimi, and oponnens digiti minimi (preferably one or more selected from the: flexor pollicis brevis, palmar interossei, abductor pollicis brevis, flexor pollicis brevis, and abductor pollicis). A chimeric clostridial neurotoxin may be administered to one or more muscles of the wrist of a subject selected from the: extensor pollicis brevis, abductor pollicis longus, extensor digiti minimi, extensor carpi ulnaris, flexor carpi ulnaris, extensor digitorum, extensor carpi radialis, and brachioradialis. A chimeric clostridial neurotoxin may be administered to one or more muscles of the knee of a subject selected from the: sartorious, vastus medialis, vastus lateralis, gastrocnemius, plantaris, semimembranosus, perineous longus, gastrocnemius, tibialis anterious, rectus femoris, peroneus longus, iliopsoas, pectineus, adductor longus, adductor magnus, gracilis, biceps femori, soleus, soleus, extensor digitorus longus, extensor hallucis longus, peroneus brevis, and flexor digitorum longus (preferably one or more selected from the: sartorious, vastus medialis, vastus lateralis, gastrocnemius, plantaris, semimembranosus, perineous longus, gastrocnemius, tibialis anterious, rectus femoris, and peroneus longus). A chimeric clostridial neurotoxin may be administered to one or more muscles of the foot of a subject selected from the: extensus digitorus brevis and extensor hallucis brevis.

The chimeric clostridial neurotoxin may be administered by intramuscular injection at at least 5, 10, 15, 20, 25 or 30 injection sites per treatment session. The chimeric clostridial neurotoxin may be administered by intramuscular injection at up to 50, 45, 40, 35, 30, 25, or 20 injection sites per treatment session. The chimeric clostridial neurotoxin may be administered by intramuscular injection at up to 20 or 15 injection sites per treatment session. Preferably, the chimeric clostridial neurotoxin may be administered by intramuscular injection at up to 10 injection sites per treatment session, for example up to 9, 8, 7, 6, 5, 4, 3 or 2. In one embodiment a chimeric clostridial neurotoxin may be administered at 1-40, 5-40, 8-38, 30-40 (e.g. 35) or 15-25 (e.g. 20) injection sites per treatment session. In one embodiment a chimeric clostridial neurotoxin may be administered

at 1-10, 3-10, 5-10 or 7-10 injection sites per treatment. In one embodiment, a chimeric clostridial neurotoxin may be administered at 25-35 (e.g. 31) injection sites per treatment session. Preferably, a chimeric clostridial neurotoxin may be administered at 25-30 (e.g. 28) injection sites per treatment session.

A chimeric clostridial neurotoxin may be administered by way of a unit dose per injection (e.g. per injection site).

A chimeric clostridial neurotoxin may be administered intraneurally, perineurally or by periganglial administration.

A chimeric clostridial neurotoxin may be administered to the trigeminal nerve, trigeminal ganglia, sphenopalatine ganglia, Gasserian ganglion, nervus intermedius, glossopharyngeal, vagus nerve, otic ganglia, and/or to the upper cervical roots via the occipital nerves. Preferably, a chimeric clostridial neurotoxin is administered to the trigeminal nerve, trigeminal ganglia, and/or sphenopalatine ganglia.

The chimeric clostridial neurotoxin may be administered intra-articularly. The chimeric clostridial neurotoxin may be administered intramuscularly and/or intradermally in the vicinity of a joint.

The chimeric clostridial neurotoxin may be administered by perivascular administration.

The dosage ranges for administration of the chimeric clostridial neurotoxin of the present invention are those to produce the desired therapeutic and/or prophylactic effect.

Fluid dosage forms are typically prepared utilising the chimeric clostridial neurotoxin and a pyrogen-free sterile vehicle. The chimeric clostridial neurotoxin, depending on the vehicle and concentration used, can be either dissolved or suspended in the vehicle. In preparing solutions the chimeric clostridial neurotoxin can be dissolved in the vehicle, the solution being made isotonic if necessary by addition of sodium chloride and sterilised by filtration through a sterile filter using aseptic techniques before filling into suitable sterile vials or ampoules and sealing. Alternatively, if solution stability is adequate, the solution in its sealed containers may be sterilised by autoclaving. Advantageously additives such as buffering, solubilising, stabilising, preservative or bactericidal, suspending or emulsifying agents and or local anaesthetic agents may be dissolved in the vehicle.

Dry powders, which are dissolved or suspended in a suitable vehicle prior to use, may be prepared by filling pre-sterilised ingredients into a sterile container using aseptic technique in a sterile area. Alternatively the ingredients may be dissolved into suitable containers using aseptic technique in a sterile area. The product is then freeze dried and the containers are sealed aseptically.

Parenteral suspensions, suitable for an administration route described herein, are prepared in substantially the same manner, except that the sterile components are suspended in the sterile vehicle, instead of being dissolved and sterilisation cannot be accomplished by filtration. The components may be isolated in a sterile state or alternatively it may be sterilised after isolation, e.g. by gamma irradiation.

Advantageously, a suspending agent for example polyvinylpyrrolidone is included in the composition(s) to facilitate uniform distribution of the components.

Administration in accordance with the present invention may take advantage of a variety of delivery technologies including microparticle encapsulation, or high-pressure aerosol impingement.

Unlike conventional clostridial neurotoxins (e.g. native BoNT/A), the chimeric clostridial neurotoxin of the invention has an improved safety profile and/or improved activity, e.g. as evidenced by an improved Safety Ratio when compared to conventional neurotoxins (*see* WO 2017/191315 A1 for additional details). In view of this, the chimeric clostridial neurotoxin may be administered at low doses while still exhibiting therapeutic efficacy and at high doses without causing unwanted toxicity-related side-effects. The present invention therefore provides a wide range of suitable dosage ranges for treating a disorder (preferably pain).

For convenience of the physician, a chimeric clostridial neurotoxin may be administered by way of a unit dose. Said unit dose may be administered at a single site or, alternatively, less than a unit dose may be administered at an administration site (e.g. where there are two or more administration sites and the dose is divided (equally or unequally) between said sites). In one embodiment, a single unit dose may be administered per muscle and/or neuron treated when carrying out the present invention.

In one embodiment at least one unit dose may be administered to a muscle and/or neuron when carrying out the present invention. For example, 1-20, 1-10, 1-7, or 1-5 unit doses may be administered to a muscle and/or neuron when carrying out the present invention.

In one embodiment, at least 0.25, 0.5, 1, or 2 unit dose(s) may be administered per injection (e.g. per injection site). For example, 0.25, 0.5, 1, or 2 unit dose(s) may be administered per injection (e.g. per injection site). Preferably, 1 unit dose is administered per injection (e.g. per injection site).

When administering a unit dose (or fraction or multiple thereof), this may mean that substantially all of the unit dose (or the fraction or multiple thereof) is administered. For example, a residual amount (e.g. up to 1%, 0.1% or 0.01%) of the unit dose (or the fraction or multiple thereof) may remain in a vial from which the chimeric clostridial neurotoxin has been taken (e.g. in which the chimeric clostridial neurotoxin has been reconstituted). However, preferably all of the unit dose (or fraction or multiple thereof) is administered (e.g. at one or more injection sites).

A suitable unit dose may be 5 pg to 17,000 pg of the chimeric clostridial neurotoxin. An upper limit of the unit dose range may be 16,500, 15,500, 14,500, 13,500, 12,500, 11,500, 10,500, 9,500, 8,500, 7,500, 6,500, 5,500, 4,500, 3,500, 2,500, 1,500 or 500 pg of chimeric clostridial neurotoxin, preferably the upper limit is 16,000 pg. A lower limit of the unit dose range may be 10, 20, 30, 50, 100, 200, 250, 350, 450, 550, 650, 750, 850, 950, 1,000, 1,500, 2,000, 2,500, 3,000, 3,500, 4,000, 4,500 or 5,000 pg of chimeric clostridial neurotoxin, preferably the lower limit is 1,000 pg or 750 pg. The lower limit of said range may be greater than 3,000 pg. Preferably, the unit dose is 750 pg to 17,000 pg of chimeric clostridial neurotoxin. The unit dose of chimeric clostridial neurotoxin may be 3,640 pg to 17,000 pg. More preferably, the unit dose of chimeric clostridial neurotoxin is 1,000 pg to 16,000 pg of chimeric clostridial neurotoxin, e.g. 4,000 pg to 6,000 pg of the chimeric clostridial neurotoxin or 1,000 to 5,500 pg such as 2,000 pg to 4,500 pg, 2,000 pg to 3,000 pg (e.g. 2,500 pg) or 3,500 to 4,500 pg of the chimeric clostridial neurotoxin. Preferably, the unit dose comprises 4,000 pg of the chimeric clostridial neurotoxin.

A suitable unit dose may be 0.2 Units up to 707 Units of chimeric clostridial neurotoxin. An upper limit of said range may be 700, 650, 600, 550, 500, 450, 400, 350, 300, 250, 200, 150 or 100 Units of chimeric clostridial neurotoxin, preferably the upper limit is 666 Units. A lower limit of said range may be 40, 45, 50, 60, 65, 70, 75, 80, 85, 90, 100, 150, 200, 250, 300,

350, 400, 450, 500, 550, 600, 650, or 700 Units of chimeric clostridial neurotoxin, preferably the lower limit is 42 Units or 31 Units. The lower limit of said range may be greater than 125 Units. Preferably, the unit dose is 31 Units to 707 Units of chimeric clostridial neurotoxin. The unit dose of chimeric clostridial neurotoxin may be 166 Units to 707 Units. More preferably, the unit dose is 42 Units to 666 Units of chimeric clostridial neurotoxin, for example 200 Units to 400 Units of the chimeric clostridial neurotoxin or 41 Units to 229 Units such as 83 Units to 188 Units, 83 Units to 125 Units (e.g. 104 Units) or 145 Units to 188 Units of the chimeric clostridial neurotoxin. Preferably, the unit dose is 166 Units of the chimeric clostridial neurotoxin. The unit dose may be 47 Units to 707 Units of chimeric clostridial neurotoxin, e.g. 187 Units to 282 Units, of the chimeric clostridial neurotoxin or 47 to 258 Units such as 94 Units to 211 Units, 94 Units to 141 Units (e.g. 117 Units) or 164 to 211 Units of the chimeric clostridial neurotoxin. The unit dose may be 188 Units of the chimeric clostridial neurotoxin.

A suitable unit dose may be 1,000 pg to 5,500 pg. An upper limit of the unit dose range may be 5,250, 5,200, 5,100, 5,000, 4,500, 4,000, 3,500, 3,000, 2,500, or 2,000 pg of chimeric clostridial neurotoxin, preferably the upper limit is 5,100 pg, more preferably 5,000 pg. A lower limit of the unit dose range may be 1,100, 1,200, 1,250, 1,300, 1,350, 1,400, or 1,450, 1,500, 2,000, 2,500, 3,000, 3,500, or 4,000 pg of chimeric clostridial neurotoxin, preferably the lower limit is 1,400 pg, more preferably 1,500 pg. The lower limit of said range may be greater than 3,000 pg. The unit dose may be 1,400 pg to 5,100 pg (e.g. 1,500 pg to 5,000 pg), 2,000 pg to 5,100 pg, 3,000 to 5,100 pg or 3,000 to 4,000 pg of chimeric clostridial neurotoxin. The unit dose may comprise greater than 3,000 pg up to 5,500 pg of chimeric clostridial neurotoxin. The unit dose of the chimeric clostridial neurotoxin may be 2,000 pg to 4,500 pg, 2,000 pg to 3,000 pg (e.g. 2,500 pg) or 3,500 to 4,500 pg of the chimeric clostridial neurotoxin. Preferably, the unit dose comprises 4,000 pg of the chimeric clostridial neurotoxin.

A suitable unit dose may be 42 Units to 258 Units (e.g. up to 229 Units). An upper limit of the unit dose range may be 225, 220, 215, 210, 205, 200, 190, 180, 170, 160, 150, 125, 100, or 83 Units of chimeric clostridial neurotoxin, preferably the upper limit is 212 Units, more preferably 208 Units. A lower limit of the unit dose range may be 46, 50, 55, 60, 65, 70, 75, 80, or 90, 100, 110, 120, 130, 140, 150, 160 or 166 Units of chimeric clostridial neurotoxin, preferably the lower limit is 58 Units, more preferably 62 Units. The lower limit of said range may be greater than 125 Units. The unit dose may be 58 Units to 212 Units (e.g. 62 Units to 208 Units), 83 Units to 212 Units, 125 to 212 Units or 125 to 166 Units of chimeric clostridial neurotoxin. The unit dose may comprise greater than 125 Units up to 229 Units of chimeric

clostridial neurotoxin. The unit dose of the chimeric clostridial neurotoxin may be 83 Units to 188 Units, 83 Units to 125 Units (e.g. 104 Units) or 146 Units to 188 Units of the chimeric clostridial neurotoxin,. Preferably, the unit dose comprises 166 Units of the chimeric clostridial neurotoxin. The unit dose may comprise 47 Units to 258 Units of chimeric clostridial neurotoxin, e.g. 94 Units to 211 Units, 94 Units to 141 Units (e.g. 117 Units) or 164 to 211 Units of the chimeric clostridial neurotoxin.. The unit dose may be 188 Units of the chimeric clostridial neurotoxin.

A total dose administered per treatment session may be up to 255,000 pg of the chimeric clostridial neurotoxin. This may correspond to 15x the unit dose. The total dose administered may be up to 255,000 pg of the chimeric clostridial neurotoxin and correspond to 28x, 31x or 39x the unit dose. In other words, the total amount of chimeric clostridial neurotoxin administered at a given treatment session may be up to 255,000 pg. The total dose may be up to 240,000, 220,000, 200,000, 180,000, 160,000, 140,000,110,000, 100,000, 90,000, 80,000, 70,000, 60,000, 50,000, 40,000, 30,000, 20,000, 10,000 or 5,000 pg. Preferably, the total dose may be up to 240,000 pg of chimeric clostridial neurotoxin. The total dose may be at least 900, 1,000, 2,000, 3,000, 4,000, 5,000, 7,500, 10,000, 12,500, 15,000, 20,000, 30,000, 40,000, 50,000, 60,000, 70,000, 80,000, 90,000, 100,000, 120,000, 150,000, 175,000, 200,000 or 220,000 pg. Preferably, the total dose may be at least 1,500 pg, more preferably at least 2,000 pg of chimeric clostridial neurotoxin, more preferably greater than 3,000pg, e.g. at least 12,000 pg. The total dose may be 3,640 pg to 255,000 pg of the chimeric clostridial neurotoxin. The total dose may be 2,000-240,000 pg, preferably 128,000-240,000 pg. More preferably, the total dose administered is 15,000-240,000 pg. The total dose may be 75,000 pg or 115,000 pg. The total dose may be 70,000 pg or 112,000 pg.

A total dose administered per treatment session may be up to 10,607 Units of the chimeric clostridial neurotoxin. This may correspond to 15x the unit dose. The total dose administered may be up to 10,607 Units of the chimeric clostridial neurotoxin and correspond to 28x, 31x or 39x the unit dose. In other words, the total amount of chimeric clostridial neurotoxin administered at a given treatment session may be up to 10,607 Units. The total dose may be up to 10,500, 10,000, 9,500, 9,000, 8,500, 8,000, 7,500 7,000, 6,500, 6,000, 5,500, 5,000, 4,500, 4,000, 3,500, 3,000, 2,500, 2,000, 1,500, 1,000, 500, or 207 Units. Preferably, the total dose may be up to 11,268 or 9,983 Units of chimeric clostridial neurotoxin. The total dose may be at least 37, 50, 100, 150, 200, 250, 500, 1,000, 1,500, 2,000, 2,500, 3,000, 3,500, 4,000, 4,500, 5,000, 5,500, 6,000, 6,500, 7,000, 7,500, 8,000, 8,500, 9,000, 9,500, 9,151, 10,000 or 10,328 Units. Preferably, the total dose may be at least 62 Units or 70

Units, more preferably at least 83 Units or 94 Units of chimeric clostridial neurotoxin, more preferably greater than 125 Units or 141 Units, e.g. at least 499 Units or 563 Units. The total dose may be 165 Units to 10,607 Units or 171 Units to 10,607 Units of the chimeric clostridial neurotoxin. The total dose may be 83-9,983 Units or 94-10,607 Units, preferably 5,324-9,983 Units or 6,009-10,607 Units. More preferably, the total dose administered is 624-9,983 Units or 704-10,607 Units. The total dose may be 3,120 or 4,784 Units. The total dose may be 2,911 or 4,659 Units. The total dose may be 3,521 Units or 5,399 Units. The total dose may be 3,286 Units or 5,258 Units.

A suitable unit dose may be 2,500 pg and the total dose may be up to 70,000 pg. For example, a suitable unit dose may be 2,500 pg and the total dose may be 70,000 pg. A suitable unit dose may be 4,000 pg and the total dose may be up to 112,000 pg. For example, a suitable unit dose may be 4,000 pg and the total dose may be 112,000 pg. A suitable unit dose may be 5,000 pg and the total dose may be up to 155,000 pg. For example, a suitable unit dose may be 5,000 pg and the total dose may be 155,000 pg.

A suitable unit dose may be 104 Units and the total dose may be up to 2,912 Units. For example, a suitable unit dose may be 104 Units and the total dose may be 2,912 Units. A suitable unit dose may be 166 Units and the total dose may be up to 4,659 Units. For example, a suitable unit dose may be 166 Units and the total dose may be 4,659 Units. A suitable unit dose may be 208 Units and the total dose may be up to 6,448 Units. For example, a suitable unit dose may be 208 Units and the total dose may be 6,448 Units.

A suitable unit dose may be 117 Units and the total dose may be up to 3,286 Units. For example, a suitable unit dose may be 117 Units and the total dose may be 3,286 Units. A suitable unit dose may be 188 Units and the total dose may be up to 5,258 Units. For example, a suitable unit dose may be 188 Units and the total dose may be 5,258 Units. A suitable unit dose may be 235 Units and the total dose may be up to 7,277 Units. For example, a suitable unit dose may be 235 Units and the total dose may be 7,277 Units.

The total number of unit doses administered in a given treatment may be up to 15x the unit dose. For example, the total number of unit doses administered may be up to 14x, 13x, 12x, 11x, 10x, 9x, 8x or 7x. The total number of unit doses administered may be at least 2x, 3x, 4x, 5x, 6x, 7x the unit dose, preferably at least 2x. The total number of unit doses administered may be 2x to 15x, 7x to 15x or 10x to 14x. Preferably, the number of unit doses administered is 15x.

The total number of unit doses administered in a given treatment may be up to 39x the unit dose (as long as the total dose administered during the treatment does not exceed the upper limit of 255,000 pg or 10,607 Units). For example, the total number of unit doses administered may be up to 35x, 31x, 30x, 29x, 28x, 27x, 26x, 25x, or 20x the unit dose, preferably the total number of unit doses administered is up to 28x the unit dose. The total number of unit doses administered may be at least 2x, 3x, 4x, 5x, 6x, 7x the unit dose, preferably at least 2x. The total number of unit doses administered may be 2x to 39x, 15x to 31x or 28x to 31x. The total number of unit doses administered may be 28x, 31x or 39x.

Thus, the total dose administered per treatment session may be up to 192,500 pg of the chimeric clostridial neurotoxin. For example, the total dose administered may be up to 180,000 pg, or up to 177,000 pg (e.g. up to 175,000 pg).

Thus, the total dose administered per treatment session may be up to 8,007 Units of the chimeric clostridial neurotoxin. For example, the total dose administered may be up to 7,488 Units, or up to 7,363 Units (e.g. up to 7,280 Units). The total dose administered per treatment session may be up to 9,037 Units of the chimeric clostridial neurotoxin. For example, the total dose administered may be up to 8,451 Units, or up to 8,310 Units (e.g. up to 8,216 Units).

The total dose administered per treatment session may be up to 110,000 pg of the chimeric clostridial neurotoxin. For example, the total dose administered may be up to 105,000 pg, up to 102,000 pg (e.g. up to 100,000 pg).

The total dose administered per treatment session may be up to 4,576 Units of the chimeric clostridial neurotoxin. For example, the total dose administered may be up to 4,368 Units, preferably up to 4,243 Units (more preferably up to 4,160 Units). The total dose administered per treatment session may be up to 5,165 Units of the chimeric clostridial neurotoxin. For example, the total dose administered may be up to 4,929 Units, preferably up to 4,789 Units (more preferably up to 4,695 Units).

The term “up to” when used in reference to a value (e.g. up to 255,000 pg) means up to and including the value recited. Thus, as an example, reference to administering “up to 255,000 pg” of chimeric clostridial neurotoxin encompasses administration of 255,000 pg of chimeric

clostridial neurotoxin as well as administration of less than 255,000 pg of chimeric clostridial neurotoxin.

Where the disorder is headache pain (e.g. migraine pain) or migraine, at least a unit dose of the chimeric clostridial neurotoxin may be administered to one or more of the frontalis, corrugator, nasalis, orbicularis oculi, temporalis, occipitalis, and trapezius. Preferably, at least a unit dose of the chimeric clostridial neurotoxin may be administered to the frontalis, corrugator, nasalis, orbicularis oculi, temporalis, occipitalis, and trapezius. In some embodiments, a plurality of unit doses are administered to one or more of: a frontalis muscle, a corrugator muscle, a nasalis muscle, an orbicularis oculi muscle, a temporalis muscle, an occipitalis muscle, and a trapezius muscle. In one embodiment, a single unit dose is administered to a corrugator muscle, a nasalis muscle, and an orbicularis oculi muscle, and a plurality of unit doses are administered to a frontalis muscle, a temporalis muscle, an occipitalis muscle, and a trapezius muscle. The plurality of unit doses may be 2-10 unit doses, e.g. 2-8 unit doses, preferably 2-5 unit doses, such as 2-4 unit doses.

More preferably, the method comprises administering the chimeric clostridial neurotoxin to at least one of a: frontalis muscle, corrugator (e.g. corrugator supercilii) muscle, nasalis muscle, orbicularis oculi muscle, temporalis muscle, occipitalis muscle, or trapezius muscle. The method may comprise administering the chimeric clostridial neurotoxin to a: frontalis muscle, corrugator (e.g. corrugator supercilii) muscle, nasalis muscle, orbicularis oculi muscle, temporalis muscle, occipitalis muscle, and trapezius muscle.

The headache pain (e.g. migraine pain) or migraine treatment may comprise administering:

(i) 2 unit doses to a frontalis muscle (preferably 2 unit doses per frontalis muscle);
(ii) 1 unit dose to a corrugator muscle (preferably 1 unit dose per corrugator muscle);
(iii) 1 unit dose to a nasalis muscle (preferably 1 unit dose per nasalis muscle);
(iv) 1 unit dose to an orbicularis oculi muscle (preferably 1 unit dose per orbicularis oculi muscle);
(v) 4 unit doses to a temporalis muscle (preferably 4 unit doses per temporalis muscle);
(vi) 3 unit doses to an occipitalis muscle (preferably 3 unit doses per occipitalis muscle); and/or
(vii) 2 unit doses to a trapezius muscle (preferably 2 unit doses per trapezius muscle)

The headache pain (e.g. migraine pain) or migraine treatment may comprise administering:

(i) 2 unit doses to a frontalis muscle (preferably 2 unit doses per frontalis muscle);

(ii) 1 unit dose to a corrugator muscle (preferably 1 unit dose per corrugator muscle);

(iii) 1 unit dose to a nasalis muscle (preferably 1 unit dose per nasalis muscle);

(iv) 1 unit dose to an orbicularis oculi muscle (preferably 1 unit dose per orbicularis oculi muscle);

(v) 4 unit doses to a temporalis muscle (preferably 4 unit doses per temporalis muscle);

(vi) 3 unit doses to an occipitalis muscle (preferably 3 unit doses per occipitalis muscle); and

(vii) 2 unit doses to a trapezius muscle (preferably 2 unit doses per trapezius muscle).

The treatment of headache pain (e.g. migraine pain) or migraine may comprise administering a unit dose of the chimeric clostridial neurotoxin bilaterally. The headache pain (e.g. migraine pain) or migraine treatment may comprise administering:

(i) 4 unit doses to the frontalis muscles (preferably 2 unit doses to a frontalis muscle at a first side of the face and 2 unit doses to a frontalis muscle at a second side of the face);

(ii) 2 unit doses to the corrugator muscles (preferably 1 unit dose to a corrugator muscle at a first side of the face and 1 unit dose to a corrugator muscle at a second side of the face);

(iii) 2 unit doses to the nasalis muscles (preferably 1 unit dose to a nasalis muscle at a first side of the face and 1 unit dose to a nasalis muscle at a second side of the face);

(iv) 2 unit doses to the orbicularis oculi muscles (preferably 1 unit dose to an orbicularis oculi muscle at a first side of the face and 1 unit dose to an orbicularis oculi muscle at a second side of the face);

(v) 8 unit doses to the temporalis muscles (preferably 4 unit doses to a temporalis muscle at a first side of the head and 4 unit doses to a temporalis muscle at a second side of the head);

(vi) 6 unit doses to the occipitalis muscles (preferably 3 unit doses to an occipitalis muscle at a first side of the head and 3 unit doses to an occipitalis muscle at a second side of the head); and/or

(vii) 4 unit doses to the trapezius muscles (preferably 2 unit doses to a trapezius muscle at a first side of the neck and 2 unit doses to a trapezius muscle at a second side of the neck).

Preferably, the headache pain (e.g. migraine pain) or migraine treatment comprises administering:

(i) 4 unit doses to the frontalis muscles (preferably 2 unit doses to a frontalis muscle at a first side of the face and 2 unit doses to a frontalis muscle at a second side of the face);

(ii) 2 unit doses to the corrugator muscles (preferably 1 unit dose to a corrugator muscle at a first side of the face and 1 unit dose to a corrugator muscle at a second side of the face);

(iii) 2 unit doses to the nasalis muscles (preferably 1 unit dose to a nasalis muscle at a first side of the face and 1 unit dose to a nasalis muscle at a second side of the face);

(iv) 2 unit doses to the orbicularis oculi muscles (preferably 1 unit dose to an orbicularis oculi muscle at a first side of the face and 1 unit dose to an orbicularis oculi muscle at a second side of the face);

(v) 8 unit doses to the temporalis muscles (preferably 4 unit doses to a temporalis muscle at a first side of the head and 4 unit doses to a temporalis muscle at a second side of the head);

(vi) 6 unit doses to the occipitalis muscles (preferably 3 unit doses to an occipitalis muscle at a first side of the head and 3 unit doses to an occipitalis muscle at a second side of the head); and

(vii) 4 unit doses to the trapezius muscles (preferably 2 unit doses to a trapezius muscle at a first side of the neck and 2 unit doses to a trapezius muscle at a second side of the neck).

When treating headache pain (e.g. migraine pain) or migraine as described in the foregoing embodiments, it is preferred that one unit dose is administered per injection (e.g. injection site). Thus, the administration of the chimeric clostridial neurotoxin may comprise:

(i) 2 injections to a frontalis muscle (preferably 2 injections per frontalis muscle);

(ii) 1 injection to a corrugator muscle (preferably 1 injection per corrugator muscle);

(iii) 1 injection to a nasalis muscle (preferably 1 injection per nasalis muscle);

(iv) 1 injection to an orbicularis oculi muscle (preferably 1 injection per orbicularis oculi muscle);

(v) 4 injections to a temporalis muscle (preferably 4 injections per temporalis muscle);

(vi) 3 injections to an occipitalis muscle (preferably 3 injections per occipitalis muscle); and/or

(vii) 2 injections to a trapezius muscle (preferably 2 injections per trapezius muscle).

The administration of the chimeric clostridial neurotoxin may comprise:

(i) 2 injections to a frontalis muscle (preferably 2 injections per frontalis muscle);

(ii) 1 injection to a corrugator muscle (preferably 1 injection per corrugator muscle);

(iii) 1 injection to a nasalis muscle (preferably 1 injection per nasalis muscle);

(iv) 1 injection to an orbicularis oculi muscle (preferably 1 injection per orbicularis oculi muscle);

(v) 4 injections to a temporalis muscle (preferably 4 injections per temporalis muscle);

(vi) 3 injections to an occipitalis muscle (preferably 3 injections per occipitalis muscle); and

(vii) 2 injections to a trapezius muscle (preferably 2 injections per trapezius muscle).

The administration of the chimeric clostridial neurotoxin may comprise:

(i) 4 injections to the frontalis muscles (preferably 2 injections to a frontalis muscle at a first side of the face and 2 injections to a frontalis muscle at a second side of the face);

(ii) 2 injections to the corrugator muscles (preferably 1 injection to a corrugator muscle at a first side of the face and 1 injection to a corrugator muscle at a second side of the face);

(iii) 2 injections to the nasalis muscles (preferably 1 injection to a nasalis muscle at a first side of the face and 1 injection to a nasalis muscle at a second side of the face;

(iv) 2 injections to the orbicularis oculi muscles (preferably 1 injection to an orbicularis oculi muscle at a first side of the face and 1 injection to an orbicularis oculi muscle at a second side of the face);

(v) 8 injections to the temporalis muscles (preferably 4 injections to a temporalis muscle at a first side of the head and 4 injections to a temporalis muscle at a second side of the head);

(vi) 6 injections to the occipitalis muscles (preferably 3 injections to an occipitalis muscle at a first side of the head and 3 injections to an occipitalis muscle at a second side of the head); and/or

(vii) 4 injections to the trapezius muscles (preferably 2 injections to a trapezius muscle at a first side of the neck and 2 injections to a trapezius muscle at a second side of the neck).

Preferably, the administration of the chimeric clostridial neurotoxin comprises:

(i) 4 injections to the frontalis muscles (preferably 2 injections to a frontalis muscle at a first side of the face and 2 injections to a frontalis muscle at a second side of the face);

(ii) 2 injections to the corrugator muscles (preferably 1 injection to a corrugator muscle at a first side of the face and 1 injection to a corrugator muscle at a second side of the face);

(iii) 2 injections to the nasalis muscles (preferably 1 injection to a nasalis muscle at a first side of the face and 1 injection to a nasalis muscle at a second side of the face;

(iv) 2 injections to the orbicularis oculi muscles (preferably 1 injection to an orbicularis oculi muscle at a first side of the face and 1 injection to an orbicularis oculi muscle at a second side of the face);

(v) 8 injections to the temporalis muscles (preferably 4 injections to a temporalis muscle at a first side of the head and 4 injections to a temporalis muscle at a second side of the head);

(vi) 6 injections to the occipitalis muscles (preferably 3 injections to an occipitalis muscle at a first side of the head and 3 injections to an occipitalis muscle at a second side of the head); and

(vii) 4 injections to the trapezius muscles (preferably 2 injections to a trapezius muscle at a first side of the neck and 2 injections to a trapezius muscle at a second side of the neck).

Where the disorder is headache pain (e.g. migraine pain) or migraine, at least a unit dose of the chimeric clostridial neurotoxin may be administered intramuscularly or intradermally to one or more of the frontalis, procerus, corrugator, temporalis, occipitalis, trapezius, and cervical paraspinal group muscle(s). Preferably, at least a unit dose of the chimeric clostridial neurotoxin may be administered intramuscularly or intradermally to the frontalis, procerus, corrugator, temporalis, occipitalis, trapezius, and cervical paraspinal group muscle(s) (e.g. at least a unit dose to each cervical paraspinal group muscle).

Where the disorder is headache pain (e.g. migraine pain) or migraine, at least a unit dose of the chimeric clostridial neurotoxin may be administered to one or more of the frontalis, procerus, corrugator, temporalis, occipitalis, trapezius, and cervical paraspinal group muscle(s). Preferably, at least a unit dose of the chimeric clostridial neurotoxin may be administered to the frontalis, procerus, corrugator, temporalis, occipitalis, trapezius, and cervical paraspinal group muscle(s) (e.g. at least a unit dose to each cervical paraspinal group muscle). In one embodiment:

(i) a single unit dose is administered to one or more of: the procerus muscle; and a corrugator muscle (preferably a single unit dose is administered to a corrugator muscle at a first side (e.g. left side) of the face and a second unit dose is administered to a corrugator muscle at a second side (e.g. right side) of the face); and/or (preferably and)

(iii) a plurality of unit doses are administered to one or more of: a frontalis muscle; a temporalis muscle; an occipitalis muscle; a trapezius muscle; and the cervical paraspinal

group (e.g. where a single or double unit dose is administered to each muscle of the cervical paraspinal group). The plurality of unit doses may be 2-8 unit doses, e.g. 2-5 unit doses.

The treatment of headache pain (e.g. migraine pain) or migraine may comprise intramuscularly or intradermally administering a unit dose of the chimeric clostridial neurotoxin bilaterally. The headache pain (e.g. migraine pain) or migraine treatment may comprise administering:

(i) 2 unit doses to a frontalis muscle at a first side of the face and/or 2 unit doses to a frontalis muscle at a second side of the face;

(ii) 1 unit dose to a procerus muscle;

(iii) 1 unit dose to a corrugator muscle at a first side of the face and/or 1 unit dose to a corrugator muscle at a second side of the face;

(iv) 4 unit doses to a temporalis muscle at a first side of the head and/or 4 unit doses to a temporalis muscle at a second side of the head;

(v) 3 unit doses to an occipitalis muscle at a first side of the neck/head (preferably head) and/or 3 unit doses to an occipitalis muscle at a second side of the neck/head (preferably head);

(vi) 3 unit doses to a trapezius muscle at a first side of the neck and/or 3 unit doses to a trapezius muscle at a second side of the neck; and/or

(vii) 4 unit doses to the cervical paraspinal group at a first side of the neck and/or 4 unit doses to a cervical paraspinal group at a second side of the neck (e.g. where 1 unit dose is administered per cervical paraspinal group muscle), or 2 unit doses to the cervical paraspinal group at a first side of the neck and/or 2 unit doses to a cervical paraspinal group at a second side of the neck (e.g. where 2 unit doses are administered per cervical paraspinal group muscle).

The headache pain (e.g. migraine pain) or migraine treatment may comprise administering:

(i) 2 unit doses to a frontalis muscle at a first side of the face and 2 unit doses to a frontalis muscle at a second side of the face;

(ii) 1 unit dose to a procerus muscle;

(iii) 1 unit dose to a corrugator muscle at a first side of the face and 1 unit dose to a corrugator muscle at a second side of the face;

(iv) 4 unit doses to a temporalis muscle at a first side of the head and 4 unit doses to a temporalis muscle at a second side of the head;

(v) 3 unit doses to an occipitalis muscle at a first side of the neck/head (preferably head) and 3 unit doses to an occipitalis muscle at a second side of the neck/head (preferably head);

(vi) 3 unit doses to a trapezius muscle at a first side of the neck and 3 unit doses to a trapezius muscle at a second side of the neck; and/or

(vii) 4 unit doses to the cervical paraspinal group at a first side of the neck and 4 unit doses to a cervical paraspinal group at a second side of the neck (e.g. where 1 unit dose is administered per cervical paraspinal group muscle), or 2 unit doses to the cervical paraspinal group at a first side of the neck and 2 unit doses to a cervical paraspinal group at a second side of the neck (e.g. where 2 unit doses are administered per cervical paraspinal group muscle).

The headache pain (e.g. migraine pain) or migraine treatment may comprise administering:

(i) 2 unit doses to a frontalis muscle at a first side of the face and 2 unit doses to a frontalis muscle at a second side of the face;

(ii) 1 unit dose to a procerus muscle;

(iii) 1 unit dose to a corrugator muscle at a first side of the face and 1 unit dose to a corrugator muscle at a second side of the face;

(iv) 4 unit doses to a temporalis muscle at a first side of the head and 4 unit doses to a temporalis muscle at a second side of the head;

(v) 3 unit doses to an occipitalis muscle at a first side of the neck/head (preferably head) and 3 unit doses to an occipitalis muscle at a second side of the neck/head (preferably head);

(vi) 3 unit doses to a trapezius muscle at a first side of the neck and 3 unit doses to a trapezius muscle at a second side of the neck; and

(vii) 4 unit doses to the cervical paraspinal group at a first side of the neck and 4 unit doses to a cervical paraspinal group at a second side of the neck (e.g. where 1 unit dose is administered per cervical paraspinal group muscle), or 2 unit doses to the cervical paraspinal group at a first side of the neck and 2 unit doses to a cervical paraspinal group at a second side of the neck (e.g. where 2 unit doses are administered per cervical paraspinal group muscle).

The headache pain (e.g. migraine pain) or migraine treatment may comprise administering:

(i) 1 unit dose to a frontalis muscle (preferably 1 unit dose per frontalis muscle);

(ii) 1 unit dose to a procerus muscle (preferably 1 unit dose per procerus muscle);

(iii) 1 unit dose to a corrugator muscle (preferably 1 unit dose per corrugator muscle);

(iv) 4 unit doses to a temporalis muscle (preferably 4 unit doses per temporalis muscle);

(v) 3 unit doses to an occipitalis muscle (preferably 3 unit doses per occipitalis muscle);

(vi) 3 unit doses to a trapezius muscle (preferably 3 unit doses per trapezius muscle); and/or

(vii) 4 unit doses to a cervical paraspinal group (preferably 4 unit doses per cervical paraspinal group), or 2 unit doses to a cervical paraspinal group (preferably 2 unit doses per cervical paraspinal group).

The headache pain (e.g. migraine pain) or migraine treatment may comprise administering:

(i) 1 unit dose to a frontalis muscle (preferably 1 unit dose per frontalis muscle);

(ii) 1 unit dose to a procerus muscle (preferably 1 unit dose per procerus muscle);

(iii) 1 unit dose to a corrugator muscle (preferably 1 unit dose per corrugator muscle);

(iv) 4 unit doses to a temporalis muscle (preferably 4 unit doses per temporalis muscle);

(v) 3 unit doses to an occipitalis muscle (preferably 3 unit doses per occipitalis muscle);

(vi) 3 unit doses to a trapezius muscle (preferably 3 unit doses per trapezius muscle); and

(vii) 4 unit doses to a cervical paraspinal group (preferably 4 unit doses per cervical paraspinal group), or 2 unit doses to a cervical paraspinal group (preferably 2 unit doses per cervical paraspinal group).

The headache pain (e.g. migraine pain) or migraine treatment may comprise administering:

(i) 4 unit doses to the frontalis muscles (preferably 2 unit doses to a frontalis muscle at a first side of the face and 2 unit doses to a frontalis muscle at a second side of the face);

(ii) 1 unit dose to a procerus muscle;

(iii) 2 unit doses to the corrugator muscles (preferably 1 unit dose to a corrugator muscle at a first side of the face and 1 unit dose to a corrugator muscle at a second side of the face);

(iv) 8 unit doses to the temporalis muscles (preferably 4 unit doses to a temporalis muscle at a first side of the head and 4 unit doses to a temporalis muscle at a second side of the head);

(v) 6 unit doses to the occipitalis muscles (preferably 3 unit doses to an occipitalis muscle at a first side of the head and 3 unit doses to an occipitalis muscle at a second side of the head);

(vi) 6 unit doses to the trapezius muscles (preferably 3 unit doses to a trapezius muscle at a first side of the neck and 3 unit doses to a trapezius muscle at a second side of the neck); and/or

(vii) 8 unit doses to the cervical paraspinal group (preferably 4 unit doses to the cervical paraspinal group at a first side of the neck and 4 unit doses to a cervical paraspinal group at a second side of the neck (e.g. where 1 unit dose is administered per cervical paraspinal group muscle)), or 4 unit doses to the cervical paraspinal group (preferably 2 unit doses to the cervical paraspinal group at a first side of the neck and 2 unit doses to a cervical paraspinal group at a second side of the neck (e.g. where 2 unit doses is administered per cervical paraspinal group muscle)).

The headache pain (e.g. migraine pain) or migraine treatment may comprise administering:

(i) 4 unit doses to the frontalis muscles (preferably 2 unit doses to a frontalis muscle at a first side of the face and 2 unit doses to a frontalis muscle at a second side of the face);

(ii) 1 unit dose to a procerus muscle;

(iii) 2 unit doses to the corrugator muscles (preferably 1 unit dose to a corrugator muscle at a first side of the face and 1 unit dose to a corrugator muscle at a second side of the face);

(iv) 8 unit doses to the temporalis muscles (preferably 4 unit doses to a temporalis muscle at a first side of the head and 4 unit doses to a temporalis muscle at a second side of the head);

(v) 6 unit doses to the occipitalis muscles (preferably 3 unit doses to an occipitalis muscle at a first side of the head and 3 unit doses to an occipitalis muscle at a second side of the head);

(vi) 6 unit doses to the trapezius muscles (preferably 3 unit doses to a trapezius muscle at a first side of the neck and 3 unit doses to a trapezius muscle at a second side of the neck); and

(vii) 8 unit doses to the cervical paraspinal group (preferably 4 unit doses to the cervical paraspinal group at a first side of the neck and 4 unit doses to a cervical paraspinal group at a second side of the neck (e.g. where 1 unit dose is administered per cervical paraspinal group muscle)), or 4 unit doses to the cervical paraspinal group (preferably 2 unit doses to the cervical paraspinal group at a first side of the neck and 2 unit doses to a cervical

paraspinal group at a second side of the neck (e.g. where 2 unit doses is administered per cervical paraspinal group muscle)).

When treating headache pain (e.g. migraine pain) or migraine as described in the foregoing embodiments, it is preferred that one unit dose is administered per injection site. Thus, the treatment may comprise administration of the chimeric clostridial neurotoxin at:

(i) 2 injection sites at a frontalis muscle at a first side of the face and/or 2 injection sites at a frontalis muscle at a second side of the face;

(ii) 1 injection site at a procerus muscle;

(iii) 1 injection site at a corrugator muscle at a first side of the face and/or 1 injection site at a corrugator muscle at a second side of the face;

(iv) 4 injection sites at a temporalis muscle at a first side of the head and/or 4 injection sites at a temporalis muscle at a second side of the head;

(v) 3 injection sites at an occipitalis muscle at a first side of the neck/head (preferably head) and/or 3 injection sites at an occipitalis muscle at a second side of the neck/head (preferably head);

(vi) 3 injection sites at a trapezius muscle at a first side of the neck and/or 3 injection sites at a trapezius muscle at a second side of the neck; and/or

(vii) 4 injection sites at the cervical paraspinal group at a first side of the neck and/or 4 injection sites at the cervical paraspinal group at a second side of the neck (e.g. where there is 1 injection site per cervical paraspinal group muscle), or 2 injection sites at the cervical paraspinal group at a first side of the neck and/or 2 injection sites at the cervical paraspinal group at a second side of the neck (e.g. where there are 2 injection sites per cervical paraspinal group muscle).

The treatment may comprise administration of the chimeric clostridial neurotoxin at:

(i) 2 injection sites at a frontalis muscle at a first side of the face and 2 injection sites at a frontalis muscle at a second side of the face;

(ii) 1 injection site at a procerus muscle;

(iii) 1 injection site at a corrugator muscle at a first side of the face and 1 injection site at a corrugator muscle at a second side of the face;

(iv) 4 injection sites at a temporalis muscle at a first side of the head and 4 injection sites at a temporalis muscle at a second side of the head;

(v) 3 injection sites at an occipitalis muscle at a first side of the neck/head (preferably head) and 3 injection sites at an occipitalis muscle at a second side of the neck/head (preferably head);

(vi) 3 injection sites at a trapezius muscle at a first side of the neck and 3 injection sites at a trapezius muscle at a second side of the neck; and/or

(vii) 4 injection sites at the cervical paraspinal group at a first side of the neck and 4 injection sites at the cervical paraspinal group at a second side of the neck (e.g. where there is 1 injection site per cervical paraspinal group muscle), or 2 injection sites at the cervical paraspinal group at a first side of the neck and 2 injection sites at the cervical paraspinal group at a second side of the neck (e.g. where there are 2 injection sites per cervical paraspinal group muscle).

The treatment may comprise administration of the chimeric clostridial neurotoxin at:

(i) 2 injection sites at a frontalis muscle at a first side of the face and 2 injection sites at a frontalis muscle at a second side of the face;

(ii) 1 injection site at a procerus muscle;

(iii) 1 injection site at a corrugator muscle at a first side of the face and 1 injection site at a corrugator muscle at a second side of the face;

(iv) 4 injection sites at a temporalis muscle at a first side of the head and 4 injection sites at a temporalis muscle at a second side of the head;

(v) 3 injection sites at an occipitalis muscle at a first side of the neck/head (preferably head) and 3 injection sites at an occipitalis muscle at a second side of the neck/head (preferably head);

(vi) 3 injection sites at a trapezius muscle at a first side of the neck and 3 injection sites at a trapezius muscle at a second side of the neck; and

(vii) 4 injection sites at the cervical paraspinal group at a first side of the neck and 4 injection sites at the cervical paraspinal group at a second side of the neck (e.g. where there is 1 injection site per cervical paraspinal group muscle), or 2 injection sites at the cervical paraspinal group at a first side of the neck and 2 injection sites at the cervical paraspinal group at a second side of the neck (e.g. where there are 2 injection site per cervical paraspinal group muscle).

The administration of the chimeric clostridial neurotoxin may comprise:

(i) 2 injections to a frontalis muscle (preferably 2 injections per frontalis muscle);

(ii) 1 injection to a procerus muscle (preferably 1 injection per procerus muscle);

(iii) 1 injection to a corrugator muscle (preferably 1 injection per corrugator muscle);

(iv) 4 injections to a temporalis muscle (preferably 4 injections per temporalis muscle);

(v) 3 injections to an occipitalis muscle (preferably 3 injections per occipitalis muscle);

(vi) 3 injections to a trapezius muscle (preferably 3 injections per trapezius muscle); and/or

(vii) 4 injections to a cervical paraspinal group (preferably 2 injections per cervical paraspinal group).

The administration of the chimeric clostridial neurotoxin may comprise:

(i) 2 injections to a frontalis muscle (preferably 2 injections per frontalis muscle);

(ii) 1 injection to a procerus muscle (preferably 1 injection per procerus muscle);

(iii) 1 injection to a corrugator muscle (preferably 1 injection per corrugator muscle);

(iv) 4 injections to a temporalis muscle (preferably 4 injections per temporalis muscle);

(v) 3 injections to an occipitalis muscle (preferably 3 injections per occipitalis muscle);

(vi) 3 injections to a trapezius muscle (preferably 3 injections per trapezius muscle); and

(vii) 4 injections to a cervical paraspinal group (preferably 2 injections per cervical paraspinal group).

The administration of the chimeric clostridial neurotoxin may comprise:

(i) 4 injections to the frontalis muscles (preferably 2 injections to a frontalis muscle at a first side of the face and 2 injections to a frontalis muscle at a second side of the face);

(ii) 1 injection to a procerus muscle;

(iii) 2 injections to the corrugator muscles (preferably 1 injection to a corrugator muscle at a first side of the face and 1 injection to a corrugator muscle at a second side of the face);

(iv) 8 injections to the temporalis muscles (preferably 4 injections to a temporalis muscle at a first side of the head and 4 injections to a temporalis muscle at a second side of the head);

(v) 6 injections to the occipitalis muscles (preferably 3 injections to an occipitalis muscle at a first side of the head and 3 injections to an occipitalis muscle at a second side of the head);

(vi) 6 injections to the trapezius muscles (preferably 3 injections to a trapezius muscle at a first side of the neck and 3 injections to a trapezius muscle at a second side of the neck); and/or

(vii) 8 injections to the cervical paraspinal group (preferably 4 injections to the cervical paraspinal group at a first side of the neck and 4 injections to the cervical paraspinal group at a second side of the neck (e.g. where there is 1 injection per cervical paraspinal group muscle)), or 4 injections to the cervical paraspinal group (preferably 2 injections to the

cervical paraspinal group at a first side of the neck and 2 injections to the cervical paraspinal group at a second side of the neck (e.g. where there are 2 injections per cervical paraspinal group muscle)).

The administration of the chimeric clostridial neurotoxin may comprise:

(i) 4 injections to the frontalis muscles (preferably 2 injections to a frontalis muscle at a first side of the face and 2 injections to a frontalis muscle at a second side of the face);

(ii) 1 injection to a procerus muscle;

(iii) 2 injections to the corrugator muscles (preferably 1 injection to a corrugator muscle at a first side of the face and 1 injection to a corrugator muscle at a second side of the face);

(iv) 8 injections to the temporalis muscles (preferably 4 injections to a temporalis muscle at a first side of the head and 4 injections to a temporalis muscle at a second side of the head);

(v) 6 injections to the occipitalis muscles (preferably 3 injections to an occipitalis muscle at a first side of the head and 3 injections to an occipitalis muscle at a second side of the head);

(vi) 6 injections to the trapezius muscles (preferably 3 injections to a trapezius muscle at a first side of the neck and 3 injections to a trapezius muscle at a second side of the neck); and

(vii) 8 injections to the cervical paraspinal group (preferably 4 injections to the cervical paraspinal group at a first side of the neck and 4 injections to the cervical paraspinal group at a second side of the neck (e.g. where there is 1 injection site per cervical paraspinal group muscle)), or 4 injections to the cervical paraspinal group (preferably 2 injections to the cervical paraspinal group at a first side of the neck and 2 injections to the cervical paraspinal group at a second side of the neck (e.g. where there are 2 injections per cervical paraspinal group muscle)).

The headache pain (e.g. migraine pain) or migraine treatment may comprise administering:

(i) 2 unit doses to a frontalis muscle (preferably 2 unit doses per frontalis muscle);

(ii) 1 unit dose to a procerus muscle;

(iii) 1 unit dose to a corrugator muscle (preferably 1 unit dose per corrugator muscle);

(iv) 5 unit doses to a temporalis muscle (preferably 5 unit doses per temporalis muscle);

(v) 4 unit doses to an occipitalis muscle (preferably 4 unit doses per occipitalis muscle);

(vi) 5 unit doses to a trapezius muscle (preferably 5 unit doses per trapezius muscle); and/or

(vii) 4 unit dose sites to a cervical paraspinal group (preferably 4 unit doses per cervical paraspinal group), or 2 unit dose sites to a cervical paraspinal group (preferably 2 unit doses per cervical paraspinal group).

The headache pain (e.g. migraine pain) or migraine treatment may comprise administering:

(i) 2 unit doses to a frontalis muscle (preferably 2 unit doses per frontalis muscle);

(ii) 1 unit dose to a procerus muscle (preferably 1 unit dose per procerus muscle);

(iii) 1 unit dose to a corrugator muscle (preferably 1 unit dose per corrugator muscle);

(iv) 5 unit doses to a temporalis muscle (preferably 5 unit doses per temporalis muscle);

(v) 4 unit doses to an occipitalis muscle (preferably 4 unit doses per occipitalis muscle);

(vi) 5 unit doses to a trapezius muscle (preferably 5 unit doses per trapezius muscle); and

(vii) 4 unit doses to a cervical paraspinal group (preferably 4 unit doses per cervical paraspinal group), or 2 unit dose sites to a cervical paraspinal group (preferably 2 unit doses per cervical paraspinal group).

The headache pain (e.g. migraine pain) or migraine treatment may comprise administering:

(i) 4 unit doses to the frontalis muscles (preferably 2 unit doses to a frontalis muscle at a first side of the face and 2 unit doses to a frontalis muscle at a second side of the face);

(ii) 1 unit dose to a procerus muscle;

(iii) 2 unit doses to the corrugator muscles (preferably 1 unit dose to a corrugator muscle at a first side of the face and 1 unit dose to a corrugator muscle at a second side of the face);

(iv) 10 unit doses to the temporalis muscles (preferably 5 unit doses to a temporalis muscle at a first side of the head and 5 unit doses to a temporalis muscle at a second side of the head);

(v) 8 unit doses to the occipitalis muscles (preferably 4 unit doses to an occipitalis muscle at a first side of the head and 4 unit doses to an occipitalis muscle at a second side of the head);

(vi) 10 unit doses to the trapezius muscles (preferably 4 unit doses to a trapezius muscle at a first side of the neck and 4 unit doses to a trapezius muscle at a second side of the neck); and/or

(vii) 4 unit doses to a cervical paraspinal group (e.g. where 1 or 2 unit doses are administered per cervical paraspinal group muscle).

The headache pain (e.g. migraine pain) or migraine treatment may comprise administering:

(i) 4 unit doses to the frontalis muscles (preferably 2 unit doses to a frontalis muscle at a first side of the face and 2 unit doses to a frontalis muscle at a second side of the face);

(ii) 1 unit dose to a procerus muscle;

(iii) 2 unit doses to the corrugator muscles (preferably 1 unit dose to a corrugator muscle at a first side of the face and 1 unit dose to a corrugator muscle at a second side of the face);

(iv) 10 unit doses to the temporalis muscles (preferably 5 unit doses to a temporalis muscle at a first side of the head and 5 unit doses to a temporalis muscle at a second side of the head);

(v) 8 unit doses to the occipitalis muscles (preferably 4 unit doses to an occipitalis muscle at a first side of the head and 4 unit doses to an occipitalis muscle at a second side of the head);

(vi) 10 unit doses to the trapezius muscles (preferably 5 unit doses to a trapezius muscle at a first side of the neck and 5 unit doses to a trapezius muscle at a second side of the neck); and

(vii) 4 unit doses to a cervical paraspinal group (e.g. where 1 or 2 unit doses are administered per cervical paraspinal group muscle).

When treating headache pain (e.g. migraine pain) or migraine, the administration of the chimeric clostridial neurotoxin may comprise:

(i) 2 injections to a frontalis muscle (preferably 2 injections per frontalis muscle);

(ii) 1 injection to a procerus muscle;

(iii) 1 injection to a corrugator muscle (preferably 1 injection per corrugator muscle);

(iv) 5 injections to a temporalis muscle (preferably 5 injections per temporalis muscle);

(v) 4 injections to an occipitalis muscle (preferably 4 injections per occipitalis muscle);

(vi) 5 injections to a trapezius muscle (preferably 5 injections per trapezius muscle); and/or

(vii) 4 injections to a cervical paraspinal group (e.g. where 1 or 2 injections are administered per cervical paraspinal group muscle).

The administration may comprise:

(i) 2 injections to a frontalis muscle (preferably 2 injections per frontalis muscle);

(ii) 1 injection to a procerus muscle;

(iii) 1 injection to a corrugator muscle (preferably 1 injection per corrugator muscle);

(iv) 5 injections to a temporalis muscle (preferably 5 injections per temporalis muscle);

(v) 4 injections to an occipitalis muscle (preferably 4 injections per occipitalis muscle);

(vi) 5 injections to a trapezius muscle (preferably 5 injections per trapezius muscle); and

(vii) 4 injections to a cervical paraspinal group (e.g. where 1 or 2 injections are administered per cervical paraspinal group muscle).

The administration may comprise:

(i) 4 injections to the frontalis muscles (preferably 2 injections to a frontalis muscle at a first side of the face and 2 injections to a frontalis muscle at a second side of the face);

(ii) 1 injection to a procerus muscle;

(iii) 2 injections to the corrugator muscles (preferably 1 injection to a corrugator muscle at a first side of the face and 1 injection to a corrugator muscle at a second side of the face);

(iv) 10 injections to the temporalis muscles (preferably 5 injections to a temporalis muscle at a first side of the head and 5 injections to a temporalis muscle at a second side of the head);

(v) 8 injections to the occipitalis muscles (preferably 4 injections to an occipitalis muscle at a first side of the head and 4 injections to an occipitalis muscle at a second side of the head);

(vi) 10 injections to the trapezius muscles (preferably 4 injections to a trapezius muscle at a first side of the neck and 4 injections to a trapezius muscle at a second side of the neck); and/or

(vii) 4 injections to a cervical paraspinal group (e.g. where 1 or 2 injections are administered per cervical paraspinal group muscle).

The administration may comprise:

(i) 4 injections to the frontalis muscles (preferably 2 injections to a frontalis muscle at a first side of the face and 2 injections to a frontalis muscle at a second side of the face);

(ii) 1 injection to a procerus muscle;

(iii) 2 injections to the corrugator muscles (preferably 1 injection to a corrugator muscle at a first side of the face and 1 injection to a corrugator muscle at a second side of the face);

(iv) 10 injections to the temporalis muscles (preferably 5 injections to a temporalis muscle at a first side of the head and 5 injections to a temporalis muscle at a second side of the head);

(v) 8 injections to the occipitalis muscles (preferably 4 injections to an occipitalis muscle at a first side of the head and 4 injections to an occipitalis muscle at a second side of the head);

(vi) 10 injections to the trapezius muscles (preferably 4 injections to a trapezius muscle at a first side of the neck and 4 injections to a trapezius muscle at a second side of the neck); and

(vii) 4 injections to a cervical paraspinal group (e.g. where 1 or 2 injections are administered per cervical paraspinal group muscle).

Where the disorder is headache pain (e.g. migraine pain) or migraine, at least a unit dose of the chimeric clostridial neurotoxin may be administered to one or more of the frontalis, corrugator, nasalis, orbicularis oculi, masseter, temporalis, occipitalis, and trapezius. In one embodiment, at least a unit dose of the chimeric clostridial neurotoxin may be administered to the frontalis, corrugator, nasalis, orbicularis oculi, masseter, temporalis, occipitalis, and trapezius. In one embodiment:

(i) a single unit dose is administered to one or more of: a frontalis muscle; a corrugator muscle (preferably a single unit dose is administered to a corrugator muscle at a first side (e.g. left side) of the face and a second unit dose is administered to a corrugator muscle at a second side (e.g. right side) of the face); an orbicularis oculi muscle; a masseter muscle; and/or (preferably and) an upper trapezius muscle;

(ii) half of a unit dose is administered to a nasalis muscle; and/or (preferably and)

(iii) a plurality of unit doses are administered to one or more of: a temporalis muscle; an occipitalis muscle; and/or (preferably and) a lower trapezius muscle. The plurality of unit doses may be 2-6 unit doses, e.g. 2-5 unit doses.

The headache pain (e.g. migraine pain) or migraine treatment may comprise administering:

(i) 1 unit dose to a frontalis muscle (preferably 1 unit dose per frontalis muscle);

(ii) 1 unit dose to a corrugator muscle (preferably 1 unit dose per corrugator muscle);

(iii) 0.5 unit doses to a nasalis muscle (preferably 0.5 unit doses per nasalis muscle);

(iv) 1 unit dose to an orbicularis oculi muscle (preferably 1 unit dose per orbicularis oculi muscle);

(v) 1 unit dose to a masseter muscle (preferably 1 unit dose per masseter muscle);

(vi) 6 unit doses to a temporalis muscle (preferably 6 unit doses per temporalis

muscle);

(vii) 6 unit doses to an occipitalis muscle (preferably 6 unit doses per occipitalis muscle);

(viii) 1 unit dose to an upper trapezius muscle (preferably 1 unit dose per upper trapezius muscle); and/or

(ix) 2 unit doses to a lower trapezius muscle (preferably 2 unit doses per lower trapezius muscle).

The headache pain (e.g. migraine pain) or migraine treatment may comprise administering:

(i) 1 unit dose to a frontalis muscle (preferably 1 unit dose per frontalis muscle);

(ii) 1 unit dose to a corrugator muscle (preferably 1 unit dose per corrugator muscle);

(iii) 0.5 unit doses to a nasalis muscle (preferably 0.5 unit doses per nasalis muscle);

(iv) 1 unit dose to an orbicularis oculi muscle (preferably 1 unit dose per orbicularis oculi muscle);

(v) 1 unit dose to a masseter muscle (preferably 1 unit dose per masseter muscle);

(vi) 6 unit doses to a temporalis muscle (preferably 6 unit doses per temporalis muscle);

(vii) 6 unit doses to an occipitalis muscle (preferably 6 unit doses per occipitalis muscle);

(viii) 1 unit dose to an upper trapezius muscle (preferably 1 unit dose per upper trapezius muscle); and

(ix) 2 unit doses to a lower trapezius muscle (preferably 2 unit doses per lower trapezius muscle).

The headache pain (e.g. migraine pain) or migraine treatment may comprise administering:

(i) 2 unit doses to the frontalis muscles (preferably 1 unit dose to a frontalis muscle at a first side of the face and 1 unit dose to a frontalis muscle at a second side of the face);

(ii) 2 unit doses to the corrugator muscles (preferably 1 unit dose to a corrugator muscle at a first side of the face and 1 unit dose to a corrugator muscle at a second side of the face);

(iii) 1 unit dose to the nasalis muscles (preferably 0.5 unit doses to a nasalis muscle at a first side of the face and 0.5 unit doses to a nasalis muscle at a second side of the face);

(iv) 2 unit doses to the orbicularis oculi muscles (preferably 1 unit dose to an orbicularis oculi muscle at a first side of the face and 1 unit dose to an orbicularis oculi muscle at a second side of the face);

(v) 2 unit doses to the masseter muscles (preferably 1 unit dose to a masseter muscle at a first side of the face and 1 unit dose to a masseter muscle at a second side of the face);

(vi) 12 unit doses to the temporalis muscles (preferably 6 unit doses to a temporalis muscle at a first side of the head and 6 unit doses to a temporalis muscle at a second side of the head);

(vii) 12 unit doses to the occipitalis muscles (preferably 6 unit doses to an occipitalis muscle at a first side of the head and 6 unit doses to an occipitalis muscle at a second side of the head);

(viii) 2 unit doses to the upper trapezius muscles (preferably 1 unit dose to an upper trapezius muscle at a first side of the neck and 1 unit dose to an upper trapezius muscle at a second side of the neck); and/or

(ix) 4 unit doses to the lower trapezius muscles (preferably 2 unit doses to a lower trapezius muscle at a first side of the neck and/or 2 unit dose to a lower trapezius muscle at a second side of the neck).

The headache pain (e.g. migraine pain) or migraine treatment may comprise administering:

(i) 2 unit doses to the frontalis muscles (preferably 1 unit dose to a frontalis muscle at a first side of the face and 1 unit dose to a frontalis muscle at a second side of the face);

(ii) 2 unit doses to the corrugator muscles (preferably 1 unit dose to a corrugator muscle at a first side of the face and 1 unit dose to a corrugator muscle at a second side of the face);

(iii) 1 unit dose to the nasalis muscles (preferably 0.5 unit doses to a nasalis muscle at a first side of the face and 0.5 unit doses to a nasalis muscle at a second side of the face);

(iv) 2 unit doses to the orbicularis oculi muscles (preferably 1 unit dose to an orbicularis oculi muscle at a first side of the face and 1 unit dose to an orbicularis oculi muscle at a second side of the face);

(v) 2 unit doses to the masseter muscles (preferably 1 unit dose to a masseter muscle at a first side of the face and 1 unit dose to a masseter muscle at a second side of the face);

(vi) 12 unit doses to the temporalis muscles (preferably 6 unit doses to a temporalis muscle at a first side of the head and 6 unit doses to a temporalis muscle at a second side of the head);

(vii) 12 unit doses to the occipitalis muscles (preferably 6 unit doses to an occipitalis muscle at a first side of the head and 6 unit doses to an occipitalis muscle at a second side of the head);

(viii) 2 unit doses to the upper trapezius muscles (preferably 1 unit dose to an upper trapezius muscle at a first side of the neck and 1 unit dose to an upper trapezius muscle at a second side of the neck); and

(ix) 4 unit doses to the lower trapezius muscles (preferably 2 unit doses to a lower trapezius muscle at a first side of the neck and/or 2 unit dose to a lower trapezius muscle at a second side of the neck).

The administration of the chimeric clostridial neurotoxin may comprise:

(i) 1 injection to a frontalis muscle (preferably 1 injection per frontalis muscle);

(ii) 1 injection to a corrugator muscle (preferably 1 injection per corrugator muscle);

(iii) 1 injection to a nasalis muscle (preferably 1 injection per nasalis muscle);

(iv) 1 injection to an orbicularis oculi muscle (preferably 1 injection per orbicularis oculi muscle);

(v) 1 injection to a masseter muscle (preferably 1 injection per masseter muscle);

(vi) 3 injections to a temporalis muscle (preferably 3 injections per temporalis muscle);

(vii) 3 injections to an occipitalis muscle (preferably 3 injections per occipitalis muscle);

(viii) 1 injection to an upper trapezius muscle (preferably 1 injection per upper trapezius muscle); and/or

(ix) 1 injection to a lower trapezius muscle (preferably 1 injection per lower trapezius muscle).

The administration of the chimeric clostridial neurotoxin may comprise:

(i) 1 injection to a frontalis muscle (preferably 1 injection per frontalis muscle);

(ii) 1 injection to a corrugator muscle (preferably 1 injection per corrugator muscle);

(iii) 1 injection to a nasalis muscle (preferably 1 injection per nasalis muscle);

(iv) 1 injection to an orbicularis oculi muscle (preferably 1 injection per orbicularis oculi muscle);

(v) 1 injection to a masseter muscle (preferably 1 injection per masseter muscle);

(vi) 3 injections to a temporalis muscle (preferably 3 injections per temporalis muscle);

(vii) 3 injections to an occipitalis muscle (preferably 3 injections per occipitalis muscle);

(viii) 1 injection to an upper trapezius muscle (preferably 1 injection per upper trapezius muscle); and

(ix) 1 injection to a lower trapezius muscle (preferably 1 injection per lower trapezius muscle).

The administration of the chimeric clostridial neurotoxin may comprise:

(i) 2 injections to the frontalis muscles (preferably 1 injection to a frontalis muscle at a first side of the face and 1 injection to a frontalis muscle at a second side of the face);

(ii) 2 injections to the corrugator muscles (preferably 1 injection to a corrugator muscle at a first side of the face and 1 injection to a corrugator muscle at a second side of the face);

(iii) 2 injections to the nasalis muscles (preferably 1 injection to a nasalis muscle at a first side of the face and 1 injection to at a nasalis muscle at a second side of the face);

(iv) 2 injections to the orbicularis oculi muscles (preferably 1 injection to an orbicularis oculi muscle at a first side of the face and 1 injection to an orbicularis oculi muscle at a second side of the face);

(v) 2 injections to the masseter muscles (preferably 1 injection to a masseter muscle at a first side of the face and 1 injection to a masseter muscle at a second side of the face);

(vi) 6 injections to the temporalis muscles (preferably 3 injections to a temporalis muscle at a first side of the head and 3 injections to a temporalis muscle at a second side of the head);

(vii) 6 injections to the occipitalis muscles (preferably 3 injections to an occipitalis muscle at a first side of the head and 3 injections to an occipitalis muscle at a second side of the head);

(viii) 2 injections to the upper trapezius muscles (preferably 1 injection to an upper trapezius muscle at a first side of the neck and 1 injection to an upper trapezius muscle at a second side of the neck); and/or

(ix) 2 injections to the lower trapezius muscles (preferably 1 injection to a lower trapezius muscle at a first side of the neck and/or 1 injection to a lower trapezius muscle at a second side of the neck).

The administration of the chimeric clostridial neurotoxin may comprise:

(i) 2 injections to the frontalis muscles (preferably 1 injection to a frontalis muscle at a first side of the face and 1 injection to a frontalis muscle at a second side of the face);

(ii) 2 injections to the corrugator muscles (preferably 1 injection to a corrugator muscle at a first side of the face and 1 injection to a corrugator muscle at a second side of the face);

(iii) 2 injections to the nasalis muscles (preferably 1 injection to a nasalis muscle at a first side of the face and 1 injection to at a nasalis muscle at a second side of the face);

(iv) 2 injections to the orbicularis oculi muscles (preferably 1 injection to an orbicularis oculi muscle at a first side of the face and 1 injection to an orbicularis oculi muscle at a second side of the face);

(v) 2 injections to the masseter muscles (preferably 1 injection to a masseter muscle at a first side of the face and 1 injection to a masseter muscle at a second side of the face);

(vi) 6 injections to the temporalis muscles (preferably 3 injections to a temporalis muscle at a first side of the head and 3 injections to a temporalis muscle at a second side of the head);

(vii) 6 injections to the occipitalis muscles (preferably 3 injections to an occipitalis muscle at a first side of the head and 3 injections to an occipitalis muscle at a second side of the head);

(viii) 2 injections to the upper trapezius muscles (preferably 1 injection to an upper trapezius muscle at a first side of the neck and 1 injection to an upper trapezius muscle at a second side of the neck); and

(ix) 2 injections to the lower trapezius muscles (preferably 1 injection to a lower trapezius muscle at a first side of the neck and/or 1 injection to a lower trapezius muscle at a second side of the neck).

Where the disorder is headache pain (e.g. migraine pain) or migraine, at least a unit dose of the chimeric clostridial neurotoxin may be administered to one or more of the frontalis, corrugator, nasalis, orbicularis oculi, masseter, temporalis, upper occipitalis, lower occipitalis, and upper and lower trapezius. At least a unit dose of the chimeric clostridial neurotoxin may be administered to the frontalis, corrugator, nasalis, orbicularis oculi, masseter, temporalis, upper occipitalis, lower occipitalis, and upper and lower trapezius. In one embodiment:

(i) a single unit dose is administered to one or more of: a frontalis muscle; a corrugator muscle (preferably a single unit dose is administered to a corrugator muscle at a first side (e.g. left side) of the face and a second unit dose is administered to a corrugator muscle at a second side (e.g. right side) of the face); a nasalis muscle; an orbicularis oculi muscle; a masseter muscle; and a lower occipitalis muscle; and/or (preferably and)

(iii) a plurality of unit doses are administered to one or more of: a temporalis muscle; an upper occipitalis muscle; and a trapezius muscle. The plurality of unit doses may be 2-8 unit doses, e.g. 2-5 unit doses.

The headache pain (e.g. migraine pain) or migraine treatment may comprise administering:

(i) 1 unit dose to a frontalis muscle (preferably 1 unit dose per frontalis muscle);

(ii) 1 unit dose to a corrugator muscle (preferably 1 unit dose per corrugator muscle);

(iii) 1 unit dose to a nasalis muscle (preferably 1 unit dose per nasalis muscle);

(iv) 1 unit dose to an orbicularis oculi muscle (preferably 1 unit dose per orbicularis oculi muscle);

(v) 1 unit dose to a masseter muscle (preferably 1 unit dose per masseter muscle);

(vi) 4 unit doses to a temporalis muscle (preferably 4 unit doses per temporalis muscle);

(vii) 2 unit doses to an upper occipitalis muscle (preferably 2 unit doses per upper occipitalis muscle);

(viii) 1 unit dose to a lower occipitalis muscle (preferably 1 unit dose per lower occipitalis muscle); and/or

(viii) 2 unit doses to a trapezius muscle (preferably 2 unit doses per trapezius muscle).

The headache pain (e.g. migraine pain) or migraine treatment may comprise administering:

(i) 1 unit dose to a frontalis muscle (preferably 1 unit dose per frontalis muscle);

(ii) 1 unit dose to a corrugator muscle (preferably 1 unit dose per corrugator muscle);

(iii) 1 unit dose to a nasalis muscle (preferably 1 unit dose per nasalis muscle);

(iv) 1 unit dose to an orbicularis oculi muscle (preferably 1 unit dose per orbicularis oculi muscle);

(v) 1 unit dose to a masseter muscle (preferably 1 unit dose per masseter muscle);

(vi) 4 unit doses to a temporalis muscle (preferably 4 unit doses per temporalis muscle);

(vii) 2 unit doses to an upper occipitalis muscle (preferably 2 unit doses per upper occipitalis muscle);

(viii) 1 unit dose to a lower occipitalis muscle (preferably 1 unit dose per lower occipitalis muscle); and

(viii) 2 unit doses to a trapezius muscle (preferably 2 unit doses per trapezius muscle).

The headache pain (e.g. migraine pain) or migraine treatment may comprise administering:

(i) 2 unit doses to the frontalis muscles (preferably 1 unit dose to a frontalis muscle at a first side of the face and 1 unit dose to a frontalis muscle at a second side of the face);

(ii) 2 unit doses to the corrugator muscles (preferably 1 unit dose to a corrugator muscle at a first side of the face and 1 unit dose to a corrugator muscle at a second side of the face);

(iii) 2 unit doses to the nasalis muscles (preferably 1 unit dose to a nasalis muscle at a first side of the face and 1 unit dose to a nasalis muscle at a second side of the face);

(iv) 2 unit doses to the orbicularis oculi muscles (preferably 1 unit dose to an orbicularis oculi muscle at a first side of the face and 1 unit dose to an orbicularis oculi muscle at a second side of the face);

(v) 2 unit doses to the masseter muscles (preferably 1 unit dose to a masseter muscle at a first side of the face and 1 unit dose to a masseter muscle at a second side of the face);

(vi) 8 unit doses to the temporalis muscles (preferably 4 unit doses to a temporalis muscle at a first side of the head and 4 unit doses to a temporalis muscle at a second side of the head);

(vii) 4 unit doses to the upper occipitalis muscles (preferably 2 unit doses to an upper occipitalis muscle at a first side of the head and 2 unit doses to an upper occipitalis muscle at a second side of the head);

(viii) 2 unit doses to the lower occipitalis muscles (preferably 1 unit dose to a lower occipitalis muscle at a first side of the head and 1 unit dose to a lower occipitalis muscle at a second side of the head); and/or

(ix) 4 unit doses to the trapezius muscles (preferably 2 unit doses to a trapezius muscle at a first side of the neck and 2 unit doses to a trapezius muscle at a second side of the neck).

The headache pain (e.g. migraine pain) or migraine treatment may comprise administering:

(i) 2 unit doses to the frontalis muscles (preferably 1 unit dose to a frontalis muscle at a first side of the face and 1 unit dose to a frontalis muscle at a second side of the face);

(ii) 2 unit doses to the corrugator muscles (preferably 1 unit dose to a corrugator muscle at a first side of the face and 1 unit dose to a corrugator muscle at a second side of the face);

(iii) 2 unit doses to the nasalis muscles (preferably 1 unit dose to a nasalis muscle at a first side of the face and 1 unit dose to a nasalis muscle at a second side of the face);

(iv) 2 unit doses to the orbicularis oculi muscles (preferably 1 unit dose to an orbicularis oculi muscle at a first side of the face and 1 unit dose to an orbicularis oculi muscle at a second side of the face);

(v) 2 unit doses to the masseter muscles (preferably 1 unit dose to a masseter muscle at a first side of the face and 1 unit dose to a masseter muscle at a second side of the face);

(vi) 8 unit doses to the temporalis muscles (preferably 4 unit doses to a temporalis muscle at a first side of the head and 4 unit doses to a temporalis muscle at a second side of the head);

(vii) 4 unit doses to the upper occipitalis muscles (preferably 2 unit doses to an upper occipitalis muscle at a first side of the head and 2 unit doses to an upper occipitalis muscle at a second side of the head);

(viii) 2 unit doses to the lower occipitalis muscles (preferably 1 unit dose to a lower occipitalis muscle at a first side of the head and 1 unit dose to a lower occipitalis muscle at a second side of the head); and

(ix) 4 unit doses to the trapezius muscles (preferably 2 unit doses to a trapezius muscle at a first side of the neck and 2 unit doses to a trapezius muscle at a second side of the neck).

The administration of the chimeric clostridial neurotoxin may comprise:

(i) 1 injection to a frontalis muscle (preferably 1 injection per frontalis muscle);

(ii) 1 injection to a corrugator muscle (preferably 1 injection per corrugator muscle);

(iii) 1 injection to a nasalis muscle (preferably 1 injection per nasalis muscle);

(iv) 1 injection to an orbicularis oculi muscle (preferably 1 injection per orbicularis oculi muscle);

(v) 1 injection to a masseter muscle (preferably 1 injection per masseter muscle);

(vi) 4 injections to a temporalis muscle (preferably 4 injections per temporalis muscle);

(vii) 2 injections to an upper occipitalis muscle (preferably 2 injections per upper occipitalis muscle);

(viii) 1 injection to a lower occipitalis muscle (preferably 1 injection per lower occipitalis muscle); and/or

(viii) 2 injections to a trapezius muscle (preferably 2 injections per trapezius muscle).

The administration of the chimeric clostridial neurotoxin may comprise:

(i) 1 injection to a frontalis muscle (preferably 1 injection per frontalis muscle);

(ii) 1 injection to a corrugator muscle (preferably 1 injection per corrugator muscle);

(iii) 1 injection to a nasalis muscle (preferably 1 injection per nasalis muscle);

(iv) 1 injection to an orbicularis oculi muscle (preferably 1 injection per orbicularis oculi muscle);

(v) 1 injection to a masseter muscle (preferably 1 injection per masseter muscle);

(vi) 4 injections to a temporalis muscle (preferably 4 injections per temporalis muscle);

(vii) 2 injections to an upper occipitalis muscle (preferably 2 injections per upper occipitalis muscle);

(viii) 1 injection to a lower occipitalis muscle (preferably 1 injection per lower occipitalis muscle); and

(viii) 2 injections to a trapezius muscle (preferably 2 injections per trapezius muscle).

The administration of the chimeric clostridial neurotoxin may comprise:

(i) 2 injections to the frontalis muscles (preferably 1 injection to a frontalis muscle at a first side of the face and 1 injection to a frontalis muscle at a second side of the face);

(ii) 2 injections to the corrugator muscles (preferably 1 injection to a corrugator muscle at a first side of the face and 1 injection to a corrugator muscle at a second side of the face);

(iii) 2 injections to the nasalis muscles (preferably 1 injection to a nasalis muscle at a first side of the face and 1 injection to a nasalis muscle at a second side of the face);

(iv) 2 injections to the orbicularis oculi muscles (preferably 1 injection to an orbicularis oculi muscle at a first side of the face and 1 injection to an orbicularis oculi muscle at a second side of the face);

(v) 2 injections to the masseter muscles (preferably 1 injection to a masseter muscle at a first side of the face and 1 injection to a masseter muscle at a second side of the face);

(vi) 8 injections to the temporalis muscles (preferably 4 injections to a temporalis muscle at a first side of the head and 4 injections to a temporalis muscle at a second side of the head);

(vii) 4 injections to the upper occipitalis muscles (preferably 2 injections to an upper occipitalis muscle at a first side of the head and 2 injections to an upper occipitalis muscle at a second side of the head);

(viii) 2 injections to the lower occipitalis muscles (preferably 1 injection to a lower occipitalis muscle at a first side of the head and 1 injection to a lower occipitalis muscle at a second side of the head); and/or

(ix) 4 injections to the trapezius muscles (preferably 2 injections to a trapezius muscle at a first side of the neck and 2 injections to a trapezius muscle at a second side of the neck).

The administration of the chimeric clostridial neurotoxin may comprise:

(i) 2 injections to the frontalis muscles (preferably 1 injection to a frontalis muscle at a first side of the face and 1 injection to a frontalis muscle at a second side of the face);

(ii) 2 injections to the corrugator muscles (preferably 1 injection to a corrugator muscle at a first side of the face and 1 injection to a corrugator muscle at a second side of the face);

(iii) 2 injections to the nasalis muscles (preferably 1 injection to a nasalis muscle at a first side of the face and 1 injection to a nasalis muscle at a second side of the face);

(iv) 2 injections to the orbicularis oculi muscles (preferably 1 injection to an orbicularis oculi muscle at a first side of the face and 1 injection to an orbicularis oculi muscle at a second side of the face);

(v) 2 injections to the masseter muscles (preferably 1 injection to a masseter muscle at a first side of the face and 1 injection to a masseter muscle at a second side of the face);

(vi) 8 injections to the temporalis muscles (preferably 4 injections to a temporalis muscle at a first side of the head and 4 injections to a temporalis muscle at a second side of the head);

(vii) 4 injections to the upper occipitalis muscles (preferably 2 injections to an upper occipitalis muscle at a first side of the head and 2 injections to an upper occipitalis muscle at a second side of the head);

(viii) 2 injections to the lower occipitalis muscles (preferably 1 injection to a lower occipitalis muscle at a first side of the head and 1 injection to a lower occipitalis muscle at a second side of the head); and

(ix) 4 injections to the trapezius muscles (preferably 2 injections to a trapezius muscle at a first side of the neck and 2 injections to a trapezius muscle at a second side of the neck).

In any of the aspects or embodiments described herein, the administration of the chimeric clostridial neurotoxin may comprise injection of the chimeric clostridial neurotoxin to a muscle directly to the muscle or indirectly to the muscle. For example, where injection of the chimeric clostridial neurotoxin to a muscle is indirectly to the muscle, the chimeric clostridial neurotoxin may be administered in the region of the muscle. In one embodiment, where injection of the chimeric clostridial neurotoxin to a muscle is directly to the muscle, the chimeric clostridial neurotoxin may be administered intramuscularly to the muscle. In one embodiment, where injection of the chimeric clostridial neurotoxin to a muscle is indirectly to the muscle, the chimeric clostridial neurotoxin may be administered intradermally.

Where the disorder is headache pain (e.g. migraine pain) or migraine, at least a unit dose of the chimeric clostridial neurotoxin may be administered intradermally to one or more of: the trigeminal ophthalmic region; the trigeminal maxillary region; the trigeminal mandibula region; and the back of the head. Preferably, at least a unit dose of the chimeric clostridial

neurotoxin may be administered intradermally to: the trigeminal ophthalmic region; the trigeminal maxillary region; the trigeminal mandibula region; and the back of the head.

The intradermal administration at one or more of said regions may target the chimeric clostridial neurotoxin to a target trigeminal nerve (e.g. target nerve terminal). A target nerve (e.g. target nerve terminal) of the trigeminal, ophthalmic region may be one or more of the: supraoribital nerve; supratrochlear nerve; and intratrochlear nerve (e.g. a nerve terminal thereof). A target nerve (e.g. target nerve terminal) of the trigeminal, maxillary region may be one or more of the: zygomaticotemporal nerve and zygomaticofacial nerve (e.g. a nerve terminal thereof). A target nerve (e.g. target nerve terminal) of the trigeminal, mandibula region may be the auriculotemporal nerve (e.g. a nerve terminal thereof). A target nerve (e.g. target nerve terminal) of the back of the head may be one or more of the: greater occipital nerve and lesser occipital nerve (e.g. a nerve terminal thereof).

The intradermal administration at one or more of said regions may target the chimeric clostridial neurotoxin to a target trigeminal nerve (e.g. target nerve terminal). A target nerve (e.g. target nerve terminal) of the trigeminal, ophthalmic region may be one or more of the: supraoribital nerve; and supratrochlear nerve (e.g. a nerve terminal thereof). A target nerve (e.g. target nerve terminal) of the trigeminal, maxillary region may be one or more of the: zygomaticotemporal nerve; and intraorbital nerve (e.g. a nerve terminal thereof). A target nerve (e.g. target nerve terminal) of the trigeminal, mandibula region may be one or more of the: auriculotemporal nerve; and mandibula nerve (e.g. a nerve terminal thereof). A target nerve (e.g. target nerve terminal) of the back of the head may be one or more of the: greater occipital nerve; lesser occipital nerve; and suboccipitalis nerve (e.g. a nerve terminal thereof).

In one embodiment:

(i) a single unit dose is administered intradermally in the region of one or more of: a supraorbital nerve (preferably a single unit dose is administered in the region of a supraorbital nerve at a first side (e.g. left side) of the face and a second unit dose is administered in the region of a supraorbital nerve at a second side (e.g. right side) of the face); a supratrochlear nerve (preferably a single unit dose is administered in the region of a supratrochlear nerve at a first side (e.g. left side) of the face and a second unit dose is administered in the region of a supratrochlear nerve at a second side (e.g. right side) of the face); an intratrochlear nerve (preferably a single unit dose is administered in the region of an intratrochlear nerve at a first side (e.g. left side) of the face and a second unit dose is

administered in the region of an intratrochelar nerve at a second side (e.g. right side) of the face); a zygomaticotemporal nerve (preferably a single unit dose is administered in the region of a zygomaticotemporal nerve at a first side (e.g. left side) of the face and a second unit dose is administered in the region of a zygomaticotemporal nerve at a second side (e.g. right side) of the face); a zygomaticofacial nerve (preferably a single unit dose is administered in the region of a zygomaticofacial nerve at a first side (e.g. left side) of the face and a second unit dose is administered in the region of a zygomaticofacial nerve at a second side (e.g. right side) of the face); a lesser occipital nerve (preferably a single unit dose is administered in the region of a lesser occipital nerve at a first side (e.g. left side) of the neck and a second unit dose is administered in the region of a lesser occipital nerve at a second side (e.g. right side) of the neck); and/or (preferably and)

(iii) a plurality of unit doses are administered in the region of one or more of: a supraorbital nerve (preferably a single unit dose is administered in the region of a supraorbital nerve at a first side (e.g. left side) of the face and a second unit dose is administered in the region of a supraorbital nerve at a second side (e.g. right side) of the face); a supratrochlear nerve (preferably a single unit dose is administered in the region of a supratrochlear nerve at a first side (e.g. left side) of the face and a second unit dose is administered in the region of a supratrochlear nerve at a second side (e.g. right side) of the face); an intratrochlear nerve (preferably a single unit dose is administered in the region of an intratrochlear nerve at a first side (e.g. left side) of the face and a second unit dose is administered in the region of an intratrochelar nerve at a second side (e.g. right side) of the face); a zygomaticotemporal nerve (preferably a single unit dose is administered in the region of a zygomaticotemporal nerve at a first side (e.g. left side) of the face and a second unit dose is administered in the region of a zygomaticotemporal nerve at a second side (e.g. right side) of the face); a zygomaticofacial nerve (preferably a single unit dose is administered in the region of a zygomaticofacial nerve at a first side (e.g. left side) of the face and a second unit dose is administered in the region of a zygomaticofacial nerve at a second side (e.g. right side) of the face); an auriculotemporal nerve; a greater occipital nerve; a lesser occipital nerve (preferably a single unit dose is administered in the region of a lesser occipital nerve at a first side (e.g. left side) of the head and a second unit dose is administered in the region of a lesser occipital nerve at a second side (e.g. right side) of the head). The plurality of unit doses may be 2-8 unit doses, e.g. 2-5 unit doses.

Preferred injection sites and numbers of injections are shown in Figure 6. In such instances one unit dose of the chimeric clostridial neurotoxin may be administered per injection site.

Preferably the injection site is in the region of a terminal of an indicated nerve.

The treatment of headache pain (e.g. migraine pain) or migraine may comprise intradermally administering a unit dose of the chimeric clostridial neurotoxin bilaterally. The headache pain (e.g. migraine pain) or migraine treatment may comprise administering:

(i) 1 unit dose in the region of a supraorbital nerve at a first side of the face and/or 1 unit dose in the region of a supraorbital nerve at a second side of the face;

(ii) 1 unit dose in the region of a supratrochlear nerve at a first side of the face and/or 1 unit dose in the region of a supratrochlear nerve at a second side of the face;

(iii) 1 unit dose in the region of an intratrochlear nerve at a first side of the face and/or 1 unit dose in the region of an intratrochlear nerve at a second side of the face;

(iv) 1 unit dose in the region of a zygomaticotemporal nerve at a first side of the face and/or 1 unit dose in the region of a zygomaticotemporal nerve at a second side of the face;

(v) 1 unit dose in the region of a zygomaticofacial nerve at a first side of the face and/or 1 unit dose in the region of a zygomaticofacial nerve at a second side of the face;

(vi) 2 unit doses in the region of an auriculotemporal nerve at a first side of the face and/or 2 unit doses in the region of an auriculotemporal nerve at a second side of the face;

(vii) 2 unit doses in the region of a greater occipital nerve at a first side of the neck and/or 2 unit doses in the region of a greater occipital nerve at a second side of the neck; and/or

(vii) 1 unit dose in the region of a lesser occipital nerve at a first side of the neck and/or 1 unit dose in the region of a lesser occipital nerve at a second side of the neck.

The treatment of headache pain (e.g. migraine pain) or migraine may comprise administering a unit dose of the chimeric clostridial neurotoxin bilaterally. The headache pain (e.g. migraine pain) or migraine treatment may comprise administering:

(i) 1 unit dose in the region of a supraorbital nerve at a first side of the face and/or 1 unit dose in the region of a supraorbital nerve at a second side of the face;

(ii) 1 unit dose in the region of a supratrochlear nerve at a first side of the face and/or 1 unit dose in the region of a supratrochlear nerve at a second side of the face;

(iii) 1 unit dose in the region of an intratrochlear nerve at a first side of the face and/or 1 unit dose in the region of an intratrochlear nerve at a second side of the face;

(iv) 1 unit dose in the region of a zygomaticotemporal nerve at a first side of the face and/or 1 unit dose in the region of a zygomaticotemporal nerve at a second side of the face;

(v) 1 unit dose in the region of a zygomaticofacial nerve at a first side of the face and/or 1 unit dose in the region of a zygomaticofacial nerve at a second side of the face;

(vi) 2 unit doses in the region of an auriculotemporal nerve at a first side of the face and/or 2 unit doses in the region of an auriculotemporal nerve at a second side of the face;

(vii) 2 unit doses in the region of a greater occipital nerve at a first side of the neck and/or 2 unit doses in the region of a greater occipital nerve at a second side of the neck; and/or

(vii) 1 unit dose in the region of a lesser occipital nerve at a first side of the neck and/or 1 unit dose in the region of a lesser occipital nerve at a second side of the neck.

Preferably, the headache pain (e.g. migraine pain) or migraine treatment may comprise administering:

(i) 1 unit dose in the region of a supraorbital nerve at a first side of the face and 1 unit dose in the region of a supraorbital nerve at a second side of the face;

(ii) 1 unit dose in the region of a supratrochlear nerve at a first side of the face and 1 unit dose in the region of a supratrochlear nerve at a second side of the face;

(iii) 1 unit dose in the region of an intratrochlear nerve at a first side of the face and 1 unit dose in the region of an intratrochlear nerve at a second side of the face;

(iv) 1 unit dose in the region of a zygomaticotemporal nerve at a first side of the face and 1 unit dose in the region of a zygomaticotemporal nerve at a second side of the face;

(v) 1 unit dose in the region of a zygomaticofacial nerve at a first side of the face and 1 unit dose in the region of a zygomaticofacial nerve at a second side of the face;

(vi) 2 unit doses in the region of an auriculotemporal nerve at a first side of the face and 2 unit doses in the region of an auriculotemporal nerve at a second side of the face;

(vii) 2 unit doses in the region of a greater occipital nerve at a first side of the neck and 2 unit doses in the region of a greater occipital nerve at a second side of the neck; and/or

(vii) 1 unit dose in the region of a lesser occipital nerve at a first side of the neck and 1 unit dose in the region of a lesser occipital nerve at a second side of the neck.

More preferably, the headache pain (e.g. migraine pain) or migraine treatment may comprise administering:

(i) 1 unit dose in the region of a supraorbital nerve at a first side of the face and 1 unit dose in the region of a supraorbital nerve at a second side of the face;

(ii) 1 unit dose in the region of a supratrochlear nerve at a first side of the face and 1 unit dose in the region of a supratrochlear nerve at a second side of the face;

(iii) 1 unit dose in the region of an intratrochlear nerve at a first side of the face and 1 unit dose in the region of an intratrochlear nerve at a second side of the face;

(iv) 1 unit dose in the region of a zygomaticotemporal nerve at a first side of the face and 1 unit dose in the region of a zygomaticotemporal nerve at a second side of the face;

(v) 1 unit dose in the region of a zygomaticofacial nerve at a first side of the face and 1 unit dose in the region of a zygomaticofacial nerve at a second side of the face;

(vi) 2 unit doses in the region of an auriculotemporal nerve at a first side of the face and 2 unit doses in the region of an auriculotemporal nerve at a second side of the face;

(vii) 2 unit doses in the region of a greater occipital nerve at a first side of the neck and 2 unit doses in the region of a greater occipital nerve at a second side of the neck; and

(vii) 1 unit dose in the region of a lesser occipital nerve at a first side of the neck and 1 unit dose in the region of a lesser occipital nerve at a second side of the neck.

The treatment of headache pain (e.g. migraine pain) or migraine may comprise intradermally administering a unit dose of the chimeric clostridial neurotoxin bilaterally. The headache pain (e.g. migraine pain) or migraine treatment may comprise administering:

(i) 2 unit doses in the region of a supraorbital nerve at a first side of the face and/or 2 unit doses in the region of a supraorbital nerve at a second side of the face;

(ii) 2 unit doses in the region of a supratrochlear nerve at a first side of the face and/or 2 unit doses in the region of a supratrochlear nerve at a second side of the face;

(iii) 2 unit doses in the region of an intratrochlear nerve at a first side of the face and/or 2 unit doses in the region of an intratrochlear nerve at a second side of the face;

(iv) 2 unit doses in the region of a zygomaticotemporal nerve at a first side of the face and/or 2 unit doses in the region of a zygomaticotemporal nerve at a second side of the face;

(v) 2 unit doses in the region of a zygomaticofacial nerve at a first side of the face and/or 2 unit doses in the region of a zygomaticofacial nerve at a second side of the face;

(vi) 4 unit doses in the region of an auriculotemporal nerve at a first side of the face and/or 4 unit doses in the region of an auriculotemporal nerve at a second side of the face;

(vii) 4 unit doses in the region of a greater occipital nerve at a first side of the neck and/or 4 unit doses in the region of a greater occipital nerve at a second side of the neck; and/or

(vii) 2 unit doses in the region of a lesser occipital nerve at a first side of the neck and/or 2 unit doses in the region of a lesser occipital nerve at a second side of the neck.

The treatment of headache pain (e.g. migraine pain) or migraine may comprise administering a unit dose of the chimeric clostridial neurotoxin bilaterally. The headache pain (e.g. migraine pain) or migraine treatment may comprise administering:

(i) 2 unit doses in the region of a supraorbital nerve at a first side of the face and/or 2 unit doses in the region of a supraorbital nerve at a second side of the face;

(ii) 2 unit doses in the region of a supratrochlear nerve at a first side of the face and/or 2 unit doses in the region of a supratrochlear nerve at a second side of the face;

(iii) 2 unit doses in the region of an intratrochlear nerve at a first side of the face and/or 2 unit doses in the region of an intratrochlear nerve at a second side of the face;

(iv) 2 unit doses in the region of a zygomaticotemporal nerve at a first side of the face and/or 2 unit doses in the region of a zygomaticotemporal nerve at a second side of the face;

(v) 2 unit doses in the region of a zygomaticofacial nerve at a first side of the face and/or 2 unit doses in the region of a zygomaticofacial nerve at a second side of the face;

(vi) 4 unit doses in the region of an auriculotemporal nerve at a first side of the face and/or 4 unit doses in the region of an auriculotemporal nerve at a second side of the face;

(vii) 4 unit doses in the region of a greater occipital nerve at a first side of the neck and/or 4 unit doses in the region of a greater occipital nerve at a second side of the neck; and/or

(vii) 2 unit doses in the region of a lesser occipital nerve at a first side of the neck and/or 2 unit doses in the region of a lesser occipital nerve at a second side of the neck.

Preferably, the headache pain (e.g. migraine pain) or migraine treatment may comprise administering:

(i) 2 unit doses in the region of a supraorbital nerve at a first side of the face and 2 unit doses in the region of a supraorbital nerve at a second side of the face;

(ii) 2 unit doses in the region of a supratrochlear nerve at a first side of the face and 2 unit doses in the region of a supratrochlear nerve at a second side of the face;

(iii) 2 unit doses in the region of an intratrochlear nerve at a first side of the face and 2 unit doses in the region of an intratrochlear nerve at a second side of the face;

(iv) 2 unit doses in the region of a zygomaticotemporal nerve at a first side of the face and 2 unit doses in the region of a zygomaticotemporal nerve at a second side of the face;

(v) 2 unit doses in the region of a zygomaticofacial nerve at a first side of the face and 2 unit doses in the region of a zygomaticofacial nerve at a second side of the face;

(vi) 4 unit doses in the region of an auriculotemporal nerve at a first side of the face and 4 unit doses in the region of an auriculotemporal nerve at a second side of the face;

(vii) 4 unit doses in the region of a greater occipital nerve at a first side of the neck and 4 unit doses in the region of a greater occipital nerve at a second side of the neck; and/or

(vii) 2 unit doses in the region of a lesser occipital nerve at a first side of the neck and 2 unit doses in the region of a lesser occipital nerve at a second side of the neck.

More preferably, the headache pain (e.g. migraine pain) or migraine treatment may comprise administering:

(i) 2 unit doses in the region of a supraorbital nerve at a first side of the face and 2 unit doses in the region of a supraorbital nerve at a second side of the face;

(ii) 2 unit doses in the region of a supratrochlear nerve at a first side of the face and 2 unit doses in the region of a supratrochlear nerve at a second side of the face;

(iii) 2 unit doses in the region of an intratrochlear nerve at a first side of the face and 2 unit doses in the region of an intratrochlear nerve at a second side of the face;

(iv) 2 unit doses in the region of a zygomaticotemporal nerve at a first side of the face and 2 unit doses in the region of a zygomaticotemporal nerve at a second side of the face;

(v) 2 unit doses in the region of a zygomaticofacial nerve at a first side of the face and 2 unit doses in the region of a zygomaticofacial nerve at a second side of the face;

(vi) 4 unit doses in the region of an auriculotemporal nerve at a first side of the face and 4 unit doses in the region of an auriculotemporal nerve at a second side of the face;

(vii) 4 unit doses in the region of a greater occipital nerve at a first side of the neck and 4 unit doses in the region of a greater occipital nerve at a second side of the neck; and

(vii) 2 unit doses in the region of a lesser occipital nerve at a first side of the neck and 2 unit doses in the region of a lesser occipital nerve at a second side of the neck.

When treating headache pain (e.g. migraine pain) or migraine as described in the foregoing embodiments, it is preferred that one unit dose is administered per injection site. Thus, the treatment may comprise administration of the chimeric clostridial neurotoxin at:

(i) 1 injection site in the region of a supraorbital nerve at a first side of the face and/or 1 injection site in the region of a supraorbital nerve at a second side of the face;

(ii) 1 injection site in the region of a supratrochlear nerve at a first side of the face and/or 1 injection site in the region of a supratrochlear nerve at a second side of the face;

(iii) 1 injection site in the region of an intratrochlear nerve at a first side of the face and/or 1 injection site in the region of an intratrochlear nerve at a second side of the face;

(iv) 1 injection site in the region of a zygomaticotemporal nerve at a first side of the face and/or 1 injection site in the region of a zygomaticotemporal nerve at a second side of the face;

(v) 1 injection site in the region of a zygomaticofacial nerve at a first side of the face and/or 1 injection site in the region of a zygomaticofacial nerve at a second side of the face;

(vi) 2 injection sites in the region of an auriculotemporal nerve at a first side of the face and/or 2 injection sites in the region of an auriculotemporal nerve at a second side of the face;

(vii) 2 injection sites in the region of a greater occipital nerve at a first side of the neck and/or 2 injection sites in the region of a greater occipital nerve at a second side of the neck; and/or

(vii) 1 injection site in the region of a lesser occipital nerve at a first side of the neck and/or 1 injection site in the region of a lesser occipital nerve at a second side of the neck.

Preferably, the treatment may comprise administration of the chimeric clostridial neurotoxin at:

(i) 1 injection site in the region of a supraorbital nerve at a first side of the face and 1 injection site in the region of a supraorbital nerve at a second side of the face;

(ii) 1 injection site in the region of a supratrochlear nerve at a first side of the face and 1 injection site in the region of a supratrochlear nerve at a second side of the face;

(iii) 1 injection site in the region of an intratrochlear nerve at a first side of the face and 1 injection site in the region of an intratrochlear nerve at a second side of the face;

(iv) 1 injection site in the region of a zygomaticotemporal nerve at a first side of the face and 1 injection site in the region of a zygomaticotemporal nerve at a second side of the face;

(v) 1 injection site in the region of a zygomaticofacial nerve at a first side of the face and 1 injection site in the region of a zygomaticofacial nerve at a second side of the face;

(vi) 2 injection sites in the region of an auriculotemporal nerve at a first side of the face and 2 injection sites in the region of an auriculotemporal nerve at a second side of the face;

(vii) 2 injection sites in the region of a greater occipital nerve at a first side of the neck and 2 injection sites in the region of a greater occipital nerve at a second side of the neck; and/or

(vii) 1 injection site in the region of a lesser occipital nerve at a first side of the neck and 1 injection site in the region of a lesser occipital nerve at a second side of the neck.

More preferably, the treatment may comprise administration of the chimeric clostridial neurotoxin at:

(i) 1 injection site in the region of a supraorbital nerve at a first side of the face and 1 injection site in the region of a supraorbital nerve at a second side of the face;

(ii) 1 injection site in the region of a supratrochlear nerve at a first side of the face and 1 injection site in the region of a supratrochlear nerve at a second side of the face;

(iii) 1 injection site in the region of an intratrochlear nerve at a first side of the face and 1 injection site in the region of an intratrochlear nerve at a second side of the face;

(iv) 1 injection site in the region of a zygomaticotemporal nerve at a first side of the face and 1 injection site in the region of a zygomaticotemporal nerve at a second side of the face;

(v) 1 injection site in the region of a zygomaticofacial nerve at a first side of the face and 1 injection site in the region of a zygomaticofacial nerve at a second side of the face;

(vi) 2 injection sites in the region of an auriculotemporal nerve at a first side of the face and 2 injection sites in the region of an auriculotemporal nerve at a second side of the face;

(vii) 2 injection sites in the region of a greater occipital nerve at a first side of the neck and 2 injection sites in the region of a greater occipital nerve at a second side of the neck; and

(vii) 1 injection site in the region of a lesser occipital nerve at a first side of the neck and 1 injection site in the region of a lesser occipital nerve at a second side of the neck.

When treating headache pain (e.g. migraine pain) or migraine as described in the foregoing embodiments, it is preferred that one unit dose is administered per injection site. Thus, the treatment may comprise administration of the chimeric clostridial neurotoxin at:

(i) 2 injection sites in the region of a supraorbital nerve at a first side of the face and/or 2 injection sites in the region of a supraorbital nerve at a second side of the face;

(ii) 2 injection sites in the region of a supratrochlear nerve at a first side of the face and/or 2 injection sites in the region of a supratrochlear nerve at a second side of the face;

(iii) 2 injection sites in the region of an intratrochlear nerve at a first side of the face and/or 2 injection sites in the region of an intratrochlear nerve at a second side of the face;

(iv) 2 injection sites in the region of a zygomaticotemporal nerve at a first side of the face and/or 2 injection sites in the region of a zygomaticotemporal nerve at a second side of the face;

(v) 2 injection sites in the region of a zygomaticofacial nerve at a first side of the face and/or 2 injection sites in the region of a zygomaticofacial nerve at a second side of the face;

(vi) 4 injection sites in the region of an auriculotemporal nerve at a first side of the face and/or 4 injection sites in the region of an auriculotemporal nerve at a second side of the face;

(vii) 4 injection sites in the region of a greater occipital nerve at a first side of the neck and/or 4 injection sites in the region of a greater occipital nerve at a second side of the neck; and/or

(vii) 2 injection sites in the region of a lesser occipital nerve at a first side of the neck and/or 2 injection sites in the region of a lesser occipital nerve at a second side of the neck.

Preferably, the treatment may comprise administration of the chimeric clostridial neurotoxin at:

(i) 2 injection sites in the region of a supraorbital nerve at a first side of the face and 2 injection sites in the region of a supraorbital nerve at a second side of the face;

(ii) 2 injection sites in the region of a supratrochlear nerve at a first side of the face and 2 injection sites in the region of a supratrochlear nerve at a second side of the face;

(iii) 2 injection sites in the region of an intratrochlear nerve at a first side of the face and 2 injection sites in the region of an intratrochlear nerve at a second side of the face;

(iv) 2 injection sites in the region of a zygomaticotemporal nerve at a first side of the face and 2 injection sites in the region of a zygomaticotemporal nerve at a second side of the face;

(v) 2 injection sites in the region of a zygomaticofacial nerve at a first side of the face and 2 injection sites in the region of a zygomaticofacial nerve at a second side of the face;

(vi) 4 injection sites in the region of an auriculotemporal nerve at a first side of the face and 4 injection sites in the region of an auriculotemporal nerve at a second side of the face;

(vii) 4 injection sites in the region of a greater occipital nerve at a first side of the neck and 4 injection sites in the region of a greater occipital nerve at a second side of the neck; and/or

(vii) 2 injection sites in the region of a lesser occipital nerve at a first side of the neck and 2 injection sites in the region of a lesser occipital nerve at a second side of the neck.

More preferably, the treatment may comprise administration of the chimeric clostridial neurotoxin at:

(i) 2 injection sites in the region of a supraorbital nerve at a first side of the face and 2 injection sites in the region of a supraorbital nerve at a second side of the face;

(ii) 2 injection sites in the region of a supratrochlear nerve at a first side of the face and 2 injection sites in the region of a supratrochlear nerve at a second side of the face;

(iii) 2 injection sites in the region of an intratrochlear nerve at a first side of the face and 2 injection sites in the region of an intratrochlear nerve at a second side of the face;

(iv) 2 injection sites in the region of a zygomaticotemporal nerve at a first side of the face and 2 injection sites in the region of a zygomaticotemporal nerve at a second side of the face;

(v) 2 injection sites in the region of a zygomaticofacial nerve at a first side of the face and 2 injection sites in the region of a zygomaticofacial nerve at a second side of the face;

(vi) 4 injection sites in the region of an auriculotemporal nerve at a first side of the face and 4 injection sites in the region of an auriculotemporal nerve at a second side of the face;

(vii) 4 injection sites in the region of a greater occipital nerve at a first side of the neck and 4 injection sites in the region of a greater occipital nerve at a second side of the neck; and

(vii) 2 injection sites in the region of a lesser occipital nerve at a first side of the neck and 2 injection sites in the region of a lesser occipital nerve at a second side of the neck.

When treating headache pain (e.g. migraine pain) or migraine as described in the foregoing embodiments, it is preferred that more than one unit dose (preferably 2 unit doses) is administered per injection site. Thus, the treatment may comprise administration of the chimeric clostridial neurotoxin at:

(i) 1 injection site in the region of a supraorbital nerve at a first side of the face and/or 1 injection site in the region of a supraorbital nerve at a second side of the face;

(ii) 1 injection site in the region of a supratrochlear nerve at a first side of the face and/or 1 injection site in the region of a supratrochlear nerve at a second side of the face;

(iii) 1 injection site in the region of an intratrochlear nerve at a first side of the face and/or 1 injection site in the region of an intratrochlear nerve at a second side of the face;

(iv) 1 injection site in the region of a zygomaticotemporal nerve at a first side of the face and/or 1 injection site in the region of a zygomaticotemporal nerve at a second side of the face;

(v) 1 injection site in the region of a zygomaticofacial nerve at a first side of the face and/or 1 injection site in the region of a zygomaticofacial nerve at a second side of the face;

(vi) 2 injection sites in the region of an auriculotemporal nerve at a first side of the face and/or 2 injection sites in the region of an auriculotemporal nerve at a second side of the face;

(vii) 2 injection sites in the region of a greater occipital nerve at a first side of the neck and/or 2 injection sites in the region of a greater occipital nerve at a second side of the neck; and/or

(vii) 1 injection site in the region of a lesser occipital nerve at a first side of the neck and/or 1 injection site in the region of a lesser occipital nerve at a second side of the neck. Preferably, the treatment may comprise administration of a the chimeric clostridial neurotoxin at:

(i) 1 injection site in the region of a supraorbital nerve at a first side of the face and 1 injection site in the region of a supraorbital nerve at a second side of the face;

(ii) 1 injection site in the region of a supratrochlear nerve at a first side of the face and 1 injection site in the region of a supratrochlear nerve at a second side of the face;

(iii) 1 injection site in the region of an intratrochlear nerve at a first side of the face and 1 injection site in the region of an intratrochlear nerve at a second side of the face;

(iv) 1 injection site in the region of a zygomaticotemporal nerve at a first side of the face and 1 injection site in the region of a zygomaticotemporal nerve at a second side of the face;

(v) 1 injection site in the region of a zygomaticofacial nerve at a first side of the face and 1 injection site in the region of a zygomaticofacial nerve at a second side of the face;

(vi) 2 injection sites in the region of an auriculotemporal nerve at a first side of the face and 2 injection sites in the region of an auriculotemporal nerve at a second side of the face;

(vii) 2 injection sites in the region of a greater occipital nerve at a first side of the neck and 2 injection sites in the region of a greater occipital nerve at a second side of the neck; and/or

(vii) 1 injection site in the region of a lesser occipital nerve at a first side of the neck and 1 injection site in the region of a lesser occipital nerve at a second side of the neck. More preferably, the treatment may comprise administration of the chimeric clostridial neurotoxin at:

(i) 1 injection site in the region of a supraorbital nerve at a first side of the face and 1 injection site in the region of a supraorbital nerve at a second side of the face;

(ii) 1 injection site in the region of a supratrochlear nerve at a first side of the face and 1 injection site in the region of a supratrochlear nerve at a second side of the face;

(iii) 1 injection site in the region of an intratrochlear nerve at a first side of the face and 1 injection site in the region of an intratrochlear nerve at a second side of the face;

(iv) 1 injection site in the region of a zygomaticotemporal nerve at a first side of the face and 1 injection site in the region of a zygomaticotemporal nerve at a second side of the face;

(v) 1 injection site in the region of a zygomaticofacial nerve at a first side of the face and 1 injection site in the region of a zygomaticofacial nerve at a second side of the face;

(vi) 2 injection sites in the region of an auriculotemporal nerve at a first side of the face and 2 injection sites in the region of an auriculotemporal nerve at a second side of the face;

(vii) 2 injection sites in the region of a greater occipital nerve at a first side of the neck and 2 injection sites in the region of a greater occipital nerve at a second side of the neck; and

(vii) 1 injection site in the region of a lesser occipital nerve at a first side of the neck and 1 injection site in the region of a lesser occipital nerve at a second side of the neck.

Thus, when treating headache pain (e.g. migraine pain) or migraine, 1-50, 5-45, or 10-38 unit doses may be administered. Preferably up to 35 unit doses are administered. Preferably, the total dose administered per treatment session may be up to 192,500 pg of the chimeric clostridial neurotoxin. For example, the total dose administered may be up to 180,000 pg, preferably up to 177,000 pg (more preferably up to 175,000 pg). Most preferably, the total dose administered may be up to 115,000 pg or 75,000 pg, e.g. up to 112,000 pg or 70,000 pg.

Thus, when treating headache pain (e.g. migraine pain) or migraine via intramuscular injection, 1-50, 5-45, or 10-38 unit doses may be administered. Preferably up to 35 unit doses are administered. Preferably, the total dose administered per treatment session may be up to 192,500 pg of the chimeric clostridial neurotoxin. For example, the total dose administered may be up to 180,000 pg, preferably up to 177,000 pg (more preferably up to 175,000 pg). Most preferably, the total dose administered may be up to 115,000 pg or 75,000 pg, e.g. up to 112,000 pg or 70,000 pg.

Thus, when treating headache pain (e.g. migraine pain) or migraine via intradermal injection, 1-35, 5-25, or 10-20 unit doses may be administered. Preferably up to 20 unit doses are administered. Preferably, the total dose administered per treatment session may be up to 110,000 pg of the chimeric clostridial neurotoxin. For example, the total dose administered may be up to 105,000 pg, preferably up to 102,000 pg (more preferably up to 100,000 pg). When treating headache pain (e.g. migraine pain) or migraine via intradermal injection, 2-70, 10-50, or 20-40 unit doses may be administered. Preferably up to 40 unit doses are administered. Preferably, the total dose administered per treatment session may be up to 220,000 pg of the chimeric clostridial neurotoxin. For example, the total dose administered may be up to 210,000 pg, preferably up to 204,000 pg (more preferably up to 200,000 pg).

In some embodiments, the treatment of headache pain (e.g. migraine pain) or migraine may be via a mixture of intramuscular and intradermal injections. For example, a subject may be administered intradermally to the neck with a chimeric clostridial neurotoxin of the invention and intramuscularly to the face with a chimeric clostridial neurotoxin of the invention. Preferably, a subject may be administered intradermally to the face with a chimeric clostridial neurotoxin of the invention and intramuscularly to the neck with a chimeric clostridial neurotoxin of the invention. The chimeric clostridial neurotoxin may be administered to the head of the subject, e.g. in addition to administration to the neck and/or face.

A preferred unit dose when treating headache pain (e.g. migraine pain) or migraine via intradermal injection or via intramuscular injection may be 1,000 pg to 5,500 pg. An upper limit of the unit dose range may be 5,250, 5,200, 5,100, 5,000, 4,500, 4,000, 3,500, 3,000, 2,500, or 2,000 pg of chimeric clostridial neurotoxin, preferably the upper limit is 5,100 pg, more preferably 5,000 pg. A lower limit of the unit dose range may be 1,100, 1,200, 1,250, 1,300, 1,350, 1,400, or 1,450, 1,500, 2,000, 2,500, 3,000, 3,500, or 4,000 pg of chimeric clostridial neurotoxin, preferably the lower limit is 1,400 pg, more preferably 1,500 pg. The lower limit of said range may be greater than 3,000 pg. The unit dose may be 1,400 pg to 5,100 pg (e.g. 1,500 pg to 5,000 pg), 2,000 pg to 5,100 pg, 3,000 to 5,100 pg or 3,000 to 4,000 pg of chimeric clostridial neurotoxin. The unit dose may comprise greater than 3,000 pg up to 5,500 pg of chimeric clostridial neurotoxin. The unit dose of the chimeric clostridial neurotoxin may be 2,000 pg to 4,500 pg, 2,000 pg to 3,000 pg (e.g. 2,500 pg) or 3,500 to 4,500 pg of the chimeric clostridial neurotoxin,. Preferably, the unit dose comprises 4,000 pg of the chimeric clostridial neurotoxin.

A preferred unit dose when treating headache pain (e.g. migraine pain) or migraine via intradermal injection or via intramuscular injection may be 42 Units to 229 Units. An upper limit of the unit dose range may be 225, 220, 215, 210, 205, 200, 190, 180, 170, 160, 150, 125, 100, or 83 Units of chimeric clostridial neurotoxin, preferably the upper limit is 212 Units, more preferably 208 Units. A lower limit of the unit dose range may be 46, 50, 55, 60, 65, 70, 75, 80, or 90, 100, 110, 120, 130, 140, 150, 160 or 166 Units of chimeric clostridial neurotoxin, preferably the lower limit is 58 Units, more preferably 62 Units. The lower limit of said range may be greater than 125 Units. The unit dose may be 58 Units to 212 Units (e.g. 62 Units to 208 Units), 83 Units to 212 Units, 125 to 212 Units or 125 to 166 Units of chimeric clostridial neurotoxin. The unit dose may comprise greater than 125 Units up to 229 Units of chimeric clostridial neurotoxin. The unit dose of the chimeric clostridial neurotoxin may be 83 Units to 188 Units, 83 Units to 125 Units (e.g. 104 Units) or 146 Units to 188 Units of the

chimeric clostridial neurotoxin. Preferably, the unit dose comprises 166 Units of the chimeric clostridial neurotoxin. The unit dose may comprise 47 Units to 258 Units of chimeric clostridial neurotoxin, e.g. 94 Units to 211 Units, 94 Units to 141 Units (e.g. 117 Units) or 164 to 211 Units of the chimeric clostridial neurotoxin.. The unit dosage form may comprise 188 Units of the chimeric clostridial neurotoxin.

When treating headache pain (e.g. migraine pain) or migraine via intramuscular injection or intradermal injection (preferably intramuscular injection), a preferred unit dose may be 2,500 pg and the total dose may be up to 70,000 pg. For example, a preferred unit dose may be 2,500 pg and the total dose may be 70,000 pg. A preferred unit dose may be 4,000 pg and the total dose may be up to 112,000 pg. For example, a preferred unit dose may be 4,000 pg and the total dose may be 112,000 pg. A suitable unit dose may be 5,000 pg and the total dose may be up to 155,000 pg. For example, a suitable unit dose may be 5,000 pg and the total dose may be 155,000 pg.

When treating headache pain (e.g. migraine pain) or migraine via intramuscular injection or intradermal injection (preferably intramuscular injection), a preferred unit dose may be 104 Units and the total dose may be up to 2,912 Units. For example, a preferred unit dose may be 104 Units and the total dose may be 2,912 Units. A preferred unit dose may be 166 Units and the total dose may be up to 4,659 Units. For example, a preferred unit dose may be 166 Units and the total dose may be 4,659 Units. A suitable unit dose may be 208 Units and the total dose may be up to 6,448 Units. For example, a suitable unit dose may be 208 Units and the total dose may be 6,448 Units.

When treating headache pain (e.g. migraine pain) or migraine via intramuscular injection or intradermal injection (preferably intramuscular injection) a preferred unit dose may be 117 Units and the total dose may be up to 3,286 Units. For example, a preferred unit dose may be 117 Units and the total dose may be 3,286 Units. A preferred unit dose may be 188 Units and the total dose may be up to 5,258 Units. For example, a preferred unit dose may be 188 Units and the total dose may be 5,258 Units. A suitable unit dose may be 235 Units and the total dose may be up to 7,277 Units. For example, a suitable unit dose may be 235 Units and the total dose may be 7,277 Units.

When treating headache pain (e.g. migraine pain) or migraine via intramuscular injection, the total dose administered per treatment session may be up to 8,007 Units of the chimeric clostridial neurotoxin. For example, the total dose administered may be up to 7,488 Units, or

up to 7,363 Units (e.g. up to 7,280 Units). Most preferably, the total dose administered may be up to 4,784 Units or 3,120 Units, e.g. up to 4,659 Units or 2,912 Units. The total dose administered per treatment session may be up to 9,037 Units of the chimeric clostridial neurotoxin. For example, the total dose administered may be up to 8,451 Units, or up to 8,310 Units (e.g. up to 8,216 Units). Most preferably, the total dose administered may be up to 5,399 Units or 3,521 Units, e.g. up to 5,258 Units or 3,286 Units.

When treating headache pain (e.g. migraine pain) or migraine via intradermal injection, the total dose administered per treatment session may be up to 4,576 Units of the chimeric clostridial neurotoxin. For example, the total dose administered may be up to 4,368 Units, or up to 4,243 Units (e.g. up to 4,160 Units). The total dose administered per treatment session may be up to 5,165 Units of the chimeric clostridial neurotoxin. For example, the total dose administered may be up to 4,929 Units, or up to 4,789 Units (e.g. up to 4,695 Units). The total dose administered per treatment session may be up to 5,165 Units of the chimeric clostridial neurotoxin. For example, the total dose administered may be up to 4,929 Units, or up to 4,789 Units (e.g. up to 4,695 Units).

In preferred embodiments, when treating pain (e.g. headache or migraine pain) or migraine with a chimeric clostridial neurotoxin, the treatment does not induce muscle paralysis. For example, in some embodiments, the unit dose of the chimeric clostridial neurotoxin may be lower than the unit dose of the chimeric clostridial neurotoxin required to induce muscle paralysis. In particular, in some embodiments, the unit dose of the chimeric clostridial neurotoxin administered at a particular site (e.g. injection site) may be lower than the unit dose of the chimeric clostridial neurotoxin required to induce muscle paralysis (e.g. at that site and/or muscle).

The headache pain mentioned above is preferably migraine pain. Said migraine pain may be episodic migraine pain or chronic migraine pain, e.g. pain caused by or otherwise associated with episodic migraine or pain caused by or otherwise associated with chronic migraine.

The chimeric clostridial neurotoxin of the invention is preferably administered iteratively (e.g. up to 5, 10, 15 or 20 times) as part of a treatment regimen (preferably on different days, e.g. with at least 1 day between successive treatments). Iterative administration means administration at least two times, e.g. at least 5, 10, 15 or 20 times. Thus, in one embodiment, a chimeric clostridial neurotoxin of the invention may be administered two or more times to treat the disorder (preferably pain) of a subject. This is particularly pertinent

for the treatment of chronic conditions, such as chronic pain, where ongoing treatment is typically necessary. In one embodiment a chimeric clostridial neurotoxin of the invention may be administered weekly, twice monthly, monthly, every two months, every six months or annually, preferably at least twice annually or annually. In one embodiment, a chimeric clostridial neurotoxin of the invention is administered two or more times in a period of 10 years, 5 years, 2 years or 1 year. Preferably, a chimeric clostridial neurotoxin of the invention is administered two or more times in a period of 1 year. Treatment may continue for at least 6 months, 1 year, 2 years, 3 years, 5 years, 10 years, 15 years, 20 years, 25 years or 30 years.

In some embodiments, following a first administration of (e.g. first treatment session with) of a chimeric clostridial neurotoxin in accordance with the invention, a subject may be subjected to a second administration of (e.g. second treatment session with) the chimeric clostridial neurotoxin. The time interval between the first and second administration may be at least 5, 6, 7, 8, 9, or 10 months. For example, the time interval between the first and second administration may be 5-10 months, 5-9 months, 5-8 months, 6-10 months, 6-9 months or 6-8 months.

It is preferred that the chimeric clostridial neurotoxin is not administered together with a further therapeutic or diagnostic agent (e.g. a nucleic acid, protein, peptide or small molecule therapeutic or diagnostic agent) additional to the light-chain and heavy-chain. For example, in one embodiment the chimeric clostridial neurotoxin is not administered with a further analgesic. In one embodiment a chimeric clostridial neurotoxin of the invention is not administered together with a covalently associated therapeutic agent. In one embodiment a chimeric clostridial neurotoxin of the invention is not administered together with a non-covalently associated therapeutic agent.

The chimeric clostridial neurotoxins are preferably for use in treating pain and may be used to treat a subject suffering from one or more types of pain.

The term “pain” as used here, means any unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage. Any associated physical disorder may or may not be apparent to a clinician.

The pain may be associated with release of a mediator (e.g. a neurotransmitter) from a neuron. The neuron is preferably a neuron to which a chimeric clostridial neurotoxin of the

invention binds. For example, a mediator may be any mediator associated with pain transmission. A mediator may be a neuropeptide, such as substance P, CGRP, or vasoactive intestinal peptide (VIP).

A mediator may be an inflammatory mediator or a non-inflammatory mediator. A mediator may be one or more of: CGRP, a neurokinin (e.g. a tachykinin, substance P, neurokinin A, neurokinin B, a hemokinin and/or an endokinin), adrenocorticotropic hormone (ACTH), glucocorticoids, vasopressin, oxytocin, a catecholamine, an opioid (e.g. an opioid peptide and/or a brain opioid), angiotensin II, an endorphin, an encephalin, vasoactive intestinal peptide (VIP), an eicosanoid (e.g. a prostaglandin such as prostaglandin E2 (PGE2), and/or a leukotriene), a tissue kininogen (e.g. bradykinin), histamine, serotonin, potassium, prostacyclin (PGI2), leukotriene B4 (LTB4), nerve growth factor (NGF), protons, ATP, adenosine, 5-hydroxytryptamine (5-HT), histamine, glutamate, norepinephrine (NE), nitric oxide (NO), γ-aminobutyric acid (GABA), glycine, acetylcholine, a cannabinoid, tissue necrosis factor alpha (TNF-α), a cytokine (e.g. interleukin (IL)-6, IL-1, and/or IL-8), a platelet activating factor (PAF), a neurotrophic growth factor (NGF), glutamate, aspartate, pituitary adenylate cyclase-activating peptide (PACAP), and a proteolytic enzyme.

A mediator may be calcitonin gene related peptide (CGRP), amylin, pituitary adenylate cyclase-activating peptide (PACAP), oxytocin, neuropeptide Y (NPY), Substance P, an angiotensin, corticotropin releasing hormone (CRH), leptin, adiponectin, an orexin, and/or melanin-concentrating hormone (MCH).

A mediator may be one or more selected from: CGRP, substance P, and glutamate.

A mediator may be one or more of: a neuropeptide (e.g. substance P, CGRP, or VIP), nitric oxide, glutamate, and aspartate.

Where the pain is headache pain (preferably migraine pain), the mediator may be one or more of: CGRP, VIP, PACAP, and a proinflammatory cytokine (e.g. IL-6, IL-8, and/or TNF-α).

Preferably, the mediator may be CGRP, substance P and/or an alternative neurokinin.

Most preferably, the mediator is CGRP. The CGRP may be α-CGRP or β-CGRP, preferably α-CGRP.

The pain may be associated with release of a pain mediator (e.g. a pain neurotransmitter) from a neuron comprising an Aδ nerve fiber or a C nerve fiber. A pain mediator may be any pain mediator released/secreted from a neuron comprising an Aδ nerve fiber or a C nerve fiber. A pain mediator may be a neuropeptide, such as substance P, CGRP, or vasoactive intestinal peptide (VIP).

A pain mediator may be an inflammatory mediator or a non-inflammatory mediator. A pain mediator may be one or more of: CGRP, a neurokinin (e.g. a tachykinin, substance P, neurokinin A, neurokinin B, a hemokinin and/or an endokinin), adrenocorticotropic hormone (ACTH), glucocorticoids, vasopressin, oxytocin, a catecholamine, an opioid (e.g. an opioid peptide and/or a brain opioid), angiotensin II, an endorphin, an encephalin, vasoactive intestinal peptide (VIP), an eicosanoid (e.g. a prostaglandin such as prostaglandin E2 (PGE2), and/or a leukotriene), a tissue kininogen (e.g. bradykinin), histamine, serotonin, potassium, prostacyclin (PGI2), leukotriene B4 (LTB4), nerve growth factor (NGF), protons, ATP, adenosine, 5-hydroxytryptamine (5-HT), histamine, glutamate, norepinephrine (NE), nitric oxide (NO), γ-aminobutyric acid (GABA), glycine, acetylcholine, a cannabinoid, tissue necrosis factor alpha (TNF-α), a cytokine (e.g. interleukin (IL)-6, IL-1, and/or IL-8), a platelet activating factor (PAF), a neurotrophic growth factor (NGF), glutamate, aspartate, pituitary adenylate cyclase-activating peptide (PACAP), and a proteolytic enzyme.

A pain mediator may be calcitonin gene related peptide (CGRP), amylin, pituitary adenylate cyclase-activating peptide (PACAP), oxytocin, neuropeptide Y (NPY), Substance P, an angiotensin, corticotropin releasing hormone (CRH), leptin, adiponectin, an orexin, and/or melanin-concentrating hormone (MCH).

A pain mediator released from a neuron comprising an Aδ nerve fiber may be one or more selected from: CGRP, substance P, and glutamate.

A pain mediator released from a neuron comprising a C nerve fiber may be one or more of: a neuropeptide (e.g. substance P, CGRP, or VIP), nitric oxide, glutamate, and aspartate.

Glutamate may be associated with the initiation of chronic pain and/or neuropathic pain.

Where the pain is headache pain (preferably migraine pain), the pain mediator may be one or more of: CGRP, VIP, PACAP, and a proinflammatory cytokine (e.g. IL-6, IL-8, and/or TNF-α).

Preferably, where a neuron comprises an Aδ nerve fiber or a C nerve fiber, the pain mediator may be CGRP, preferably where a neuron comprises a C fiber, the pain mediator is CGRP. Where a neuron comprises a C fiber, the pain mediator may be substance P and/or an alternative neurokinin.

Most preferably, the pain mediator is CGRP. The CGRP may be α-CGRP or β-CGRP, preferably α-CGRP.

Thus, the chimeric clostridial neurotoxin of the invention may inhibit release of CGRP from a sensory neuron comprising an Aδ nerve fiber or a C nerve fiber.

Where the pain mediator is CGRP, the pain may be CGRP-associated pain.

The term "CGRP-associated pain" as used here, means pain that is associated with CGRP release from a neuron and any effect thereof. A CGRP-induced pain may be a CGRP-dependent pain. In one embodiment a CGRP-associated pain is a CGRP-induced pain that has been induced by CGRP release from a neuron and any effect thereof.

Examples of CGRP-associated pain include migraine and itch.

In one embodiment, a therapeutic use or method of the invention excludes treating pain associated with any pain mediator other than CGRP. A therapeutic use or method of the invention may exclude treating pain associated with one or more of: a neurokinin (e.g. a tachykinin, substance P, neurokinin A, neurokinin B, a hemokinin and/or an endokinin), adrenocorticotropic hormone (ACTH), glucocorticoids, vasopressin, oxytocin, a catecholamine, an opioid (e.g. an opioid peptide and/or a brain opioid), angiotensin II, an endorphin, an encephalin, vasoactive intestinal peptide (VIP), an eicosanoid (e.g. a prostaglandin such as prostaglandin E2 (PGE2), and/or a leukotriene), a tissue kininogen (e.g. bradykinin), histamine, serotonin, potassium, prostacyclin (PGI2), leukotriene B4 (LTB4), nerve growth factor (NGF), protons, ATP, adenosine, 5-hydroxytryptamine (5-HT), histamine, glutamate, norepinephrine (NE), nitric oxide (NO), γ-aminobutyric acid (GABA), glycine, acetylcholine, a cannabinoid, tissue necrosis factor alpha (TNF-α), a cytokine (e.g. interleukin (IL)-6, IL-1, and/or IL-8), a platelet activating factor (PAF), a neurotrophic growth factor (NGF), glutamate, aspartate, pituitary adenylate cyclase-activating peptide (PACAP), and a proteolytic enzyme.

Pain may be chronic or acute. An “acute pain” is a pain of short duration having a sudden onset. One type of acute pain, for example, is cutaneous pain felt on injury to the skin or other superficial tissues, such as caused by a cut or a burn. Cutaneous nociceptors terminate just below the skin, and due to the high concentration of nerve endings, produce a well-defined, localized pain of short duration. “Chronic pain” is a pain other than an acute pain.

Thus, the pain may be chronic or acute pain. The pain may be one or more selected from the following four categories of pain: nociceptive pain; neuropathic pain; mixed pain; and pain of an unknown origin. nociceptive pain may be caused by a known noxious stimulus to a nociceptor (pain receptor) and may be somatic or visceral. Neuropathic pain may be pain initiated or caused by a primary lesion or dysfunction in the nervous system. Mixed pain may be a combination of nociceptive pain and neuropathic pain.

Pain (e.g. chronic pain) may be one or more selected from: neuropathic pain, inflammatory pain, headache pain, somatic pain, visceral pain, referred pain, allodynia, mixed pain, and post-operative pain. However, preferably the pain is not post-operative pain.

In one embodiment a pain is not visceral pain. In one embodiment a disorder is not a visceral pain disorder.

The somatic pain may be one or more selected from: headache pain (e.g. post traumatic headache, head injury headache or post-traumatic brain injury headache), arthritic pain (e.g. osteo arthritis pain and/or rheumatoid arthritis pain), exercise pain, degenerative disc disease pain, carpal tunnel compression pain, soft tissue injury pain, temporomandibular joint pain, musculoskeletal pain, somatic pain caused by or associated with a vascular disorder (e.g. Raynaud’s syndrome, Buerger’s disease, peripheral venous disease, peripheral arterial disease, varicose veins, blood clots in the veins, blood clotting disorders or lymphedema), facial pain, somatic pain caused by or associated with trigeminal autonomic cephalalgia; somatic pain caused by or associated with trigeminal neuralgia; and bone pain (e.g. cancer-induced bone pain, such as CGRP-associated cancer-induced bone pain).

The pain is preferably headache pain. More preferably, the pain is migraine pain.

The visceral pain may be one or more selected from: endometriosis pain, pancreatitis pain, gastrointestinal pain, and visceral pain caused by or associated with a vascular disorder.

The inflammatory pain may be one or more selected from chronic pain, wound healing pain, pruritus pain, and burn pain.

The neuropathic pain may be one or more selected from: post herpetic neuralgia pain, diabetes pain, chronic neuropathic pain, and Morton's neuroma pain.

Pain and conditions that may be treated by a chimeric clostridial neurotoxin of the invention are described in more detail below.

The chimeric clostridial neurotoxin of the invention may be used to treat pain caused by or otherwise associated with any of the following neuropathic pain conditions. "Neuropathic pain" means abnormal sensory input, resulting in discomfort, from the peripheral nervous system, central nervous systems, or both. Symptoms of neuropathic pain can involve persistent, spontaneous pain, as well as allodynia (a painful response to a stimulus that normally is not painful), hyperalgesia (an accentuated response to a painful stimulus that usually causes only a mild discomfort, such as a pin prick), or hyperpathia (where a short discomfort becomes a prolonged severe pain). Neuropathic pain may be caused by any of the following:

1. A traumatic insult, such as, for example, a nerve compression injury (e.g., a nerve crush, a nerve stretch, a nerve entrapment or an incomplete nerve transsection); a spinal cord injury (e.g., a hemisection of the spinal cord); a limb amputation; a contusion; an inflammation (e.g., an inflammation of the spinal cord); or a surgical procedure.
2. An ischemic event, including, for example, a stroke and heart attack.
3. An infectious agent.
4. Exposure to a toxic agent, including, for example, a drug, an alcohol, a heavy metal (e.g., lead, arsenic, mercury), an industrial agent (e.g., a solvent, fumes from a glue) or nitrous oxide.
5. A disease, including, for example, an inflammatory disorder, a neoplastic tumour, an acquired immune deficiency syndrome (AIDS), Lymes disease, a leprosy, a metabolic disease, a peripheral nerve disorder, like neuroma, a mononeuropathy or a polyneuropathy.

Types of neuropathic pain include the following:

1. Neuralgia.

A neuralgia is a pain that radiates along the course of one or more specific nerves usually without any demonstrable pathological change in the nerve structure. The causes of neuralgia are varied. Chemical irritation, inflammation, trauma (including surgery), compression by nearby structures (for instance, tumours), and infections may all lead to neuralgia. In many cases, however, the cause is unknown or unidentifiable. Neuralgia is most common in elderly persons, but it may occur at any age. A neuralgia, includes, without limitation, a trigeminal neuralgia, a post-herpetic neuralgia, a postherpetic neuralgia, a glossopharyngeal neuralgia, a sciatica and an atypical facial pain.

Neuralgia is pain in the distribution of a nerve or nerves. Examples are trigeminal neuralgia, atypical facial pain, and postherpetic neuralgia (caused by shingles or herpes). The affected nerves are responsible for sensing touch, temperature, and pressure in the facial area from the jaw to the forehead. The disorder generally causes short episodes of excruciating pain, usually for less than two minutes and on only one side of the face. The pain can be described in a variety of ways such as "stabbing," "sharp," "like lightning," "burning," and even "itchy". In the atypical form of TN, the pain can also present as severe or merely aching and last for extended periods. The pain associated with TN is recognized as one the most excruciating pains that can be experienced.

Simple stimuli such as eating, talking, washing the face, or any light touch or sensation can trigger an attack (even the sensation of a gentle breeze). The attacks can occur in clusters or as an isolated attack.

Symptoms include sharp, stabbing pain or constant, burning pain located anywhere, usually on or near the surface of the body, in the same location for each episode; pain along the path of a specific nerve; impaired function of an affected body part due to pain, or muscle weakness due to concomitant motor nerve damage; increased sensitivity of the skin or numbness of the affected skin area (feeling similar to a local anaesthetic such as a Novocaine shot); and any touch or pressure is interpreted as pain. Movement may also be painful.

Trigeminal neuralgia is the most common form of neuralgia. It affects the main sensory nerve of the face, the trigeminal nerve ("trigeminal" literally means "three origins", referring to the division of the nerve into 3 branches). This condition involves sudden and short attacks of severe pain on the side of the face, along the area supplied by the trigeminal nerve on that side. The pain attacks may be severe enough to cause a facial grimace, which is classically referred to as a painful tic (tic douloureux). Sometimes, the cause of trigeminal neuralgia is a blood vessel or small tumour pressing on the nerve. Disorders such as multiple sclerosis (an inflammatory disease affecting the brain and spinal cord), certain forms of arthritis, and diabetes (high blood sugar) may also cause trigeminal neuralgia, but a cause is not always identified. In this condition, certain movements such as chewing, talking, swallowing, or touching an area of the face may trigger a spasm of excruciating pain.

A related but rather uncommon neuralgia affects the glosso-pharyngeal nerve, which provides sensation to the throat. Symptoms of this neuralgia are short, shock-like episodes of pain located in the throat.

Neuralgia may occur after infections such as shingles, which is caused by the varicella-zoster virus, a type of herpesvirus. This neuralgia produces a constant burning pain after the shingles rash has healed. The pain is worsened by movement of or contact with the affected area. Not all of those diagnosed with shingles go on to experience postherpetic neuralgia, which can be more painful than shingles. The pain and sensitivity can last for months or even years. The pain is usually in the form of an intolerable sensitivity to any touch but especially light touch. Postherpetic neuralgia is not restricted to the face; it can occur anywhere on the body but usually occurs at the location of the shingles rash. Depression is not uncommon due to the pain and social isolation during the illness.

Postherpetic neuralgia may be debilitating long after signs of the original herpes infection have disappeared. Other infectious diseases that may cause neuralgia are syphilis and Lyme disease.

Diabetes is another common cause of neuralgia. This very common medical problem affects almost 1 out of every 20 Americans during adulthood. Diabetes damages the tiny arteries that supply circulation to the nerves, resulting in nerve fibre malfunction and sometimes nerve loss. Diabetes can produce almost any neuralgia, including trigeminal neuralgia, carpal tunnel syndrome (pain and numbness of the hand and wrist), and meralgia paresthetica (numbness and pain in the thigh due to damage to the lateral femoral cutaneous

nerve). Strict control of blood sugar may prevent diabetic nerve damage and may accelerate recovery in subjects who do develop neuralgia.

Other medical conditions that may be associated with neuralgias are chronic renal insufficiency and porphyria - a hereditary disease in which the body cannot rid itself of certain substances produced after the normal breakdown of blood in the body. Certain drugs may also cause this problem.

2. Deafferentation.

Deafferentation indicates a loss of the sensory input from a portion of the body, and can be caused by interruption of either peripheral sensory fibres or nerves from the central nervous system. A deafferentation pain syndrome, includes, without limitation, an injury to the brain or spinal cord, a post-stroke pain, a phantom pain, a paraplegia, a brachial plexus avulsion injuries, lumbar radiculopathies.

3. Complex regional pain syndromes (CRPSs)

CRPS is a chronic pain syndrome resulting from sympathetically-maintained pain, and presents in two forms. CRPS 1 currently replaces the term "reflex sympathetic dystrophy syndrome". It is a chronic nerve disorder that occurs most often in the arms or legs after a minor or major injury. CRPS 1 is associated with severe pain; changes in the nails, bone, and skin; and an increased sensitivity to touch in the affected limb. CRPS 2 replaces the term causalgia, and results from an identified injury to the nerve. A CRPS, includes, without limitation, a CRPS Type I (reflex sympathetic dystrophy) and a CRPS Type II (causalgia).

4. Neuropathy.

A neuropathy is a functional or pathological change in a nerve and is characterized clinically by sensory or motor neuron abnormalities.

Central neuropathy is a functional or pathological change in the central nervous system.

Peripheral neuropathy is a functional or pathological change in one or more peripheral nerves. The peripheral nerves relay information from the central nervous system (brain and spinal cord) to muscles and other organs and from the skin, joints, and other organs back to the brain. Peripheral neuropathy occurs when these nerves fail to carry information to and from the brain and spinal cord, resulting in pain, loss of sensation, or inability to control muscles. In some cases, the failure of nerves that control blood vessels, intestines, and

other organs results in abnormal blood pressure, digestion problems, and loss of other basic body processes. Risk factors for neuropathy include diabetes, heavy alcohol use, and exposure to certain chemicals and drugs. Some people have a hereditary predisposition for neuropathy. Prolonged pressure on a nerve is another risk for developing a nerve injury. Pressure injury may be caused by prolonged immobility (such as a long surgical procedure or lengthy illness) or compression of a nerve by casts, splints, braces, crutches, or other devices. Polyneuropathy implies a widespread process that usually affects both sides of the body equally. The symptoms depend on which type of nerve is affected. The three main types of nerves are sensory, motor, and autonomic. Neuropathy can affect any one or a combination of all three types of nerves. Symptoms also depend on whether the condition affects the whole body or just one nerve (as from an injury). The cause of chronic inflammatory polyneuropathy is an abnormal immune response. The specific antigens, immune processes, and triggering factors are variable and in many cases are unknown. It may occur in association with other conditions such as HIV, inflammatory bowel disease, lupus erythematosus, chronic active hepatitis, and blood cell abnormalities.

Peripheral neuropathy may involve a functional or pathological change to a single nerve or nerve group (mononeuropathy) or a functional or pathological change affecting multiple nerves (polyneuropathy).

Peripheral neuropathies may include the following:

Hereditary disorders

- Charcot-Marie-Tooth disease
- Friedreich's ataxia

Systemic or metabolic disorders

- Diabetes (diabetic neuropathy)
- Dietary deficiencies (especially vitamin B-12)
- Excessive alcohol use (alcoholic neuropathy)
- Uremia (from kidney failure)
- Cancer

Infectious or inflammatory conditions

- AIDS
- Hepatitis
- Colorado tick fever
- diphtheria
- Guillain-Barre syndrome

HIV infection without development of AIDS
leprosy
Lyme
polyarteritis nodosa
rheumatoid arthritis
sarcoidosis
Sjogren syndrome
syphilis
systemic lupus erythematosus
amyloid

Exposure to toxic compounds

sniffing glue or other toxic compounds
nitrous oxide
industrial agents - especially solvents
heavy metals (lead, arsenic, mercury, etc.)
Neuropathy secondary to drugs like analgesic nephropathy

Miscellaneous causes

ischemia (decreased oxygen/decreased blood flow)
prolonged exposure to cold temperature

a. Polyneuropathy

Polyneuropathy is a peripheral neuropathy involving the loss of movement or sensation to an area caused by damage or destruction to multiple peripheral nerves. Polyneuropathic pain, includes, without limitation, post-polio syndrome, postmastectomy syndrome, diabetic neuropathy, alcohol neuropathy, amyloid, toxins, AIDS, hypothyroidism, uremia, vitamin deficiencies, chemotherapy-induced pain, 2',3'-didexoycytidine (ddC) treatment, Guillain-Barré syndrome or Fabry's disease.

b. Mononeuropathy

Mononeuropathy is a peripheral neuropathy involving loss of movement or sensation to an area caused by damage or destruction to a single peripheral nerve or nerve group. Mononeuropathy is most often caused by damage to a local area resulting from injury or trauma, although occasionally systemic disorders may cause isolated nerve damage (as with mononeuritis multiplex). The usual causes are direct trauma, prolonged pressure on the nerve, and compression of the nerve by swelling or injury to nearby body structures. The damage includes destruction of the myelin sheath (covering) of the nerve or of part of the nerve cell (the axon). This damage slows or prevents conduction of impulses through the nerve. Mononeuropathy may involve any part of the body. Mononeuropathic pain, includes,

without limitation, a sciatic nerve dysfunction, a common peroneal nerve dysfunction. a radial nerve dysfunction, an ulnar nerve dysfunction, a cranial mononeuropathy VI, a cranial mononeuropathy VII, a cranial mononeuropathy III (compression type), a cranial mononeuropathy III (diabetic type), an axillary nerve dysfunction, a carpal tunnel syndrome, a femoral nerve dysfunction, a tibial nerve dysfunction, a Bell's palsy, a thoracic outlet syndrome, a carpal tunnel syndrome and a sixth (abducent) nerve palsy.

c. Generalized peripheral neuropathies

Generalized peripheral neuropathies are symmetrical, and usually due to various systematic illnesses and disease processes that affect the peripheral nervous system in its entirety. They are further subdivided into several categories:

i. Distal axonopathies are the result of some metabolic or toxic derangement of neurons. They may be caused by metabolic diseases such as diabetes, renal failure, deficiency syndromes such as malnutrition and alcoholism, or the effects of toxins or drugs. Distal axonopathy (aka dying back neuropathy) is a type of peripheral neuropathy that results from some metabolic or toxic derangement of peripheral nervous system (PNS) neurons. It is the most common response of nerves to metabolic or toxic disturbances, and as such may be caused by metabolic diseases such as diabetes, renal failure, deficiency syndromes such as malnutrition and alcoholism, or the effects of toxins or drugs. The most common cause of distal axonopathy is diabetes, and the most common distal axonopathy is diabetic neuropathy.

ii. Myelinopathies are due to a primary attack on myelin causing an acute failure of impulse conduction. The most common cause is acute inflammatory demyelinating polyneuropathy (AIDP; aka Guillain-Barré syndrome), though other causes include chronic inflammatory demyelinating syndrome (CIDP), genetic metabolic disorders (e.g., leukodystrophy), or toxins. Myelinopathy is due to primary destruction of myelin or the myelinating Schwann cells, which leaves the axon intact, but causes an acute failure of impulse conduction. This demyelination slows down or completely blocks the conduction of electrical impulses through the nerve. The most common cause is acute inflammatory demyelinating polyneuropathy (AIDP, better known as Guillain-Barré syndrome), though other causes include chronic inflammatory demyelinating polyneuropathy (CIDP), genetic metabolic disorders (e.g., leukodystrophy or Charcot-Marie-Tooth disease), or toxins.

iii. Neuronopathies are the result of destruction of peripheral nervous system (PNS) neurons. They may be caused by motor neurone diseases, sensory neuronopathies (e.g., Herpes zoster), toxins or autonomic dysfunction. Neurotoxins may cause neuronopathies, such as the chemotherapy agent vincristine. Neuronopathy is dysfunction due to damage to neurons of the peripheral nervous system (PNS), resulting in a peripheral

neuropathy. It may be caused by motor neurone diseases, sensory neuronopathies (e.g., Herpes zoster), toxic substances or autonomic dysfunction. A person with neuronopathy may present in different ways, depending on the cause, the way it affects the nerve cells, and the type of nerve cell that is most affected.

iv. Focal entrapment neuropathies (e.g., carpal tunnel syndrome).

The chimeric clostridial neurotoxin of the invention may be used to treat pain caused by or otherwise associated with any of the following inflammatory conditions.

A. Arthritic disorder

Arthritic disorders include, for example, a rheumatoid arthritis; a juvenile rheumatoid arthritis; a systemic lupus erythematosus (SLE); a gouty arthritis; a scleroderma; an osteoarthritis; a psoriatic arthritis; an ankylosing spondylitis; a Reiter's syndrome (reactive arthritis); an adult Still's disease; an arthritis from a viral infection; an arthritis from a bacterial infection, such as, e.g., a gonococcal arthritis and a non-gonococcal bacterial arthritis (septic arthritis); a Tertiary Lyme disease; a tuberculous arthritis; and an arthritis from a fungal infection, such as, e,g. a blastomycosis.

B. Autoimmune diseases

Autoimmune diseases include, for example, a Guillain-Barré syndrome, a Hashimoto's thyroiditis, a pernicious anemia, an Addison's disease, a type I diabetes, a systemic lupus erythematosus, a dermatomyositis, a Sjogren's syndrome, a lupus erythematosus, a multiple sclerosis, a myasthenia gravis, a Reiter's syndrome and a Grave's disease.

C. Connective tissue disorder

Connective tissue disorders include, for example, a spondyloarthritis a dermatomyositis, and a fibromyalgia.

D. Injury

Inflammation caused by injury, including, for example, a crush, puncture, stretch of a tissue or joint, may cause chronic inflammatory pain.

E. Infection

Inflammation caused by infection, including, for example, a tuberculosis or an interstitial keratitis may cause chronic inflammatory pain.

F. Neuritis

Neuritis is an inflammatory process affecting a nerve or group of nerves. Symptoms depend on the nerves involved, but may include pain, paresthesias, paresis, or hypesthesia (numbness).

Examples include:

a. Brachial neuritis

b. Retrobulbar neuropathy, an inflammatory process affecting the part of the optic nerve lying immediately behind the eyeball.

c. Optic neuropathy, an inflammatory process affecting the optic nerve causing sudden, reduced vision in the affected eye. The cause of optic neuritis is unknown. The sudden inflammation of the optic nerve (the nerve connecting the eye and the brain) leads to swelling and destruction of the myelin sheath. The inflammation may occasionally be the result of a viral infection, or it may be caused by autoimmune diseases such as multiple sclerosis. Risk factors are related to the possible causes.

d. Vestibular neuritis, a viral infection causing an inflammatory process affecting the vestibular nerve.

G. Joint inflammation

Inflammation of the joint, such as that caused by bursitis or tendonitis, for example, may cause chronic inflammatory pain.

H. Sunburn and/or UV-induced damage

The chimeric clostridial neurotoxin of the invention may be used to treat pain caused by or otherwise associated with any of the following headache conditions. A headache (medically known as cephalgia) is a condition of mild to severe pain in the head; sometimes neck or upper back pain may also be interpreted as a headache. It may indicate an underlying local or systemic disease or be a disorder in itself.

A. Muscular/myogenic headache

Muscular/myogenic headaches appear to involve the tightening or tensing of facial and neck muscles; they may radiate to the forehead. Tension headache is the most common form of myogenic headache.

A tension headache is a condition involving pain or discomfort in the head, scalp, or neck, usually associated with muscle tightness in these areas. Tension headaches result from the contraction of neck and scalp muscles. One cause of this muscle contraction is a response to stress, depression or anxiety. Any activity that causes the head to be held in one position for a long time without moving can cause a headache. Such activities include typing or use of computers, fine work with the hands, and use of a microscope. Sleeping in a cold room or sleeping with the neck in an abnormal position may also trigger this type of headache. A tension-type headache, includes, without limitation, an episodic tension headache and a chronic tension headache.

B. Vascular headache

The most common type of vascular headache is migraine. Other kinds of vascular headaches include cluster headaches, which cause repeated episodes of intense pain, and headaches resulting from high blood pressure.

1. Migraine

A migraine is a heterogeneous disorder that generally involves recurring headaches. Migraines are different from other headaches because they occur with other symptoms, such as, e.g., nausea, vomiting, or sensitivity to light. In most people, a throbbing pain is felt only on one side of the head. Clinical features such as type of aura symptoms, presence of prodromes, or associated symptoms such as vertigo, may be seen in subgroups of subjects with different underlying pathophysiological and genetic mechanisms. A migraine headache, includes, without limitation, a migraine without aura (common migraine), a migraine with aura (classic migraine), a menstrual migraine, a migraine equivalent (acephalic headache), a complicated migraine, an abdominal migraine and a mixed tension migraine.

2. Cluster headache

Cluster headaches affect one side of the head (unilateral) and may be associated with tearing of the eyes and nasal congestion. They occurs in clusters, happening repeatedly every day at the same time for several weeks and then remitting.

D. High blood pressure headache

E. Traction and inflammatory headache

Traction and inflammatory headaches are usually symptoms of other disorders, ranging from stroke to sinus infection.

F. Hormone headache

G. Rebound headache

Rebound headaches, also known as medication overuse headaches, occur when medication is taken too frequently to relieve a headache. Rebound headaches frequently occur daily and can be very painful.

H. Chronic sinusitis headache

Sinusitis is inflammation, either bacterial, fungal, viral, allergic or autoimmune, of the paranasal sinuses. Chronic sinusitis is one of the most common complications of the common cold. Symptoms include: nasal congestion; facial pain; headache; fever; general malaise; thick green or yellow discharge; feeling of facial 'fullness' worsening on bending over. In a small number of cases, chronic maxillary sinusitis can also be brought on by the spreading of bacteria from a dental infection. Chronic hyperplastic eosinophilic sinusitis is a noninfective form of chronic sinusitis.

I. An organic headache

J. Ictal headaches

Ital headaches are headaches associated with seizure activity.

The chimeric clostridial neurotoxin of the invention may be used to treat pain caused by or otherwise associated with any of the following somatic pain conditions. Somatic pain originates from ligaments, tendons, bones, blood vessels, and even nerves themselves. It is detected with somatic nociceptors. The scarcity of pain receptors in these areas produces a dull, poorly-localized pain of longer duration than cutaneous pain; examples include sprains and broken bones. Additional examples include the following.

A. Excessive muscle tension

Excessive muscle tension can be caused, for example, by a sprain or a strain.

B. Repetitive motion disorders

Repetitive motion disorders can result from overuse of the hands, wrists, elbows, shoulders, neck, back, hips, knees, feet, legs, or ankles.

C. Muscle disorders

Muscle disorders causing somatic pain include, for example, a polymyositis, a dermatomyositis, a lupus, a fibromyalgia, a polymyalgia rheumatica, and a rhabdomyolysis.

D. Myalgia

Myalgia is muscle pain and is a symptom of many diseases and disorders. The most common cause for myalgia is either overuse or over-stretching of a muscle or group of muscles. Myalgia without a traumatic history is often due to viral infections. Longer-term myalgias may be indicative of a metabolic myopathy, some nutritional deficiencies or chronic fatigue syndrome.

E. Infection

Infection can cause somatic pain. Examples of such infection include, for example, an abscess in the muscle, a trichinosis, an influenza, a Lyme disease, a malaria, a Rocky Mountain spotted fever, Avian influenza, the common cold, community-acquired pneumonia, meningitis, monkeypox, Severe Acute Respiratory Syndrome, toxic shock syndrome, trichinosis, typhoid fever, and upper respiratory tract infection.

F. Drugs

Drugs can cause somatic pain. Such drugs include, for example, cocaine, a statin for lowering cholesterol (such as atorvastatin, simvastatin, and lovastatin), and an ACE inhibitor for lowering blood pressure (such as enalapril and captopril).

The chimeric clostridial neurotoxin of the invention may be used to treat pain caused by or otherwise associated with any of the following visceral pain conditions. Visceral pain originates from body's viscera, or organs. Visceral nociceptors are located within body organs and internal cavities. The even greater scarcity of nociceptors in these areas produces pain that is usually more aching and of a longer duration than somatic pain. Visceral pain is extremely difficult to localise, and several injuries to visceral tissue exhibit "referred" pain, where the sensation is localised to an area completely unrelated to the site of injury. Examples of visceral pain include the following.

A. Functional visceral pain

Functional visceral pain includes, for example, an irritable bowel syndrome and a chronic functional abdominal pain (CFAP), a functional constipation and a functional dyspepsia, a non-cardiac chest pain (NCCP) and a chronic abdominal pain.

B. Chronic gastrointestinal inflammation

Chronic gastrointestinal inflammation includes, for example, a gastritis, an inflammatory bowel disease, like, e.g., a Crohn's disease, an ulcerative colitis, a microscopic colitis, a diverticulitis and a gastroenteritis; an interstitial cystitis; an intestinal ischemia; a cholecystitis; an appendicitis; a gastroesophageal reflux; an ulcer, a nephrolithiasis, an urinary tract infection, a pancreatitis and a hernia.

C. Autoimmune pain

Autoimmune pain includes, for example, a sarcoidosis and a vasculitis.

D. Organic visceral pain

Organic visceral pain includes, for example, pain resulting from a traumatic, inflammatory or degenerative lesion of the gut or produced by a tumour impinging on sensory innervation.

E. Treatment-induced visceral pain

Treatment-induced visceral pain includes, for example, a pain attendant to chemotherapy therapy or a pain attendant to radiation therapy.

The chimeric clostridial neurotoxin of the invention may be used to treat pain caused by or otherwise associated with any of the following referred pain conditions.

Referred pain arises from pain localized to an area separate from the site of pain stimulation. Often, referred pain arises when a nerve is compressed or damaged at or near its origin. In this circumstance, the sensation of pain will generally be felt in the territory that the nerve serves, even though the damage originates elsewhere. A common example occurs in intervertebral disc herniation, in which a nerve root arising from the spinal cord is compressed by adjacent disc material. Although pain may arise from the damaged disc itself, pain will also be felt in the region served by the compressed nerve (for example, the thigh, knee, or foot). Relieving the pressure on the nerve root may ameliorate the referred pain, provided that permanent nerve damage has not occurred. Myocardial ischaemia (the loss of blood flow to a part of the heart muscle tissue) is possibly the best known example of referred pain; the sensation can occur in the upper chest as a restricted feeling, or as an ache in the left shoulder, arm or even hand.

The chimeric clostridial neurotoxin of the invention may be used to treat post-operative pain. However, it is preferred that the pain in accordance with the invention is not post-operative pain.

Post-operative (e.g. post-surgical) pain is an unpleasant sensation that results from a surgical procedure. Post-operative pain may be caused by damage to tissue by an incision, the procedure itself, the closing of the wound, and any force that is applied during the procedure. Pain after surgery (e.g. post-operative pain) can also stem from factors that accompany surgery. For example, a subject may suffer back pain due to the way the subject was positioned on the surgical table, or chest pain may be due to an incision in the chest area. Throat pain may also occur after general anesthesia because the insertion of the breathing tube can cause irritation. However, most common is post-operative pain caused by cutting into the skin and muscle from a surgical incision. Post-operative pain may also include pain caused by or associated with a post-operative scar (e.g. post-operative scar pain).

For example, the surgical procedure (or more particularly, surgical incision) may represent a 'noxious stimulus' causing pain. Noxious stimuli, stimuli which can elicit tissue damage, can activate the release of pain mediators from nociceptive afferent terminals and from sensory terminals (e.g. release of CGRP therefrom). The noxious information is then transduced from the peripheral nervous system to the central nervous system, where pain is perceived by the individual.

Post-operative pain can be caused by the combination of inflammation and neural tissue damage. For example, degranulation of activated mast cells in response to tissue injury can result in the release of various substances including proteases, cytokines, serotonin and extracellular space. These substances can sensitize (activate at a lower threshold) primary afferent neurons to produce pain hypersensitivity. As tissue is extensively innervated, any region of the body is susceptible to nerve damage from surgery.

Reference to surgery means a medical procedure involving the treatment of an injury or disease in a subject comprising subjecting a part of the body to an incision (optionally removing or repairing a damaged part of the body). Although the level of invasiveness (e.g. level of surgical incision required) may vary amongst surgery types, surgery having a level of invasiveness that causes pain in the subject once surgery is complete is intended to be encompassed.

The surgery may comprise an incision to skin and/or fascia and/or muscle. Preferably, the surgery comprises an incision to the skin.

The surgery is not limited to that which may be carried out by a physician, but also includes for example dental surgery. Non-limiting examples of surgery include appendectomy, breast biopsy, breast augmentation or reduction, facelift, cholecystectomy, coronary artery bypass, debridement (e.g. of a wound, a burn, or infection), skin graft, organ transplant and tonsillectomy.

Preferably, “post-operative” may refer to a time period beginning at most one day subsequent to surgery (e.g. post-surgery). In other words, the term “post-operative” may refer to a time period beginning not greater than one day post-surgery. For example, the term “post-operative” may refer to a time point beginning 1-20 hours post-surgery; optionally 2-15 hours post-surgery; optionally 5-10 hours post-surgery. Such time may represent a time period beginning at the chronological interface at which the analgesic effects from a surgical anaesthetic administered to a subject diminish (e.g. taper) and thus the subject begins to perceive pain.

Furthermore, the term “post-operative” may be used interchangeably with the term “post-surgical”, as ‘operative’ is used in the sense of ‘surgery’ herein.

Similarly, the term “post-operative pain” may refer to pain that is perceived (or more particularly, begins to be perceived) for a time period beginning at most one day subsequent to surgery (e.g. post-surgery). In other words, the term “post-operative pain” may refer to pain that is perceived by a subject for a time period beginning not greater than one day post-surgery. For example, the term “post-operative pain” may refer to pain that is perceived for a time period beginning 1-20 hours post-surgery; optionally 2-15 hours post-surgery; optionally 5-10 hours post-surgery.

Said time period may be 1-50 weeks; for example 5-45 weeks, 10-40 weeks or 10-35 weeks post-surgery.

This contrasts with the term “peri-operative”, which may refer, for example, to a time period at or around the time that a subject is undergoing surgery (e.g. the time when the subject is

in the operating theatre), suitably a period beginning at least 1 hour pre-surgery and/or ending less than 1 hour post-surgery.

The present invention addresses a wide range of pain conditions, e.g. chronic pain conditions. In some embodiments, the chimeric clostridial neurotoxin of the invention is used for treating cancerous and/or non-cancerous pain.

Preferably, the chimeric clostridial neurotoxin of the invention is used to treat bladder pain syndrome (e.g. bladder pain), phantom limb pain, or migraine pain. The bladder pain syndrome (e.g. bladder pain) may be caused by or associated with interstitial cystitis.

In a particularly preferred embodiment, the pain is bladder pain, e.g. caused by or associated with interstitial cystitis.

Treating pain preferably means reducing pain. In other words, in one embodiment, administration of a chimeric clostridial neurotoxin of the invention reduces pain in a subject.

In more detail, reference to “reduced” or “reducing” (in terms of pain) preferably means a lower level of pain is perceived by the subject after administration with a chimeric clostridial neurotoxin of the invention (post-administration) when compared with a level of pain perceived by the subject prior to administration (pre-administration). For example, the level of pain perceived may be reduced by at least 15%, 25%, 35%, 45%, 55%, 65%, 75%, 85% or 95% post-administration relative to pre-administration. For example, the level of pain perceived may be reduced by at least 75%; preferably at least 85%; more preferably at least 95% post-administration.

A variety of means for assessing pain perception are known to those skilled in the art. For example, evaluation of mechanical allodynia (either static or dynamic) is routinely used in human pain studies as described in Pogatzki-Zahn *et. al.* (Pain Rep. 2017 Mar; 2(2): e588), incorporated herein by reference.

A suitable (albeit non-limiting) method for assessing pain perception in a subject includes the following: Numerical Rating Scale (NRS) score; although the skilled person is aware of other methods which may be used additionally or alternatively such as sensory threshold, pain perception threshold, static mechanical allodynia, dynamic mechanical allodynia, temporal

summation, pressure pain threshold, conditioned pain modulation, and temperature threshold.

Other non-limiting examples of pain perception measures include: change from baseline in SF-36 scores at each scheduled time point; amount of rescue medication taken during the study and time to first intake of rescue medication. These may be considered "exploratory" endpoints or pain perception assessment measures.

Thus, in a preferred embodiment, following the administration of a chimeric clostridial neurotoxin of the invention, pain perception may be assessed by one or more of: (a) a Numerical Rating Scale (NRS); (b) a stimulus-evoked NRS; (c) temperature of the painful area; (d) size of the painful area; (e) time to onset of analgesic effect; (f) peak analgesic effect; (g) time to peak analgesic effect; (h) duration of analgesic effect; and (i) an SF-36 quality of life assessment.

The skilled person is aware of such methods for assessing pain perception. For convenience, further description of the Numerical Rating Score and Quality of Life questionnaire Short Form-36 are provided below.

Numerical Rating Scale (NRS): Typically pain perception according to the present invention uses the Numerical Rating Scale (NRS). The NRS is an 11-point scale to assess subject pain perception. Subjects are asked to give a number between 0 and 10 that fits best to their pain intensity. Zero represents 'no pain at all' whereas the upper limit, 10, represents 'the worst pain possible'.

The NRS can be used to assess numerous facets of pain, including spontaneous average pain, spontaneous worst pain, and spontaneous current pain. Spontaneous average pain is assessed by asking a subject to select a number that best describes the subject's average pain (e.g. perceived pain) over a period of time, for example at least 6 hours, 12 hours, 24 hours, or at least 48 hours. Spontaneous worst pain is assessed by asking a subject to select a number that best describes the subject's pain at its worst during a specified period, e.g. at least the previous 6 hours, 12 hours, 24 hours or previous 48 hours. Spontaneous current pain is assessed by asking a subject to select a number that best describes how much pain the subject is in at the time of assessment.

The NRS can also be used to assess a subject's pain perception in response to a variety of different stimuli. To assess pain perception in response to a stimulus, the subject will be subjected to stimuli of various nature applied to the painful area. Subjects will be asked what are their current NRS scores pre-dose and post-stimulus.

Examples of stimuli used include: (i) light touch (which can be assessed by measuring pain on the surface of the painful area on radial spokes following application of a von Frey filament as described herein); (ii) pressure (pressure pain threshold), which can be assessed by asking the subject to give a NRS score as increasing pressure is applied using a pressure algometer; and (iii) temperature (which can be assessed by asking the subject for an NRS score for warm, cold and hot stimulation using a thermode applied to the painful area).

Preferably, administration of a chimeric clostridial neurotoxin of the invention reduces the subject's NRS score post-administration (e.g. from a rating of ≥7 to a rating of ≤6) when compared with the subject's NRS score pre-administration.

Quality of Life questionnaire Short Form-36 (SF-36): The SF-36 quality of life questionnaire may be used to assess a subject's pain perception. The SF-36 is a 36-item, subject-reported survey of subject health. The SF-36 consists of eight scaled scores (vitality, physical functioning, bodily pain, general health perceptions, physical role functioning, emotional role functioning, social role functioning and mental health). Each scale is directly transformed into a 0-100 scale on the assumption that each question carries equal weight. The higher the score recorded in the SF-36, the less disability.

Relevant parameters commonly tested in clinical trials for the treatment of pain are known in the art and could be readily selected by one of ordinary skill in the art. Examples of such parameters include, but are not limited to NRS; stimulus-evoked NRS; temperature of the painful area; size of the painful area; time to onset of analgesic effect; peak analgesic effect; time to peak analgesic effect; duration of analgesic effect; and/or SF-36 quality of life as described herein. Methods for assessing these parameters are also known in the art and can be carried out by one of ordinary skill using routine methods and procedures.

Preferably, administration of a chimeric clostridial neurotoxin of the invention increases the subject's SF-36 score post-administration (e.g. from a score of ≤50 to a score of ≥50) when compared with the subject's SF-36 score pre-administration.

The present invention may further (e.g. additionally or alternatively) be directed to the treatment of any sensory disorder that can be treated by a chimeric clostridial neurotoxin binding to a neuron comprising the Aδ nerve fiber or the C nerve fiber, respectively, and inhibiting release of a mediator therefrom. Without wishing to be bound by theory, it is believed that said disorder can be treated analogously to pain, as described herein. Thus, all of the embodiments described above in respect of treating pain may be equally valid in the context of treating sensory disorders. The mediator may be any mediator involved in sensation (e.g. a sensory mediator), in some cases said mediator may be a pain mediator described herein. The mediator may be a neurotransmitter. The inhibition of release of the mediator from the neuron may be partial or complete inhibition, preferably complete inhibition. For example, the chimeric clostridial neurotoxin may inhibit at least 80%, 90%, 95% or 99% of the mediator being released from the neuron. Preferably, the chimeric clostridial neurotoxin inhibits 100% of the mediator being released from the neuron. The chimeric clostridial neurotoxin may inhibit the release of a plurality of mediators from a neuron. A sensory disorder may be sensory modulation disorder (e.g. sensory over-responsivity) and/or a disorder of abnormal sensory processing (e.g. fibromyalgia).

Thus, in one aspect, the invention provides a chimeric clostridial neurotoxin for use in treating a sensory disorder by inhibiting release of a mediator from a neuron comprising an Aδ nerve fiber or a C nerve fiber, wherein the chimeric clostridial neurotoxin binds to the neuron comprising the Aδ nerve fiber or the C nerve fiber, respectively, and wherein the chimeric clostridial neurotoxin comprises a botulinum neurotoxin A (BoNT/A) light-chain and translocation domain (H_N domain), and a BoNT/B receptor binding domain (H_C domain).

In a related aspect, the invention provides a method for treating a sensory disorder by inhibiting release of a mediator from a neuron comprising an Aδ nerve fiber or a C nerve fiber, the method comprising administering to a subject a chimeric clostridial neurotoxin, wherein the chimeric clostridial neurotoxin binds to the neuron comprising the Aδ nerve fiber or the C nerve fiber, respectively, and wherein the chimeric clostridial neurotoxin comprises a botulinum neurotoxin A (BoNT/A) light-chain and translocation domain (H_N domain), and a BoNT/B receptor binding domain (H_C domain).

In another related aspect, the invention provides the use of a chimeric clostridial neurotoxin in the manufacture of a medicament for treating a sensory disorder by inhibiting release of a mediator from a neuron comprising an Aδ nerve fiber or a C nerve fiber, wherein the chimeric clostridial neurotoxin binds to the neuron comprising the Aδ nerve fiber or the C nerve fiber,

respectively, and wherein the chimeric clostridial neurotoxin comprises a botulinum neurotoxin A (BoNT/A) light-chain and translocation domain (H_N domain), and a BoNT/B receptor binding domain (H_C domain).

In another aspect, the invention provides a kit comprising:

(a) the unit dosage form according to the present invention; and

(b) instructions for use of the same in treating pain; and

(c) optionally a diluent.

CLAUSES:

1. A chimeric clostridial neurotoxin for use in treating pain by inhibiting release of a pain mediator from a neuron comprising an Aδ nerve fiber or a C nerve fiber, wherein the chimeric clostridial neurotoxin binds to the neuron comprising the Aδ nerve fiber or the C nerve fiber, respectively, and wherein the chimeric clostridial neurotoxin comprises a botulinum neurotoxin A (BoNT/A) light-chain and translocation domain (H_N domain), and a BoNT/B receptor binding domain (H_C domain).
2. The chimeric clostridial neurotoxin for use according to clause 1, wherein the pain mediator is one or more selected from: calcitonin gene-related peptide (CGRP); substance P; and a neurokinin.
3. The chimeric clostridial neurotoxin for use according to clause 1 or 2, wherein the pain mediator is CGRP and the pain is CGRP-associated pain.
4. The chimeric clostridial neurotoxin for use according to clause 3, wherein the CGRP-associated pain is CGRP-associated headache pain.
5. The chimeric clostridial neurotoxin for use according to clause 3 or 4, wherein the CGRP-associated pain is CGRP-associated migraine pain.
6. The chimeric clostridial neurotoxin for use according to any one of clauses 3-5, wherein the CGRP-associated pain is:
   (a) CGRP-associated somatic pain selected from: headache pain (e.g. post traumatic headache, head injury headache or post-traumatic brain injury headache), arthritic pain (e.g. osteo arthritis pain and/or rheumatoid arthritis pain), exercise pain, degenerative disc disease pain, carpal tunnel compression pain, soft tissue injury pain, temporomandibular joint pain, musculoskeletal pain, CGRP-associated somatic pain caused by or associated with a vascular disorder (e.g. Raynaud's syndrome, Buerger's disease, peripheral venous disease, peripheral arterial disease, varicose veins, blood clots in the veins, blood clotting disorders or lymphedema), facial

pain, CGRP-associated somatic pain caused by or associated with trigeminal autonomic cephalalgia, CGRP-associated somatic pain caused by or associated with trigeminal neuralgia, and CGRP-associated cancer-induced pain (e.g. CGRP-associated cancer-induced bone pain);

(b) CGRP-associated visceral pain selected from: endometriosis pain, pancreatitis pain, gastrointestinal pain, and CGRP-associated visceral pain caused by or associated with a vascular disorder;

(c) CGRP-associated inflammatory pain selected from: chronic pain, wound healing pain, pruritus pain, and burn pain; and/or

(d) CGRP-associated neuropathic pain selected from: post herpetic neuralgia pain, diabetes pain, chronic neuropathic pain, and Morton's neuroma pain.

7. The chimeric clostridial neurotoxin for use according to any one of the preceding clauses, wherein the neuron is the trigeminal ganglion.

8. The chimeric clostridial neurotoxin for use according to any one of the preceding clauses, wherein the chimeric clostridial neurotoxin is administered to the face, neck, and/or skull.

9. The chimeric clostridial neurotoxin for use according to any one of the preceding clauses, wherein the chimeric clostridial neurotoxin is administered intradermally.

10. The chimeric clostridial neurotoxin for use according to any one of the preceding clauses, wherein the chimeric clostridial neurotoxin is administered by intradermal injection at up to 10 injection sites per treatment session.

11. The chimeric clostridial neurotoxin for use according to any one of clauses 1-8, wherein the chimeric clostridial neurotoxin is administered intramuscularly.

12. The chimeric clostridial neurotoxin for use according to any one of clauses 1-8 or 11, wherein the chimeric clostridial neurotoxin is administered to one or more muscles of a subject selected from the: frontalis, corrugator (e.g. corrugator supercilii), procerus (e.g. procerus nasalis), occipitalis, temporalis, trapezius, masseter, nasalis, orbicularis oculi, cervical paraspinal muscles, temporal fascia, auricularis superior, auricularis anterior, auricularis posterior, sternocleidomastoid, platysma, dilatator naris anterior, dilatator naris posterior, depressor septi, mentalis, orbicularis oris, zygomaticus, risorius, buccinator, occipitofrontalis, levator labii superioris, depressor labii inferioris, depressor anguli oris, thyrohyoid, omohyoid, sternohyoid, splenius cervicis, splenius capitis, semispinalis cervicis, semispinalis capitis, levator scapulae, digastric, or scalene muscle(s);

preferably wherein the chimeric clostridial neurotoxin is administered to one or more muscles of a subject selected from the: frontalis, corrugator, procerus (e.g. procerus

nasalis), occipitalis, temporalis, trapezius, masseter, nasalis, orbicularis oculi, cervical paraspinal muscles, temporal fascia, auricularis superior, auricularis anterior, auricularis posterior, sternocleidomastoid, platysma, dilatator naris anterior, dilatator naris posterior, depressor septi, mentalis, orbicularis oris, zygomaticus, risorius, buccinator, occipitofrontalis, levator labii superioris, depressor labii inferioris, depressor anguli oris, thyrohyoid, omohyoid, sternohyoid, splenius cervicis, levator scapulae, digastric, and scalene muscle(s).

13. The chimeric clostridial neurotoxin for use according to any one of clauses 1-8 or 11-12, wherein the chimeric clostridial neurotoxin is administered by intramuscular injection at up to 10 injection sites per treatment session.

14. The chimeric clostridial neurotoxin for use according to any one of clauses 1-8, wherein the chimeric clostridial neurotoxin is administered intraneurally, perineurally or by periganglial administration.

15. The chimeric clostridial neurotoxin for use according to any one of clauses 1-8 or 14, wherein the chimeric clostridial neurotoxin is administered to the trigeminal nerve, Gasserian ganglion, nervus intermedius, glossopharyngeal, vagus nerve, and/or to the upper cervical roots via the occipital nerves.

16. The chimeric clostridial neurotoxin for use according to any one of clauses 1-8, wherein the chimeric clostridial neurotoxin is administered by perivascular administration.

17. The chimeric clostridial neurotoxin for use according to any one of the preceding clauses, wherein the chimeric clostridial neurotoxin is administered by way of a unit dose of 5 pg to 17,000 pg of the chimeric clostridial neurotoxin.

18. The chimeric clostridial neurotoxin for use according to any one of the preceding clauses, wherein the chimeric clostridial neurotoxin is administered by way of a unit dose of 500 pg to 17,000 pg.

19. The chimeric clostridial neurotoxin for use according to any one of the preceding clauses, wherein the chimeric clostridial neurotoxin is administered by way of a unit dose of 1,000 pg to 17,000 pg.

20. The chimeric clostridial neurotoxin for use according to any one of the preceding clauses, wherein the total dose administered per treatment session is up to 255,000 pg of the chimeric clostridial neurotoxin, e.g. 3,640-255,000 pg.

21. The chimeric clostridial neurotoxin for use according to any one of the preceding clauses, wherein the chimeric clostridial neurotoxin is administered by way of a unit dose of 3,640 pg to 17,000 pg.

22. The chimeric clostridial neurotoxin for use according to any one of the preceding clauses, wherein the chimeric clostridial neurotoxin has a Safety Ratio of greater than 7 (preferably a Safety Ratio of at least 10), wherein the Safety Ratio is calculated as: dose of toxin required for ~10% bodyweight change measured as pg/mouse divided by DAS ED_{50} measured as pg/mouse, wherein ED_{50} = dose required to produce a DAS score of 2.

23. The chimeric clostridial neurotoxin for use according to any one of the preceding clauses, wherein the C-terminal amino acid residue of said H_N domain corresponds to the first amino acid residue of the 3_{10} helix separating the H_N and H_C domains in BoNT/A, and wherein the N-terminal amino acid residue of said H_C domain corresponds to the second amino acid residue of the 3_{10} helix separating the H_N and H_C domains in BoNT/B.

24. The chimeric clostridial neurotoxin for use according to any one of the preceding clauses, wherein the chimeric clostridial neurotoxin comprises a polypeptide sequence having at least 70% sequence identity to SEQ ID NO: 1.

25. The chimeric clostridial neurotoxin for use according to any one of the preceding clauses, wherein the BoNT/B H_C domain comprises one or more substitution mutation(s) selected from the group consisting of: E1191M; S1199Y; V1118M; Y1183M; E1191I; E1191Q; E1191T; S1199F; S1199L; S1201V; and combinations thereof, preferably wherein the BoNT/B H_C domain comprises substitution mutations at E1191M and S1199Y.

Embodiments related to the various therapeutic uses of the invention are intended to be applied equally to methods, compositions (e.g. unit dosage forms), and kits of the invention and *vice versa*.

SEQUENCE HOMOLOGY

Any of a variety of sequence alignment methods can be used to determine percent identity, including, without limitation, global methods, local methods and hybrid methods, such as, e.g., segment approach methods. Protocols to determine percent identity are routine procedures within the scope of one skilled in the art. Global methods align sequences from the beginning to the end of the molecule and determine the best alignment by adding up scores of individual residue pairs and by imposing gap penalties. Non-limiting methods include, e.g., CLUSTAL W, see, e.g., Julie D. Thompson et al., CLUSTAL W: Improving the Sensitivity of Progressive Multiple Sequence Alignment Through Sequence Weighting, Position- Specific Gap Penalties and Weight Matrix Choice, 22(22) Nucleic Acids Research 4673-4680 (1994); and iterative refinement, see, e.g., Osamu Gotoh, Significant

Improvement in Accuracy of Multiple Protein. Sequence Alignments by Iterative Refinement as Assessed by Reference to Structural Alignments, 264(4) J. Mol. Biol. 823-838 (1996). Local methods align sequences by identifying one or more conserved motifs shared by all of the input sequences. Non-limiting methods include, e.g., Match-box, see, e.g., Eric Depiereux and Ernest Feytmans, Match-Box: A Fundamentally New Algorithm for the Simultaneous Alignment of Several Protein Sequences, 8(5) CABIOS 501 -509 (1992); Gibbs sampling, see, e.g., C. E. Lawrence et al., Detecting Subtle Sequence Signals: A Gibbs Sampling Strategy for Multiple Alignment, 262(5131) Science 208-214 (1993); Align-M, see, e.g., Ivo Van Walle et al., Align-M - A New Algorithm for Multiple Alignment of Highly Divergent Sequences, 20(9) Bioinformatics:1428-1435 (2004).

Thus, percent sequence identity is determined by conventional methods. See, for example, Altschul et al., Bull. Math. Bio. 48: 603-16, 1986 and Henikoff and Henikoff, Proc. Natl. Acad. Sci. USA 89:10915-19, 1992. Briefly, two amino acid sequences are aligned to optimize the alignment scores using a gap opening penalty of 10, a gap extension penalty of 1, and the "blosum 62" scoring matrix of Henikoff and Henikoff (ibid.) as shown below (amino acids are indicated by the standard one-letter codes); preferably this method is used to align a sequence with a subject sequence herein (e.g. SEQ ID NO: 7) to define amino acid position numbering as described herein.

The "percent sequence identity" between two or more nucleic acid or amino acid sequences is a function of the number of identical positions shared by the sequences. Thus, % identity may be calculated as the number of identical nucleotides / amino acids divided by the total number of nucleotides / amino acids, multiplied by 100. Calculations of % sequence identity may also take into account the number of gaps, and the length of each gap that needs to be introduced to optimize alignment of two or more sequences. Sequence comparisons and the determination of percent identity between two or more sequences can be carried out using specific mathematical algorithms, such as BLAST, which will be familiar to a skilled person.

ALIGNMENT SCORES FOR DETERMINING SEQUENCE IDENTITY

	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
A	4																			
R	-1	5																		
N	-2	0	6																	
D	-2	-2	1	6																
C	0	-3	-3	-3	9															
Q	-1	1	0	0	-3	5														
E	-1	0	0	2	-4	2	5													
G	0	-2	0	-1	-3	-2	-2	6												
H	-2	0	1	-1	-3	0	0	-2	8											
I	-1	-3	-3	-3	-1	-3	-3	-4	-3	4										
L	-1	-2	-3	-4	-1	-2	-3	-4	-3	2	4									
K	-1	2	0	-1	-3	1	1	-2	-1	-3	-2	5								
M	-1	-1	-2	-3	-1	0	-2	-3	-2	1	2	-1	5							
F	-2	-3	-3	-3	-2	-3	-3	-3	-1	0	0	-3	0	6						
P	-1	-2	-2	-1	-3	-1	-1	-2	-2	-3	-3	-1	-2	-4	7					
S	1	-1	1	0	-1	0	0	0	-1	-2	-2	0	-1	-2	-1	4				
T	0	-1	0	-1	-1	-1	-1	-2	-2	-1	-1	-1	-1	-2	-1	1	5			
W	-3	-3	-4	-4	-2	-2	-3	-2	-2	-3	-2	-3	-1	1	-4	-3	-2	11		
Y	-2	-2	-2	-3	-2	-1	-2	-3	2	-1	-1	-2	-1	3	-3	-2	-2	2	7	
V	0	-3	-3	-3	-1	-2	-2	-3	-3	3	1	-2	1	-1	-2	-2	0	-3	-1	4

The percent identity is then calculated as:

$$\frac{\text{Total number of identical matches}}{[\text{length of the longer sequence plus the number of gaps introduced into the longer sequence in order to align the two sequences}]} \times 100$$

Substantially homologous polypeptides are characterized as having one or more amino acid substitutions, deletions or additions. These changes are preferably of a minor nature, that is conservative amino acid substitutions (see below) and other substitutions that do not significantly affect the folding or activity of the polypeptide; small deletions, typically of one to about 30 amino acids; and small amino- or carboxyl-terminal extensions, such as an amino-

terminal methionine residue, a small linker peptide of up to about 20-25 residues, or an affinity tag.

CONSERVATIVE AMINO ACID SUBSTITUTIONS

Basic:	arginine
	lysine
	histidine
Acidic:	glutamic acid
	aspartic acid
Polar:	glutamine
	asparagine
Hydrophobic:	leucine
	isoleucine
	valine
Aromatic:	phenylalanine
	tryptophan
	tyrosine
Small:	glycine
	alanine
	serine
	threonine
	methionine

In addition to the 20 standard amino acids, non-standard amino acids (such as 4-hydroxyproline, 6-N-methyl lysine, 2-aminoisobutyric acid, isovaline and α -methyl serine) may be substituted for amino acid residues of the polypeptides of the present invention. A limited number of non-conservative amino acids, amino acids that are not encoded by the genetic code, and unnatural amino acids may be substituted for polypeptide amino acid residues. The polypeptides of the present invention can also comprise non-naturally occurring amino acid residues.

Non-naturally occurring amino acids include, without limitation, trans-3-methylproline, 2,4-methano-proline, cis-4-hydroxyproline, trans-4-hydroxy-proline, N-methylglycine, allo-threonine, methyl-threonine, hydroxy-ethylcysteine, hydroxyethylhomo-cysteine, nitro-glutamine, homoglutamine, pipecolic acid, tert-leucine, norvaline, 2-azaphenylalanine, 3-azaphenyl-alanine, 4-azaphenyl-alanine, and 4-fluorophenylalanine. Several methods are

known in the art for incorporating non-naturally occurring amino acid residues into proteins. For example, an in vitro system can be employed wherein nonsense mutations are suppressed using chemically aminoacylated suppressor tRNAs. Methods for synthesizing amino acids and aminoacylating tRNA are known in the art. Transcription and translation of plasmids containing nonsense mutations is carried out in a cell free system comprising an E. coli S30 extract and commercially available enzymes and other reagents. Proteins are purified by chromatography. See, for example, Robertson et al., J. Am. Chem. Soc. 113:2722, 1991; Ellman et al., Methods Enzymol. 202:301, 1991; Chung et al., Science 259:806-9, 1993; and Chung et al., Proc. Natl. Acad. Sci. USA 90:10145-9, 1993). In a second method, translation is carried out in Xenopus oocytes by microinjection of mutated mRNA and chemically aminoacylated suppressor tRNAs (Turcatti et al., J. Biol. Chem. 271:19991-8, 1996). Within a third method, E. coli cells are cultured in the absence of a natural amino acid that is to be replaced (e.g., phenylalanine) and in the presence of the desired non-naturally occurring amino acid(s) (e.g., 2-azaphenylalanine, 3-azaphenylalanine, 4-azaphenylalanine, or 4-fluorophenylalanine). The non-naturally occurring amino acid is incorporated into the polypeptide in place of its natural counterpart. See, Koide et al., Biochem. 33:7470-6, 1994. Naturally occurring amino acid residues can be converted to non-naturally occurring species by in vitro chemical modification. Chemical modification can be combined with site-directed mutagenesis to further expand the range of substitutions (Wynn and Richards, Protein Sci. 2:395-403, 1993).

A limited number of non-conservative amino acids, amino acids that are not encoded by the genetic code, non-naturally occurring amino acids, and unnatural amino acids may be substituted for amino acid residues of polypeptides of the present invention.

Essential amino acids in the polypeptides of the present invention can be identified according to procedures known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (Cunningham and Wells, Science 244: 1081-5, 1989). Sites of biological interaction can also be determined by physical analysis of structure, as determined by such techniques as nuclear magnetic resonance, crystallography, electron diffraction or photoaffinity labeling, in conjunction with mutation of putative contact site amino acids. See, for example, de Vos et al., Science 255:306-12, 1992; Smith et al., J. Mol. Biol. 224:899-904, 1992; Wlodaver et al., FEBS Lett. 309:59-64, 1992. The identities of essential amino acids can also be inferred from analysis of homologies with related components (e.g. the translocation or protease components) of the polypeptides of the present invention.

Multiple amino acid substitutions can be made and tested using known methods of mutagenesis and screening, such as those disclosed by Reidhaar-Olson and Sauer (Science 241:53-7, 1988) or Bowie and Sauer (Proc. Natl. Acad. Sci. USA 86:2152-6, 1989). Briefly, these authors disclose methods for simultaneously randomizing two or more positions in a polypeptide, selecting for functional polypeptide, and then sequencing the mutagenized polypeptides to determine the spectrum of allowable substitutions at each position. Other methods that can be used include phage display (e.g., Lowman et al., Biochem. 30:10832-7, 1991; Ladner et al., U.S. Patent No. 5,223,409; Huse, WIPO Publication WO 92/06204) and region-directed mutagenesis (Derbyshire et al., Gene 46:145, 1986; Ner et al., DNA 7:127, 1988).

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. Singleton, et al., DICTIONARY OF MICROBIOLOGY AND MOLECULAR BIOLOGY, 20 ED., John Wiley and Sons, New York (1994), and Hale & Marham, THE HARPER COLLINS DICTIONARY OF BIOLOGY, Harper Perennial, NY (1991) provide the skilled person with a general dictionary of many of the terms used in this disclosure.

This disclosure is not limited by the exemplary methods and materials disclosed herein, and any methods and materials similar or equivalent to those described herein can be used in the practice or testing of embodiments of this disclosure. Numeric ranges are inclusive of the numbers defining the range. Unless otherwise indicated, any nucleic acid sequences are written left to right in 5' to 3' orientation; amino acid sequences are written left to right in amino to carboxy orientation, respectively.

The headings provided herein are not limitations of the various aspects or embodiments of this disclosure.

Amino acids are referred to herein using the name of the amino acid, the three letter abbreviation or the single letter abbreviation. The term “protein", as used herein, includes proteins, polypeptides, and peptides. As used herein, the term “amino acid sequence” is synonymous with the term “polypeptide” and/or the term “protein”. In some instances, the term “amino acid sequence” is synonymous with the term “peptide”. In some instances, the term “amino acid sequence” is synonymous with the term “enzyme”. The terms "protein" and "polypeptide" are used interchangeably herein. In the present disclosure and claims, the conventional one-letter and three-letter codes for amino acid residues may be used. The 3-

letter code for amino acids as defined in conformity with the IUPACIUB Joint Commission on Biochemical Nomenclature (JCBN). It is also understood that a polypeptide may be coded for by more than one nucleotide sequence due to the degeneracy of the genetic code.

Other definitions of terms may appear throughout the specification. Before the exemplary embodiments are described in more detail, it is to be understood that this disclosure is not limited to particular embodiments described, and as such may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present disclosure will be defined only by the appended claims.

Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limits of that range is also specifically disclosed. Each smaller range between any stated value or intervening value in a stated range and any other stated or intervening value in that stated range is encompassed within this disclosure. The upper and lower limits of these smaller ranges may independently be included or excluded in the range, and each range where either, neither or both limits are included in the smaller ranges is also encompassed within this disclosure, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in this disclosure.

It must be noted that as used herein and in the appended claims, the singular forms “a”, “an”, and “the” include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to “a chimeric clostridial neurotoxin” includes a plurality of such candidate agents and reference to “the chimeric clostridial neurotoxin” includes reference to one or more chimeric clostridial neurotoxins and equivalents thereof known to those skilled in the art, and so forth.

The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that such publications constitute prior art to the claims appended hereto.

BRIEF DESCRIPTION OF THE DRAWINGS

Embodiments of the invention will now be described, by way of example only, with reference to the following Figures and Examples. Many of the Figures submitted herein are better

understood in colour. The colour versions of the drawings are part of the application as filed and the right to present colour images of the drawings in later proceedings is hereby reserved.

Figure 1 shows a representative single image (n=3) of untreated aDRG neurons stained with an antibody able to recognize cleaved SNAP25 (green) and DAPI for nuclear staining. The bottom image represents a merge of the two channels.

Figure 2 shows a representative single image (n=3) of aDRG neurons treated with 1 nM rBoNT/A for 24 hours and stained with an antibody able to recognize cleaved SNAP25 (green), antibodies for the aDRG markers NF200, CGRP, P2X3, TrkA (red) and DAPI for nuclear staining. Arrows indicate the specific areas in which the co-localization is more evident or not present. The bottom image represents a merge of the two channels.

Figure 3 shows a representative single image (n=3) of aDRG neurons treated with 1 nM mrBoNT/AB for 24 hours and stained with an antibody able to recognize cleaved SNAP25 (green), antibodies for the aDRG markers NF200, CGRP, P2X3, TrkA (red) and DAPI for nuclear staining. Arrows indicate the specific areas in which the co-localization is more evident or not present. The bottom image represents a merge of the two channels.

Figure 4 shows % CGRP release by aDRG neurons treated with BoNT for 24 hours before stimulation with KCl. CGRP release was assayed by EIA. Basal CGRP release was subtracted from stimulated release, and the results were normalised to no-BoNT control cells. Nonlinear curves were fitted to individual experiment dose responses (variable slope – four parameter where the bottom of the curves were constrained to 20%) to determine the IC_{50}. Displayed are representative curves fitted to KCl stimulated aDRGs treated with mrBoNT/AB (n = 3) or rBoNT/A (n = 3) mean ±SEM are shown. Individual experiments were run in triplicate.

Figure 5 shows the maximal % CGRP release inhibition from aDRGs treated with 1 nM BoNT for 24 hours. One-Way ANOVA with post-hoc Tukey test for multiple comparisons: ** - $p \leq 0.01$. mrBoNT/AB (n = 3) and rBoNT/A (n = 3). Individual experiments were run in triplicate (mean ±SEM).

Figure 6 shows the positions of preferred intradermal injection sites. The Table indicates the number of injections per side of the face for a given target nerve terminal as well as the total number of injections for that target nerve terminal.

Figure 7 shows concentration response curves based on CGRP release (pg/ml) for cells treated with: (**A**) rBoNT/A; or (**B**) mrBoNT/AB. Each average value (▲), represents data from 6 (or 5 for rBoNT/A) samples from the three different plates +/- standard deviation. All individual data points are also included (+).

Figure 8 shows pEC_{50} values for SNAP25 cleavage following treatment with mrBoNT/AB (□; 12.14) or rBoNT/A (o; 9.67) for 24 h in rat primary TG neurons *in vitro*. Data are the means ± sem of n=3 experiments. **** $p<0.0001$ (Student's unpaired t-test).

Figure 9 shows concentration-response curve values for SNAP25 cleavage following treatment with mrBoNT/AB (pEC_{50} = 12.85) or rBoNT/A (pEC_{50} = 10.73) for 24 h in sensory neurons derived from hiPSCs *in vitro*.

Figure 10 shows the proportion (%) of the area of the spinal trigeminal sensory nuclei in the brainstem (left) or trigeminal motor nuclei (right) that stained positive for cleaved SNAP25 in rats administered 300 pg/kg of mrBoNT/AB via intramuscular (IM) or intradermal (ID) injection. *$p<0.05$; Mann-Whitney test.

Figure 11 shows the amount of cleaved SNAP25 in the dorsal horn (sensory) in the cervical spinal cord (left) or ventral horn (motor) (right) in rats administered 300 pg/kg of mrBoNT/AB via intramuscular (IM) or intradermal (ID) injection. The amount is represented by way of a scoring system ("c-SNAP25 IHC score") as explained in Example 12.

Figure 12 shows the amount of cleaved SNAP25 in the axons of the trigeminal ganglia in rats administered 300 pg/kg of mrBoNT/AB via intramuscular (IM) or intradermal (ID) injection or administered Botox via IM injection. The "c-SNAP25 IHC score" was assigned based on a scoring system as explained in Example 12. **$p<0.01$; Kruskal-Wallis test followed by Dunn's multiple comparisons test.

SEQUENCE LISTING

Where an initial Met amino acid residue is indicated in any of the following SEQ ID NOs, said residue is optional.

SEQ ID NO: 1 - Polypeptide Sequence of Chimeric Clostridial Neurotoxin 1 (mrBoNT/AB)

SEQ ID NO: 2 - Polypeptide Sequence of Chimeric Clostridial Neurotoxin 2

SEQ ID NO: 3 - Polypeptide Sequence of Chimeric Clostridial Neurotoxin 3

SEQ ID NO: 4 - Polypeptide Sequence of Chimeric Clostridial Neurotoxin 4

SEQ ID NO: 5 - Polypeptide Sequence of Chimeric Clostridial Neurotoxin 5

SEQ ID NO: 6 - Polypeptide Sequence of Native BoNT/A (rBoNT/A)

SEQ ID NO: 7 - Polypeptide Sequence of BoNT/B

SEQ ID NO: 8 - Polypeptide Sequence of BoNT/C

SEQ ID NO: 9 - Polypeptide Sequence of BoNT/D

SEQ ID NO: 10 - Polypeptide Sequence of BoNT/E

SEQ ID NO: 11 - Polypeptide Sequence of BoNT/F

SEQ ID NO: 12 - Polypeptide Sequence of BoNT/G

SEQ ID NO: 13 - Polypeptide Sequence of BoNT/X

SEQ ID NO: 14 – Polypeptide Sequence of TeNT

SEQ ID NO: 15 – C-terminal L-chain Fragment

SEQ ID NO: 16 – C-terminal L-chain Fragment 2

SEQ ID NO: 17 – Di-Chain L-Chain 1

SEQ ID NO: 18 – Di-Chain L-Chain 2

SEQ ID NO: 19 – Di-Chain H-Chain

SEQ ID NO: 1 - Polypeptide Sequence of Chimeric Clostridial Neurotoxin 1 (mrBoNT/AB)

MPFVNKQFNYKDPVNGVDIAYIKIPNAGQMQPVKAFKIHNKIWVIPERDTFTNPEEGDLNPPPEAKQVPVSYYDS
TYLSTDNEKDNYLKGVTKLFERIYSTDLGRMLLTSIVRGIPFWGGSTIDTELKVIDTNCINVIQPDGSYRSEELN
LVIIGPSADIIQFECKSFGHEVLNLTRNGYGSTQYIRFSPDFTFGFEESLEVDTNPLLGAGKFATDPAVTLAHEL
IHAGHRLYGIAINPNRVFKVNTNAYYEMSGLEVSFEELRTFGGHDAKFIDSLQENEFRLYYYNKFKDIASTLNKA
KSIVGTTASLQYMKNVFKEKYLLSEDTSGKFSVDKLKFDKLYKMLTEIYTEDNFVKFFKVLNRKTYLNFDKAVFK
INIVPKVNYTIYDGFNLRNTNLAANFNGQNTEINNMNFTKLKNFTGLFEFYKLLCVRGIITSKTKSLDKGYNKAL
NDLCIKVNNWDLFFSPSEDNFTNDLNKGEEITSDTNIEAAEENISLDLIQQYYLTFNFDNEPENISIENLSSDII
GQLELMPNIERFPNGKKYELDKYTMFHYLRAQEFEHGKSRIALTNSVNEALLNPSRVYTFFSSDYVKKVNKATEA
AMFLGWVEQLVYDFTDETSEVSTTDKIADITIIIPYIGPALNIGNMLYKDDFVGALIFSGAVILLEFIPEIAIPV
LGTFALVSYIANKVLTVQTIDNALSKRNEKWDEVYKYIVTNWLAKVNTQIDLIRKKMKEALENQAEATKAIINYQ
YNQYTEEEKNNINFNIDDLSSKLNESINKAMININKFLNQCSVSYLMNSMIPYGVKRLEDFDASLKDALLKYIYD
NRGTLIGQVDRLKDKVNNTLSTDIPFQLSKYVDNQRLLSTFTEYIKNILNNIILNLRYKDNNLIDLSGYGAKVEV
YDGVELNDKNQFKLTSSANSKIRVTQNQNIIFNSVFLDFSVSFWIRIPKYKNDGIQNYIHNEYTIINCMKNNSGW
KISIRGNRIIWTLIDINGKTKSVFFEYNIREDISEYINRWFFVTITNNLNNAKIYINGKLESNTDIKDIREVIAN
GEIIFKLDGDIDRTQFIWMKYFSIFNTELSQSNIEERYKIQSYSEYLKDFWGNPLMYNKEYYMFNAGNKNSYIKL
KKDSPVGEILTRSKYNQNSKYINYRDLYIGEKFIIRRKSNSQSINDDIVRKEDYIYLDFFNLNQEWRVYTYKYFK
KEEMKLFLAPIYDSDEFYNTIQIKEYDEQPTYSCQLLFKKDEESTDEIGLIGIHRFYESGIVFEEYKDYFCISKW
YLKEVKRKPYNLKLGCNWQFIPKDEGWTE

SEQ ID NO: 2 - Polypeptide Sequence of Chimeric Clostridial Neurotoxin 2

MPFVNKQFNYKDPVNGVDIAYIKIPNAGQMQPVKAFKIHNKIWVIPERDTFTNPEEGDLN
PPPEAKQVPVSYYDSTYLSTDNEKDNYLKGVTKLFERIYSTDLGRMLLTSIVRGIPFWGG
STIDTELKVIDTNCINVIQPDGSYRSEELNLVIIGPSADIIQFECKSFGHEVLNLTRNGY
GSTQYIRFSPDFTFGFEESLEVDTNPLLGAGKFATDPAVTLAHELIHAGHRLYGIAINPN
RVFKVNTNAYYEMSGLEVSFEELRTFGGHDAKFIDSLQENEFRLYYYNKFKDIASTLNKA
KSIVGTTASLQYMKNVFKEKYLLSEDTSGKFSVDKLKFDKLYKMLTEIYTEDNFVKFFKV
LNRKTYLNFDKAVFKINIVPKVNYTIYDGFNLRNTNLAANFNGQNTEINNMNFTKLKNFT
GLFEFYKLLCVRGIITSKTKSLDKGYNKALNDLCIKVNNWDLFFSPSEDNFTNDLNKGEE
ITSDTNIEAAEENISLDLIQQYYLTFNFDNEPENISIENLSSDIIGQLELMPNIERFPNG
KKYELDKYTMFHYLRAQEFEHGKSRIALTNSVNEALLNPSRVYTFFSSDYVKKVNKATEA
AMFLGWVEQLVYDFTDETSEVSTTDKIADITIIIPYIGPALNIGNMLYKDDFVGALIFSG
AVILLEFIPEIAIPVLGTFALVSYIANKVLTVQTIDNALSKRNEKWDEVYKYIVTNWLAK
VNTQIDLIRKKMKEALENQAEATKAIINYQYNQYTEEEKNNINFNIDDLSSKLNESINKA
MININKFLNQCSVSYLMNSMIPYGVKRLEDFDASLKDALLKYIYDNRGTLIGQVDRLKDK
VNNTLSTDIPFQLSKYVDNQRLLSTFTEYIKSEILNNIILNLRYKDNNLIDLSGYGAKVE
VYDGVELNDKNQFKLTSSANSKIRVTQNQNIIFNSVFLDFSVSFWIRIPKYKNDGIQNYI
HNEYTIINCMKNNSGWKISIRGNRIIWTLIDINGKTKSVFFEYNIREDISEYINRWFFVT
ITNNLNNAKIYINGKLESNTDIKDIREVIANGEIIFKLDGDIDRTQFIWMKYFSIFNTEL
SQSNIEERYKIQSYSEYLKDFWGNPLMYNKEYYMFNAGNKNSYIKLKKDSPVGEILTRSK
YNQNSKYINYRDLYIGEKFIIRRKSNSQSINDDIVRKEDYIYLDFFNLNQEWRVYTYKYF
KKEEMKLFLAPIYDSDEFYNTIQIKEYDEQPTYSCQLLFKKDEESTDEIGLIGIHRFYES
GIVFEEYKDYFCISKWYLKEVKRKPYNLKLGCNWQFIPKDEGWTEHHHHHHHHHH

SEQ ID NO: 3 - Polypeptide Sequence of Chimeric Clostridial Neurotoxin 3

MPFVNKQFNYKDPVNGVDIAYIKIPNAGQMQPVKAFKIHNKIWVIPERDTFTNPEEGDLN
PPPEAKQVPVSYYDSTYLSTDNEKDNYLKGVTKLFERIYSTDLGRMLLTSIVRGIPFWGG
STIDTELKVIDTNCINVIQPDGSYRSEELNLVIIGPSADIIQFECKSFGHEVLNLTRNGY
GSTQYIRFSPDFTFGFEESLEVDTNPLLGAGKFATDPAVTLAHELIHAGHRLYGIAINPN
RVFKVNTNAYYEMSGLEVSFEELRTFGGHDAKFIDSLQENEFRLYYYNKFKDIASTLNKA
KSIVGTTASLQYMKNVFKEKYLLSEDTSGKFSVDKLKFDKLYKMLTEIYTEDNFVKFFKV
LNRKTYLNFDKAVFKINIVPKVNYTIYDGFNLRNTNLAANFNGQNTEINNMNFTKLKNFT
GLFEFYKLLCVRGIITSKTKSLDKGYNKALNDLCIKVNNWDLFFSPSEDNFTNDLNKGEE
ITSDTNIEAAEENISLDLIQQYYLTFNFDNEPENISIENLSSDIIGQLELMPNIERFPNG
KKYELDKYTMFHYLRAQEFEHGKSRIALTNSVNEALLNPSRVYTFFSSDYVKKVNKATEA
AMFLGWVEQLVYDFTDETSEVSTTDKIADITIIIPYIGPALNIGNMLYKDDFVGALIFSG
AVILLEFIPEIAIPVLGTFALVSYIANKVLTVQTIDNALSKRNEKWDEVYKYIVTNWLAK
VNTQIDLIRKKMKEALENQAEATKAIINYQYNQYTEEEKNNINFNIDDLSSKLNESINKA
MININKFLNQCSVSYLMNSMIPYGVKRLEDFDASLKDALLKYIYDNRGTLIGQVDRLKDK
VNNTLSTDIPFQLSKYVDNQRLLSTFTEYIKNIIELGGGGSELSEILNNIILNLRYKDNN
LIDLSGYGAKVEVYDGVELNDKNQFKLTSSANSKIRVTQNQNIIFNSVFLDFSVSFWIRI
PKYKNDGIQNYIHNEYTIINCMKNNSGWKISIRGNRIIWTLIDINGKTKSVFFEYNIRED
ISEYINRWFFVTITNNLNNAKIYINGKLESNTDIKDIREVIANGEIIFKLDGDIDRTQFI
WMKYFSIFNTELSQSNIEERYKIQSYSEYLKDFWGNPLMYNKEYYMFNAGNKNSYIKLKK
DSPVGEILTRSKYNQNSKYINYRDLYIGEKFIIRRKSNSQSINDDIVRKEDYIYLDFFNL
NQEWRVYTYKYFKKEEMKLFLAPIYDSDEFYNTIQIKEYDEQPTYSCQLLFKKDEESTDE
IGLIGIHRFYESGIVFEEYKDYFCISKWYLKEVKRKPYNLKLGCNWQFIPKDEGWTEHHH
HHHHHHH

SEQ ID NO: 4 - Polypeptide Sequence of Chimeric Clostridial Neurotoxin 4

MPFVNKQFNYKDPVNGVDIAYIKIPNAGQMQPVKAFKIHNKIWVIPERDTFTNPEEGDLN

PPPEAKQVPVSYYDSTYLSTDNEKDNYLKGVTKLFERIYSTDLGRMLLTSIVRGIPFWGG
STIDTELKVIDTNCINVIQPDGSYRSEELNLVIIGPSADIIQFECKSFGHEVLNLTRNGY
GSTQYIRFSPDFTFGFEESLEVDTNPLLGAGKFATDPAVTLAHELIHAGHRLYGIAINPN
RVFKVNTNAYYEMSGLEVSFEELRTFGGHDAKFIDSLQENEFRLYYYNKFKDIASTLNKA
KSIVGTTASLQYMKNVFKEKYLLSEDTSGKFSVDKLKFDKLYKMLTEIYTEDNFVKFFKV
LNRKTYLNFDKAVFKINIVPKVNYTIYDGFNLRNTNLAANFNGQNTEINNMNFTKLKNFT
GLFEFYKLLCVRGIITSKTKSLDKGYNKALNDLCIKVNNWDLFFSPSEDNFTNDLNKGEE
ITSDTNIEAAEENISLDLIQQYYLTFNFDNEPENISIENLSSDIIGQLELMPNIERFPNG
KKYELDKYTMFHYLRAQEFEHGKSRIALTNSVNEALLNPSRVYTFFSSDYVKKVNKATEA
AMFLGWVEQLVYDFTDETSEVSTTDKIADITIIIPYIGPALNIGNMLYKDDFVGALIFSG
AVILLEFIPEIAIPVLGTFALVSYIANKVLTVQTIDNALSKRNEKWDEVYKYIVTNWLAK
VNTQIDLIRKKMKEALENQAEATKAIINYQYNQYTEEEKNNINFNIDDLSSKLNESINKA
MININKFLNQCSVSYLMNSMIPYGVKRLEDFDASLKDALLKYIYDNRGTLIGQVDRLKDK
VNNTLSTDIPFQLSKYVDNQRLLSTFTEYIKNILNNIILNLRYKDNNLIDLSGYGAKVEV
YDGVELNDKNQFKLTSSANSKIRVTQNQNIIFNSVFLDFSVSFWIRIPKYKNDGIQNYIH
NEYTIINCMKNNSGWKISIRGNRIIWTLIDINGKTKSVFFEYNIREDISEYINRWFFVTI
TNNLNNAKIYINGKLESNTDIKDIREVIANGEIIFKLDGDIDRTQFIWMKYFSIFNTELS
QSNIEERYKIQSYSEYLKDFWGNPLMYNKEYYMFNAGNKNSYIKLKKDSPVGEILTRSKY
NQNSKYINYRDLYIGEKFIIRRKSNSQSINDDIVRKEDYIYLDFFNLNQEWRVYTYKYFK
KEEMKLFLAPIYDSDEFYNTIQIKEYDEQPTYSCQLLFKKDEESTDEIGLIGIHRFYESG
IVFEEYKDYFCISKWYLKEVKRKPYNLKLGCNWQFIPKDEGWTEHHHHHHHHHH

SEQ ID NO: 5 - Polypeptide Sequence of Chimeric Clostridial Neurotoxin 5

MPFVNKQFNYKDPVNGVDIAYIKIPNAGQMQPVKAFKIHNKIWVIPERDTFTNPEEGDLN
PPPEAKQVPVSYYDSTYLSTDNEKDNYLKGVTKLFERIYSTDLGRMLLTSIVRGIPFWGG
STIDTELKVIDTNCINVIQPDGSYRSEELNLVIIGPSADIIQFECKSFGHEVLNLTRNGY
GSTQYIRFSPDFTFGFEESLEVDTNPLLGAGKFATDPAVTLAHELIHAGHRLYGIAINPN
RVFKVNTNAYYEMSGLEVSFEELRTFGGHDAKFIDSLQENEFRLYYYNKFKDIASTLNKA
KSIVGTTASLQYMKNVFKEKYLLSEDTSGKFSVDKLKFDKLYKMLTEIYTEDNFVKFFKV
LNRKTYLNFDKAVFKINIVPKVNYTIYDGFNLRNTNLAANFNGQNTEINNMNFTKLKNFT
GLFEFYKLLCVRGIITSKTKSLDKGYNKALNDLCIKVNNWDLFFSPSEDNFTNDLNKGEE
ITSDTNIEAAEENISLDLIQQYYLTFNFDNEPENISIENLSSDIIGQLELMPNIERFPNG
KKYELDKYTMFHYLRAQEFEHGKSRIALTNSVNEALLNPSRVYTFFSSDYVKKVNKATEA
AMFLGWVEQLVYDFTDETSEVSTTDKIADITIIIPYIGPALNIGNMLYKDDFVGALIFSG
AVILLEFIPEIAIPVLGTFALVSYIANKVLTVQTIDNALSKRNEKWDEVYKYIVTNWLAK
VNTQIDLIRKKMKEALENQAEATKAIINYQYNQYTEEEKNNINFNIDDLSSKLNESINKA
MININKFLNQCSVSYLMNSMIPYGVKRLEDFDASLKDALLKYIYDNRGTLIGQVDRLKDK
VNNTLSTDIPFQLSKYVDNQRLLSTFTEYIKNILNNIILNLRYKDNNLIDLSGYGAKVEV
YDGVELNDKNQFKLTSSANSKIRVTQNQNIIFNSVFLDFSVSFWIRIPKYKNDGIQNYIH
NEYTIINCMKNNSGWKISIRGNRIIWTLIDINGKTKSVFFEYNIREDISEYINRWFFVTI
TNNLNNAKIYINGKLESNTDIKDIREVIANGEIIFKLDGDIDRTQFIWMKYFSIFNTELS
QSNIEERYKIQSYSEYLKDFWGNPLMYNKEYYMFNAGNKNSYIKLKKDSPVGEILTRSKY
NQNSKYINYRDLYIGEKFIIRRKSNSQSINDDIVRKEDYIYLDFFNLNQEWRVYTYKYFK
KEEEKLFLAPISDSDEFYNTIQIKEYDEQPTYSCQLLFKKDEESTDEIGLIGIHRFYESG
IVFEEYKDYFCISKWYLKEVKRKPYNLKLGCNWQFIPKDEGWTE

SEQ ID NO: 6 - Polypeptide Sequence of Native BoNT/A (rBoNT/A)

MPFVNKQFNYKDPVNGVDIAYIKIPNAGQMQPVKAFKIHNKIWVIPERDTFTNPEEGDLNPPPEAKQVPVSYYDS
TYLSTDNEKDNYLKGVTKLFERIYSTDLGRMLLTSIVRGIPFWGGSTIDTELKVIDTNCINVIQPDGSYRSEELN
LVIIGPSADIIQFECKSFGHEVLNLTRNGYGSTQYIRFSPDFTFGFEESLEVDTNPLLGAGKFATDPAVTLAHEL
IHAGHRLYGIAINPNRVFKVNTNAYYEMSGLEVSFEELRTFGGHDAKFIDSLQENEFRLYYYNKFKDIASTLNKA
KSIVGTTASLQYMKNVFKEKYLLSEDTSGKFSVDKLKFDKLYKMLTEIYTEDNFVKFFKVLNRKTYLNFDKAVFK
INIVPKVNYTIYDGFNLRNTNLAANFNGQNTEINNMNFTKLKNFTGLFEFYKLLCVRGIITSKTKSLDKGYNKAL
NDLCIKVNNWDLFFSPSEDNFTNDLNKGEEITSDTNIEAAEENISLDLIQQYYLTFNFDNEPENISIENLSSDII
GQLELMPNIERFPNGKKYELDKYTMFHYLRAQEFEHGKSRIALTNSVNEALLNPSRVYTFFSSDYVKKVNKATEA

AMFLGWVEQLVYDFTDETSEVSTTDKIADITIIIPYIGPALNIGNMLYKDDFVGALIFSGAVILLEFIPEIAIPV
LGTFALVSYIANKVLTVQTIDNALSKRNEKWDEVYKYIVTNWLAKVNTQIDLIRKKMKEALENQAEATKAIINYQ
YNQYTEEEKNNINFNIDDLSSKLNESINKAMININKFLNQCSVSYLMNSMIPYGVKRLEDFDASLKDALLKYIYD
NRGTLIGQVDRLKDKVNNTLSTDIPFQLSKYVDNQRLLSTFTEYIKNIINTSILNLRYESNHLIDLSRYASKINI
GSKVNFDPIDKNQIQLFNLESSKIEVILKNAIVYNSMYENFSTSFWIRIPKYFNSISLNNEYTIINCMENNSGWK
VSLNYGEIIWTLQDTQEIKQRVVFKYSQMINISDYINRWIFVTITNNRLNNSKIYINGRLIDQKPISNLGNIHAS
NNIMFKLDGCRDTHRYIWIKYFNLFDKELNEKEIKDLYDNQSNSGILKDFWGDYLQYDKPYYMLNLYDPNKYVDV
NNVGIRGYMYLKGPRGSVMTTNIYLNSSLYRGTKFIIKKYASGNKDNIVRNNDRVYINVVVKNKEYRLATNASQA
GVEKILSALEIPDVGNLSQVVVMKSKNDQGITNKCKMNLQDNNGNDIGFIGFHQFNNIAKLVASNWYNRQIERSS
RTLGCSWEFIPVDDGWGERPL

SEQ ID NO: 7 - Polypeptide Sequence of BoNT/B

MPVTINNFNYNDPIDNNNIIMMEPPFARGTGRYYKAFKITDRIWIIPERYTFGYKPEDFN
KSSGIFNRDVCEYYDPDYLNTNDKKNIFLQTMIKLFNRIKSKPLGEKLLEMIINGIPYLG
DRRVPLEEFNTNIASVTVNKLISNPGEVERKKGIFANLIIFGPGPVLNENETIDIGIQNH
FASREGFGGIMQMKFCPEYVSVFNNVQENKGASIFNRRGYFSDPALILMHELIHVLHGLY
GIKVDDLPIVPNEKKFFMQSTDAIQAEELYTFGGQDPSIITPSTDKSIYDKVLQNFRGIV
DRLNKVLVCISDPNININIYKNKFKDKYKFVEDSEGKYSIDVESFDKLYKSLMFGFTETN
IAENYKIKTRASYFSDSLPPVKIKNLLDNEIYTIEEGFNISDKDMEKEYRGQNKAINKQA
YEEISKEHLAVYKIQMCKSVKAPGICIDVDNEDLFFIADKNSFSDDLSKNERIEYNTQSN
YIENDFPINELILDTDLISKIELPSENTESLTDFNVDVPVYEKQPAIKKIFTDENTIFQY
LYSQTFPLDIRDISLTSSFDDALLFSNKVYSFFSMDYIKTANKVVEAGLFAGWVKQIVND
FVIEANKSNTMDKIADISLIVPYIGLALNVGNETAKGNFENAFEIAGASILLEFIPELLI
PVVGAFLLESYIDNKNKIIKTIDNALTKRNEKWSDMYGLIVAQWLSTVNTQFYTIKEGMY
KALNYQAQALEEIIKYRYNIYSEKEKSNINIDFNDINSKLNEGINQAIDNINNFINGCSV
SYLMKKMIPLAVEKLLDFDNTLKKNLLNYIDENKLYLIGSAEYEKSKVNKYLKTIMPFDL
SIYTNDTILIEMFNKYNSEILNNIILNLRYKDNNLIDLSGYGAKVEVYDGVELNDKNQFK
LTSSANSKIRVTQNQNIIFNSVFLDFSVSFWIRIPKYKNDGIQNYIHNEYTIINCMKNNS
GWKISIRGNRIIWTLIDINGKTKSVFFEYNIREDISEYINRWFFVTITNNLNNAKIYING
KLESNTDIKDIREVIANGEIIFKLDGDIDRTQFIWMKYFSIFNTELSQSNIEERYKIQSY
SEYLKDFWGNPLMYNKEYYMFNAGNKNSYIKLKKDSPVGEILTRSKYNQNSKYINYRDLY
IGEKFIIRRKSNSQSINDDIVRKEDYIYLDFFNLNQEWRVYTYKYFKKEEEKLFLAPISD
SDEFYNTIQIKEYDEQPTYSCQLLFKKDEESTDEIGLIGIHRFYESGIVFEEYKDYFCIS
KWYLKEVKRKPYNLKLGCNWQFIPKDEGWTE

SEQ ID NO: 8 - Polypeptide Sequence of BoNT/C

MPITINNFNYSDPVDNKNILYLDTHLNTLANEPEKAFRITGNIWVIPDRFSRNSNPNLNK
PPRVTSPKSGYYDPNYLSTDSDKDPFLKEIIKLFKRINSREIGEELIYRLSTDIPFPGNN
NTPINTFDFDVDFNSVDVKTRQGNNWVKTGSINPSVIITGPRENIIDPETSTFKLTNNTF
AAQEGFGALSIISISPRFMLTYSNATNDVGEGRFSKSEFCMDPILILMHELNHAMHNLYG
IAIPNDQTISSVTSNIFYSQYNVKLEYAEIYAFGGPTIDLIPKSARKYFEEKALDYYRSI
AKRLNSITTANPSSFNKYIGEYKQKLIRKYRFVVESSGEVTVNRNKFVELYNELTQIFTE
FNYAKIYNVQNRKIYLSNVYTPVTANILDDNVYDIQNGFNIPKSNLNVLFMGQNLSRNPA
LRKVNPENMLYLFTKFCHKAIDGRSLYNKTLDCRELLVKNTDLPFIGDISDVKTDIFLRK
DINEETEVIYYPDNVSVDQVILSKNTSEHGQLDLLYPSIDSESEILPGENQVFYDNRTQN
VDYLNSYYYLESQKLSDNVEDFTFTRSIEEALDNSAKVYTYFPTLANKVNAGVQGGLFLM
WANDVVEDFTTNILRKDTLDKISDVSAIIPYIGPALNISNSVRRGNFTEAFAVTGVTILL
EAFPEFTIPALGAFVIYSKVQERNEIIKTIDNCLEQRIKRWKDSYEWMMGTWLSRIITQF
NNISYQMYDSLNYQAGAIKAKIDLEYKKYSGSDKENIKSQVENLKNSLDVKISEAMNNIN
KFIRECSVTYLFKNMLPKVIDELNEFDRNTKAKLINLIDSHNIILVGEVDKLKAKVNNSF
QNTIPFNIFSYTNNSLLKDIINEYFNNINDSKILSLQNRKNTLVDTSGYNAEVSEEGDVQ
LNPIFPFDFKLGSSGEDRGKVIVTQNENIVYNSMYESFSISFWIRINKWVSNLPGYTIID
SVKNNSGWSIGIISNFLVFTLKQNEDSEQSINFSYDISNNAPGYNKWFFVTVTNNMMGNM
KIYINGKLIDTIKVKELTGINFSKTITFEINKIPDTGLITSDSDNINMWIRDFYIFAKEL
DGKDINILFNSLQYTNVVKDYWGNDLRYNKEYYMVNIDYLNRYMYANSRQIVFNTRRNNN

DFNEGYKIIIKRIRGNTNDTRVRGGDILYFDMTINNKAYNLFMKNETMYADNHSTEDIYA
IGLREQTKDINDNIIFQIQPMNNTYYYASQIFKSNFNGENISGICSIGTYRFRLGGDWYR
HNYLVPTVKQGNYASLLESTSTHWGFVPVSE

SEQ ID NO: 9 - Polypeptide Sequence of BoNT/D

MTWPVKDFNYSDPVNDNDILYLRIPQNKLITTPVKAFMITQNIWVIPERFSSDTNPSLSK
PPRPTSKYQSYYDPSYLSTDEQKDTFLKGIIKLFKRINERDIGKKLINYLVVGSPFMGDS
STPEDTFDFTRHTTNIAVEKFENGSWKVTNIITPSVLIFGPLPNILDYTASLTLQGQQSN
PSFEGFGTLSILKVAPEFLLTFSDVTSNQSSAVLGKSIFCMDPVIALMHELTHSLHQLYG
INIPSDKRIRPQVSEGFFSQDGPNVQFEELYTFGGLDVEIIPQIERSQLREKALGHYKDI
AKRLNNINKTIPSSWISNIDKYKKIFSEKYNFDKDNTGNFVVNIDKFNSLYSDLTNVMSE
VVYSSQYNVKNRTHYFSRHYLPVFANILDDNIYTIRDGFNLTNKGFNIENSGQNIERNPA
LQKLSSESVVDLFTKVCLRLTKNSRDDSTCIKVKNNRLPYVADKDSISQEIFENKIITDE
TNVQNYSDKFSLDESILDGQVPINPEIVDPLLPNVNMEPLNLPGEEIVFYDDITKYVDYL
NSYYYLESQKLSNNVENITLTTSVEEALGYSNKIYTFLPSLAEKVNKGVQAGLFLNWANE
VVEDFTTNIMKKDTLDKISDVSVIIPYIGPALNIGNSALRGNFNQAFATAGVAFLLEGFP
EFTIPALGVFTFYSSIQEREKIIKTIENCLEQRVKRWKDSYQWMVSNWLSRITTQFNHIN
YQMYDSLSYQADAIKAKIDLEYKKYSGSDKENIKSQVENLKNSLDVKISEAMNNINKFIR
ECSVTYLFKNMLPKVIDELNKFDLRTKTELINLIDSHNIILVGEVDRLKAKVNESFENTM
PFNIFSYTNNSLLKDIINEYFNSINDSKILSLQNKKNALVDTSGYNAEVRVGDNVQLNTI
YTNDFKLSSSGDKIIVNLNNNILYSAIYENSSVSFWIKISKDLTNSHNEYTIINSIEQNS
GWKLCIRNGNIEWILQDVNRKYKSLIFDYSESLSHTGYTNKWFFVTITNNIMGYMKLYIN
GELKQSQKIEDLDEVKLDKTIVFGIDENIDENQMLWIRDFNIFSKELSNEDINIVYEGQI
LRNVIKDYWGNPLKFDTEYYIINDNYIDRYIAPESNVLVLVQYPDRSKLYTGNPITIKSV
SDKNPYSRILNGDNIILHMLYNSRKYMIIRDTDTIYATQGGECSQNCVYALKLQSNLGNY
GIGIFSIKNIVSKNKYCSQIFSSFRENTMLLADIYKPWRFSFKNAYTPVAVTNYETKLLS
TSSFWKFISRDPGWVE

SEQ ID NO: 10 - Polypeptide Sequence of BoNT/E

MPKINSFNYNDPVNDRTILYIKPGGCQEFYKSFNIMKNIWIIPERNVIGTTPQDFHPPTS
LKNGDSSYYDPNYLQSDEEKDRFLKIVTKIFNRINNNLSGGILLEELSKANPYLGNDNTP
DNQFHIGDASAVEIKFSNGSQDILLPNVIIMGAEPDLFETNSSNISLRNNYMPSNHRFGS
IAIVTFSPEYSFRFNDNCMNEFIQDPALTLMHELIHSLHGLYGAKGITTKYTITQKQNPL
ITNIRGTNIEEFLTFGGTDLNIITSAQSNDIYTNLLADYKKIASKLSKVQVSNPLLNPYK
DVFEAKYGLDKDASGIYSVNINKFNDIFKKLYSFTEFDLRTKFQVKCRQTYIGQYKYFKL
SNLLNDSIYNISEGYNINNLKVNFRGQNANLNPRIITPITGRGLVKKIIRFCKNIVSVKG
IRKSICIEINNGELFFVASENSYNDDNINTPKEIDDTVTSNNNYENDLDQVILNFNSESA
PGLSDEKLNLTIQNDAYIPKYDSNGTSDIEQHDVNELNVFFYLDAQKVPEGENNVNLTSS
IDTALLEQPKIYTFFSSEFINNVNKPVQAALFVSWIQQVLVDFTTEANQKSTVDKIADIS
IVVPYIGLALNIGNEAQKGNFKDALELLGAGILLEFEPELLIPTILVFTIKSFLGSSDNK
NKVIKAINNALKERDEKWKEVYSFIVSNWMTKINTQFNKRKEQMYQALQNQVNAIKTIIE
SKYNSYTLEEKNELTNKYDIKQIENELNQKVSIAMNNIDRFLTESSISYLMKIINEVKIN
KLREYDENVKTYLLNYIIQHGSILGESQQELNSMVTDTLNNSIPFKLSSYTDDKILISYF
NKFFKRIKSSSVLNMRYKNDKYVDTSGYDSNININGDVYKYPTNKNQFGIYNDKLSEVNI
SQNDYIIYDNKYKNFSISFWVRIPNYDNKIVNVNNEYTIINCMRDNNSGWKVSLNHNEII
WTFEDNRGINQKLAFNYGNANGISDYINKWIFVTITNDRLGDSKLYINGNLIDQKSILNL
GNIHVSDNILFKIVNCSYTRYIGIRYFNIFDKELDETEIQTLYSNEPNTNILKDFWGNYL
LYDKEYYLLNVLKPNNFIDRRKDSTLSINNIRSTILLANRLYSGIKVKIQRVNNSSTNDN
LVRKNDQVYINFVASKTHLFPLYADTATTNKEKTIKISSSGNRFNQVVVMNSVGNCTMNF
KNNNGNNIGLLGFKADTVVASTWYYTHMRDHTNSNGCFWNFISEEHGWQEK

SEQ ID NO: 11 - Polypeptide Sequence of BoNT/F

MPVVINSFNYNDPVNDDTILYMQIPYEEKSKKYYKAFEIMRNVWIIPERNTIGTDPSDFD
PPASLENGSSAYYDPNYLTTDAEKDRYLKTTIKLFKRINSNPAGEVLLQEISYAKPYLGN
EHTPINEFHPVTRTTSVNIKSSTNVKSSIILNLLVLGAGPDIFENSSYPVRKLMDSGGVY
DPSNDGFGSINIVTFSPEYEYTFNDISGGYNSSTESFIADPAISLAHELIHALHGLYGAR

GVTYKETIKVKQAPLMIAEKPIRLEEFLTFGGQDLNIITSAMKEKIYNNLLANYEKIATR
LSRVNSAPPEYDINEYKDYFQWKYGLDKNADGSYTVNENKFNEIYKKLYSFTEIDLANKF
KVKCRNTYFIKYGFLKVPNLLDDDIYTVSEGFNIGNLAVNNRGQNIKLNPKIIDSIPDKG
LVEKIVKFCKSVIPRKGTKAPPRLCIRVNNRELFFVASESSYNENDINTPKEIDDTTNLN
NNYRNNLDEVILDYNSETIPQISNQTLNTLVQDDSYVPRYDSNGTSEIEEHNVVDLNVFF
YLHAQKVPEGETNISLTSSIDTALSEESQVYTFFSSEFINTINKPVHAALFISWINQVIR
DFTTEATQKSTFDKIADISLVVPYVGLALNIGNEVQKENFKEAFELLGAGILLEFVPELL
IPTILVFTIKSFIGSSENKNKIIKAINNSLMERETKWKEIYSWIVSNWLTRINTQFNKRK
EQMYQALQNQVDAIKTVIEYKYNNYTSDERNRLESEYNINNIREELNKKVSLAMENIERF
ITESSIFYLMKLINEAKVSKLREYDEGVKEYLLDYISEHRSILGNSVQELNDLVTSTLNN
SIPFELSSYTNDKILILYFNKLYKKIKDNSILDMRYENNKFIDISGYGSNISINGDVYIY
STNRNQFGIYSSKPSEVNIAQNNDIIYNGRYQNFSISFWVRIPKYFNKVNLNNEYTIIDC
IRNNNSGWKISLNYNKIIWTLQDTAGNNQKLVFNYTQMISISDYINKWIFVTITNNRLGN
SRIYINGNLIDEKSISNLGDIHVSDNILFKIVGCNDTRYVGIRYFKVFDTELGKTEIETL
YSDEPDPSILKDFWGNYLLYNKRYYLLNLLRTDKSITQNSNFLNINQQRGVYQKPNIFSN
TRLYTGVEVIIRKNGSTDISNTDNFVRKNDLAYINVVDRDVEYRLYADISIAKPEKIIKL
IRTSNSNNSLGQIIVMDSIGNNCTMNFQNNNGGNIGLLGFHSNNLVASSWYYNNIRKNTS
SNGCFWSFISKEHGWQEN

SEQ ID NO: 12 - Polypeptide Sequence of BoNT/G

MPVNIKNFNYNDPINNDDIIMMEPFNDPGPGTYYKAFRIIDRIWIVPERFTYGFQPDQFNASTGVFSK
DVYEYYDPTYLKTDAEKDKFLKTMIKLFNRINSKPSGQRLLDMIVDAIPYLGNASTPPDKFAANVANV
SINKKIIQPGAEDQIKGLMTNLIIFGPGPVLSDNFTDSMIMNGHSPISEGFGARMMIRFCPSCLNVFN
NVQENKDTSIFSRRAYFADPALTLMHELIHVLHGLYGIKISNLPITPNTKEFFMQHSDPVQAEELYTF
GGHDPSVISPSTDMNIYNKALQNFQDIANRLNIVSSAQGSGIDISLYKQIYKNKYDFVEDPNGKYSVD
KDKFDKLYKALMFGFTETNLAGEYGIKTRYSYFSEYLPPIKTEKLLDNTIYTQNEGFNIASKNLKTEF
NGQNKAVNKEAYEEISLEHLVIYRIAMCKPVMYKNTGKSEQCIIVNNEDLFFIANKDSFSKDLAKAET
IAYNTQNNTIENNFSIDQLILDNDLSSGIDLPNENTEPFTNFDDIDIPVYIKQSALKKIFVDGDSLFE
YLHAQTFPSNIENLQLTNSLNDALRNNNKVYTFFSTNLVEKANTVVGASLFVNWVKGVIDDFTSESTQ
KSTIDKVSDVSIIIPYIGPALNVGNETAKENFKNAFEIGGAAILMEFIPELIVPIVGFFTLESYVGNK
GHIIMTISNALKKRDQKWTDMYGLIVSQWLSTVNTQFYTIKERMYNALNNQSQAIEKIIEDQYNRYSE
EDKMNINIDFNDIDFKLNQSINLAINNIDDFINQCSISYLMNRMIPLAVKKLKDFDDNLKRDLLEYID
TNELYLLDEVNILKSKVNRHLKDSIPFDLSLYTKDTILIQVFNNYISNISSNAILSLSYRGGRLIDSS
GYGATMNVGSDVIFNDIGNGQFKLNNSENSNITAHQSKFVVYDSMFDNFSINFWVRTPKYNNNDIQTY
LQNEYTIISCIKNDSGWKVSIKGNRIIWTLIDVNAKSKSIFFEYSIKDNISDYINKWFSITITNDRLG
NANIYINGSLKKSEKILNLDRINSSNDIDFKLINCTDTTKFVWIKDFNIFGRELNATEVSSLYWIQSS
TNTLKDFWGNPLRYDTQYYLFNQGMQNIYIKYFSKASMGETAPRTNFNNAAINYQNLYLGLRFIIKKA
SNSRNINNDNIVREGDYIYLNIDNISDESYRVYVLVNSKEIQTQLFLAPINDDPTFYDVLQIKKYYEK
TTYNCQILCEKDTKTFGLFGIGKFVKDYGYVWDTYDNYFCISQWYLRRISENINKLRLGCNWQFIPVD
EGWTE

SEQ ID NO: 13 - Polypeptide Sequence of BoNT/X

MKLEINKFNYNDPIDGINVITMRPPRHSDKINKGKGPFKAFQVIKNIWIVPERYNFTNNT
NDLNIPSEPIMEADAIYNPNYLNTPSEKDEFLQGVIKVLERIKSKPEGEKLLELISSSIP
LPLVSNGALTLSDNETIAYQENNNIVSNLQANLVIYGPGPDIANNATYGLYSTPISNGEG
TLSEVSFSPFYLKPFDESYGNYRSLVNIVNKFVKREFAPDPASTLMHELVHVTHNLYGIS
NRNFYYNFDTGKIETSRQQNSLIFEELLTFGGIDSKAISSLIIKKIIETAKNNYTTLISE
RLNTVTVENDLLKYIKNKIPVQGRLGNFKLDTAEFEKKLNTILFVLNESNLAQRFSILVR
KHYLKERPIDPIYVNILDDNSYSTLEGFNISSQGSNDFQGQLLESSYFEKIESNALRAFI
KICPRNGLLYNAIYRNSKNYLNNIDLEDKKTTSKTNVSYPCSLLNGCIEVENKDLFLISN
KDSLNDINLSEEKIKPETTVFFKDKLPPQDITLSNYDFTEANSIPSISQQNILERNEELY
EPIRNSLFEIKTIYVDKLTTFHFLEAQNIDESIDSSKIRVELTDSVDEALSNPNKVYSPF
KNMSNTINSIETGITSTYIFYQWLRSIVKDFSDETGKIDVIDKSSDTLAIVPYIGPLLNI
GNDIRHGDFVGAIELAGITALLEYVPEFTIPILVGLEVIGGELAREQVEAIVNNALDKRD
QKWAEVYNITKAQWWGTIHLQINTRLAHTYKALSRQANAIKMNMEFQLANYKGNIDDKAK
IKNAISETEILLNKSVEQAMKNTEKFMIKLSNSYLTKEMIPKVQDNLKNFDLETKKTLDK

FIKEKEDILGTNLSSSLRRKVSIRLNKNIAFDINDIPFSEFDDLINQYKNEIEDYEVLNL
GAEDGKIKDLSGTTSDINIGSDIELADGRENKAIKIKGSENSTIKIAMNKYLRFSATDNF
SISFWIKHPKPTNLLNNGIEYTLVENFNQRGWKISIQDSKLIWYLRDHNNSIKIVTPDYI
AFNGWNLITITNNRSKGSIVYVNGSKIEEKDISSIWNTEVDDPIIFRLKNNRDTQAFTLL
DQFSIYRKELNQNEVVKLYNYYFNSNYIRDIWGNPLQYNKKYYLQTQDKPGKGLIREYWS
SFGYDYVILSDSKTITFPNNIRYGALYNGSKVLIKNSKKLDGLVRNKDFIQLEIDGYNMG
ISADRFNEDTNYIGTTYGTTHDLTTDFEIIQRQEKYRNYCQLKTPYNIFHKSGLMSTETS
KPTFHDYRDWVYSSAWYFQNYENLNLRKHTKTNWYFIPKDEGWDED

SEQ ID NO: 14 – Polypeptide Sequence of TeNT

MPITINNFRYSDPVNNDTIIMMEPPYCKGLDIYYKAFKITDRIWIVPERYEFGTKPEDFN
PPSSLIEGASEYYDPNYLRTDSDKDRFLQTMVKLFNRIKNNVAGEALLDKIINAIPYLGN
SYSLLDKFDTNSNSVSFNLLEQDPSGATTKSAMLTNLIIFGPGPVLNKNEVRGIVLRVDN
KNYFPCRDGFGSIMQMAFCPEYVPTFDNVIENITSLTIGKSKYFQDPALLLMHELIHVLH
GLYGMQVSSHEIIPSKQEIYMQHTYPISAEELFTFGGQDANLISIDIKNDLYEKTLNDYK
AIANKLSQVTSCNDPNIDIDSYKQIYQQKYQFDKDSNGQYIVNEDKFQILYNSIMYGFTE
IELGKKFNIKTRLSYFSMNHDPVKIPNLLDDTIYNDTEGFNIESKDLKSEYKGQNMRVNT
NAFRNVDGSGLVSKLIGLCKKIIPPTNIRENLYNRTASLTDLGGELCIKIKNEDLTFIAE
KNSFSEEPFQDEIVSYNTKNKPLNFNYSLDKIIVDYNLQSKITLPNDRTTPVTKGIPYAP
EYKSNAASTIEIHNIDDNTIYQYLYAQKSPTTLQRITMTNSVDDALINSTKIYSYFPSVI
SKVNQGAQGILFLQWVRDIIDDFTNESSQKTTIDKISDVSTIVPYIGPALNIVKQGYEGN
FIGALETTGVVLLLEYIPEITLPVIAALSIAESSTQKEKIIKTIDNFLEKRYEKWIEVYK
LVKAKWLGTVNTQFQKRSYQMYRSLEYQVDAIKKIIDYEYKIYSGPDKEQIADEINNLKN
KLEEKANKAMININIFMRESSRSFLVNQMINEAKKQLLEFDTQSKNILMQYIKANSKFIG
ITELKKLESKINKVFSTPIPFSYSKNLDCWVDNEEDIDVILKKSTILNLDINNDIISDIS
GFNSSVITYPDAQLVPGINGKAIHLVNNESSEVIVHKAMDIEYNDMFNNFTVSFWLRVPK
VSASHLEQYGTNEYSIISSMKKHSLSIGSGWSVSLKGNNLIWTLKDSAGEVRQITFRDLP
DKFNAYLANKWVFITITNDRLSSANLYINGVLMGSAEITGLGAIREDNNITLKLDRCNNN
NQYVSIDKFRIFCKALNPKEIEKLYTSYLSITFLRDFWGNPLRYDTEYYLIPVASSSKDV
QLKNITDYMYLTNAPSYTNGKLNIYYRRLYNGLKFIIKRYTPNNEIDSFVKSGDFIKLYV
SYNNNEHIVGYPKDGNAFNNLDRILRVGYNAPGIPLYKKMEAVKLRDLKTYSVQLKLYDD
KNASLGLVGTHNGQIGNDPNRDILIASNWYFNHLKDKILGCDWYFVPTDEGWTND

SEQ ID NO: 15 – C-terminal L-chain Fragment

TKSLDKGYNK

SEQ ID NO: 16 – C-terminal L-chain Fragment 2

SLDKGYNK

SEQ ID NO: 17 – Di-Chain L-Chain 1

PFVNKQFNYKDPVNGVDIAYIKIPNAGQMQPVKAFKIHNKIWVIPERDTFTNPEEGDLNPPPEAKQVPVSYYDST
YLSTDNEKDNYLKGVTKLFERIYSTDLGRMLLTSIVRGIPFWGGSTIDTELKVIDTNCINVIQPDGSYRSEELNL
VIIGPSADIIQFECKSFGHEVLNLTRNGYGSTQYIRFSPDFTFGFEESLEVDTNPLLGAGKFATDPAVTLAHELI
HAGHRLYGIAINPNRVFKVNTNAYYEMSGLEVSFEELRTFGGHDAKFIDSLQENEFRLYYYNKFKDIASTLNKAK
SIVGTTASLQYMKNVFKEKYLLSEDTSGKFSVDKLKFDKLYKMLTEIYTEDNFVKFFKVLNRKTYLNFDKAVFKI
NIVPKVNYTIYDGFNLRNTNLAANFNGQNTEINNMNFTKLKNFTGLFEFYKLLCVRGIITSK

SEQ ID NO: 18 – Di-Chain L-Chain 2

PFVNKQFNYKDPVNGVDIAYIKIPNAGQMQPVKAFKIHNKIWVIPERDTFTNPEEGDLNPPPEAKQVPVSYYDST
YLSTDNEKDNYLKGVTKLFERIYSTDLGRMLLTSIVRGIPFWGGSTIDTELKVIDTNCINVIQPDGSYRSEELNL
VIIGPSADIIQFECKSFGHEVLNLTRNGYGSTQYIRFSPDFTFGFEESLEVDTNPLLGAGKFATDPAVTLAHELI

HAGHRLYGIAINPNRVFKVNTNAYYEMSGLEVSFEELRTFGGHDAKFIDSLQENEFRLYYYNKFKDIASTLNKAK
SIVGTTASLQYMKNVFKEKYLLSEDTSGKFSVDKLKFDKLYKMLTEIYTEDNFVKFFKVLNRKTYLNFDKAVFKI
NIVPKVNYTIYDGFNLRNTNLAANFNGQNTEINNMNFTKLKNFTGLFEFYKLLCVRGIITSKTK

SEQ ID NO: 19 – Di-Chain H-Chain

ALNDLCIKVNNWDLFFSPSEDNFTNDLNKGEEITSDTNIEAAEENISLDLIQQYYLTFNFDNEPENISIENLSSD
IIGQLELMPNIERFPNGKKYELDKYTMFHYLRAQEFEHGKSRIALTNSVNEALLNPSRVYTFFSSDYVKKVNKAT
EAAMFLGWVEQLVYDFTDETSEVSTTDKIADITIIIPYIGPALNIGNMLYKDDFVGALIFSGAVILLEFIPEIAI
PVLGTFALVSYIANKVLTVQTIDNALSKRNEKWDEVYKYIVTNWLAKVNTQIDLIRKKMKEALENQAEATKAIIN
YQYNQYTEEEKNNINFNIDDLSSKLNESINKAMININKFLNQCSVSYLMNSMIPYGVKRLEDFDASLKDALLKYI
YDNRGTLIGQVDRLKDKVNNTLSTDIPFQLSKYVDNQRLLSTFTEYIKNILNNIILNLRYKDNNLIDLSGYGAKV
EVYDGVELNDKNQFKLTSSANSKIRVTQNQNIIFNSVFLDFSVSFWIRIPKYKNDGIQNYIHNEYTIINCMKNNS
GWKISIRGNRIIWTLIDINGKTKSVFFEYNIREDISEYINRWFFVTITNNLNNAKIYINGKLESNTDIKDIREVI
ANGEIIFKLDGDIDRTQFIWMKYFSIFNTELSQSNIEERYKIQSYSEYLKDFWGNPLMYNKEYYMFNAGNKNSYI
KLKKDSPVGEILTRSKYNQNSKYINYRDLYIGEKFIIRRKSNSQSINDDIVRKEDYIYLDFFNLNQEWRVYTYKY
FKKEEMKLFLAPIYDSDEFYNTIQIKEYDEQPTYSCQLLFKKDEESTDEIGLIGIHRFYESGIVFEEYKDYFCIS
KWYLKEVKRKPYNLKLGCNWQFIPKDEGWTE

EXAMPLES

EXAMPLE 1

Chimeric Clostridial Neurotoxin BoNT/AB Targets a Different Type of Neuron to BoNT/A

A study was designed to determine the subtypes of neurons intoxicated by various clostridial neurotoxins. An adult rat dorsal route ganglia (aDRG) *in vitro* model was employed. During the characterization of this model, different neuronal subtypes were found. These subtypes reflected the characterization described by Usoskin, D., A. Furlan, S. Islam, H. Abdo, P. Lonnerberg, D. Lou, J. Hjerling-Leffler, J. Haeggstrom, O. Kharchenko, P. V. Kharchenko, S. Linnarsson and P. Ernfors (2015). "Unbiased classification of sensory neuron types by large-scale single-cell RNA sequencing." Nat Neurosci 18(1): 145-153.

Materials & Methods

aDRG cultures

aDRG neurons were generated on glass coverslips. Briefly, adult rat DRG tissue was dissected from 2-3 month-old CD (Sprague Dawley) rats. Dissected tissue was digested using papain followed by dispase/collagenase and plated onto poly-D-lysine and laminin coated glass coverslips. The proliferation of glial cell types in the culture was inhibited by using anti-mitotic agents. From DIV 7, the neuronal cultures were determined to be ready for use in the assay.

Immunofluorescence

aDRG neurons were treated with 1 nM of native recombinant BoNT/A ("rBoNT/A" – SEQ ID NO: 6 [converted into a di-chain form]) or a BoNT/AB chimera ("mrBoNT/AB" – SEQ ID NO: 1 [converted into a di-chain form]) at DIV7-8 for 24 hours. Control samples were left untreated. After treatment, neurons were washed twice in culture media and fixed in 4% PFA for 30 minutes. Neurons were then permeabilized using 0.1% Triton X-100 in 1X PBS for 15 minutes prior to blocking in 10% donkey serum for 30 minutes. Primary antibody and secondary antibody staining was performed as shown in the table below.

Table 1. Primary and secondary antibody staining.

Ab anti-	Company	Cat. N.	Specie	Mono/Poly	1st/2nd	Dilution
DEAN10	Ipsen	N/A	Rabbit	Poly	1st	1:400
C. SNAP25	Origene	AM31671SU-N	Mouse	Mono	1st	1:100
NF200(N52)	Sigma	N0142-.2ML	Mouse	Mono	1st	1:200
NF200	Abcam	ab8135	Rabbit	Poly	1st	1:200
CGRP	Stratech	311-CGRP-PP5	Mouse	Mono	1st	1:150
CGRP	Sigma	C8198	Rabbit	Poly	1st	1:150
P2X3	Stratech	GP10108-NEU	Guinea Pig	Poly	1st	1:150
TrkA	Fisher	15710644	Goat	Poly	1st	1:100
Mouse AlexaFluor594	Fischer	15960296	Donkey	Poly	2nd	5X 1st
Mouse AlexaFluor488	Fischer	16051752	Donkey	Poly	2nd	5X 1st
Rabbit AlexaFluor594	Fischer	15910767	Donkey	Poly	2nd	5X 1st
Rabbit AlexaFluor488	Thermo	A32790	Donkey	Poly	2nd	5X 1st
Guinea Pig AlexaFluor594	Thermo	A-11076	Goat	Poly	2nd	5X 1st
Goat AlexaFluor594	Fischer	16331974	Donkey	Poly	2nd	5X 1st

Coverslips were then mounted on glass slides using ProLong Diamond mounting media containing 1 ug/ml DAPI for nuclear staining.

Imaging and co-localization analysis

Images were taken using the LSM800 Zeiss confocal microscope with a magnification of 40X. A minimum of 3 images for each specific sample and antibody combination were taken and 3 biological replicates were performed. The intoxicated neurons were determined by using two different antibodies which detect the cleaved isoform of SNAP25. The aDRG subtype markers used are well characterized for the aDRG cultures. These are NF200 for the NF category, CGRP for PEP neurons, P2X3 for NP neurons, TrkA for both the NP and PEP neurons. Co-localization was performed by merging the 2 colours of interest (usually green colour (488) for cleaved SNAP25 and red colour (594) for the specific neuronal marker).

Results

All the images presented in Figure 1-3 show one representative example (at least 3 images were taken for each biological experiment) of the single color image for both the cleaved SNAP25 signal and the specific marker and the colored merge of the two channels with the addition of the nuclear staining in blue. Arrows, when present, indicate the areas of interest for the specific marker/cleaved SNAP25 co-localizations. The untreated control of Figure 1 showed a small amount of background given by the antibodies used to detect cleaved SNAP25. This background staining was taken into consideration when analyzing the treated samples.

Native recombinant BoNT/A targets Aβ fibers

Figure 2 shows the results obtained when aDRG neurons were contacted with rBoNT/A (SEQ ID NO: 6 [converted into a di-chain form]). In particular, clear co-localization between the Aβ fibers (NF200) and cleaved SNAP25 was evident. The expression of cleaved SNAP25 and NF200 was high in all images analyzed. For the Aδ and C fibers (NP, PEP) no co-localization was seen. Neurons expressing the specific markers (CGRP, P2X3, TrkA) did not express high levels of cleaved SNAP25. In the images, instances in which cleaved SNAP25 is expressed can be seen, however, the amount of signal is not higher than the background signal shown in Figure 1 and is clearly lower than the signal given by neurons expressing high levels of cleaved SNAP25. In conclusion, it was found that the intoxication of rBoNT/A in aDRG neurons occurs in Aβ fibers (NF200).

Chimeric BoNT/AB targets Aδ fibers and C fibers

Figure 3 shows the results obtained when aDRG neurons were contacted with mrBoNT/AB (SEQ ID NO: 1 [converted into a di-chain form]). In particular, no co-localization between the Aβ fibers (NF200) and cleaved SNAP25. The portions of the neurons highlighted with the arrows show the high expression of NF200 without cleaved SNAP25 presence. For the Aδ and C fibers (NP, PEP) co-localization was seen. In particular, strong co-localization was seen in CGRP and TrkA expressing neurons. In conclusion, the intoxication of mrBoNT/AB in aDRG neurons occurs in Aδ (PEP) and C fibers (NP). This is the opposite of that seen for rBoNT/A (Figure 2).

Conclusions

Immunofluorescence studies on aDRG primary neurons were able to determine the subtypes of neurons intoxicated by rBoNT/A and mrBoNT/AB. The study showed a clear difference between the subtype of neurons intoxicated by mrBoNT/AB when compared to rBoNT/A.

rBoNT/A cleaves SNAP25 in Aβ fibers, whereas mrBoNT/AB cleaves SNAP25 in Aδ and C fibers. These last two subpopulations represent particularly important pain targets given their role in nociception. The table below summarizes these differences.

Table 2. Schematic summary of the aDRG fiber type targeted by the different toxins.

Molecule	Fiber Type
rBoNT/A	Aβ
mrBoNT/AB	Aδ
mrBoNT/AB	C

In conclusion, the data show that the BoNT/AB chimera targets cells involved in nociception and thus is likely to exhibit analgesic effects, such as improved analgesic effects when compared to rBoNT/A.

EXAMPLE 2

Chimeric Clostridial Neurotoxin BoNT/AB is Effective at Inhibiting Calcitonin Gene Related Peptide (CGRP) Release from Aδ and C Fibers

Following on from the findings presented in Example 1, experiments were carried out to validate the BoNT/AB chimera's role as an analgesic by determining whether its targeting to Aδ and C fibers was able to inhibit Calcitonin Gene Related Peptide (CGRP) release. CGRP is a neuropeptide found primarily in a subset of C and Aδ sensory fibres arising from dorsal root and trigeminal ganglia. Recent studies have implicated CGRP in the development of peripheral sensitisation and enhanced pain, neuroinflammation, and neuropathic pain. In support of this, blockade of CGRP function has been shown to alleviate migraine.

Materials & Methods

aDRG cultures

aDRG neurons were plated on 96-well half-volume plates following a slightly modified form of the procedure presented in Example 1.

CGRP release assay

aDRG neurons were treated at DIV7-14 with Log_{10} dilutions of clostridial neurotoxin from 10 nM-1 pM for 24 hours. Toxins used: rBoNT/A (SEQ ID NO: 6 [converted into a di-chain form]) and mrBoNT/AB (SEQ ID NO: 1 [converted into a di-chain form]). Control samples were left untreated. After treatment, neurons were washed twice in HBS (110 mM sodium chloride, 3 mM potassium chloride, 2 mM calcium chloride, 1 mM magnesium chloride, 10mM HEPES, 20 mM glucose, pH 7.2) and placed back in the incubator at 37°C for 1 hour. The plate of cells was then transferred to a prewarmed heat block and was washed with HBS one more time. The HBS was removed and replaced with 50 µL HBS + 0.03% BSA. After 5 minutes, the HBS/BSA superfusate was removed and stored in a separate plate. Immediately after the superfusates were removed, 50 µL of the stimulation media (100 nM Capsaicin HBS + 0.03% BSA or 65 nM potassium chloride (KCl) HBS + 0.03% BSA) was added to appropriate wells. After 5 minutes, the superfusate was collected. After collection, the superfusates were either immediately used in the CGRP EIA assay, or stored at -20°C.

CGRP Enzyme Immunoassay (EIA) assay

The CGRP immunoassay reagents were purchased as part of a commercially-available kit (Bertin Pharma, France, #A05482) and prepared as per the manufacturer's instructions. The plate was washed 5 times with Wash Buffer before addition of 40 µL standards and samples. 100 µL CGRP tracer (prepared as follows: stock vial (#A10482) diluted in 10ml of EIA buffer (vial of stock EIA buffer #A07000 reconstituted in 50 ml distilled water)) was then added to each well. The plate was then covered with an adhesive strip and incubated between 16 and 20 hours at 4°C. Following incubation, the plate contents were discarded, and the wells were washed with Wash Buffer (prepared as follows: 1 ml wash buffer stock (#A17000), diluted in 400 ml distilled water and with addition of 200 µl Tween-20 (#A12000)) 3 times before a 2-minute shaking step in wash buffer, followed by 3 further washes. After the removal of wash buffer, 200 µL Ellman's reagent (prepared as follows: stock Ellman's reagent vial #A09000 diluted in 1 ml stock wash buffer #A17000 and 49 ml distilled water) was added per well. The plate was then covered with foil and incubated in the dark for 4 hours at room temperature. Finally, the plate was read at 410 nm using a Clariostar plate reader. The

standards were plotted on an X-Y graph in Prism V8 (Graphpad) and the sample values were interpolated from the standard curve.

Results

Figure 4 shows that mrBoNT/AB was much more effective at inhibiting CGRP release than rBoNT/A. The average pIC_{50} values for rBoNT/A and mrBoNT/AB were respectively: 6.87 ±0.44 (7.34 μM for rBoNT/A) and 9.99 ±0.16 (9.73 nM for mrBoNT/AB) (mean ±SEM).

In confirmation of the results shown in Figure 4, comparison of maximal inhibition showed that there was a significant difference between the CGRP release inhibition elicited by 1 nM mrBoNT/AB when compared to 1 nM rBoNT/A. Specifically, mrBoNT/AB was statistically-significantly better at inhibiting CGRP release than rBoNT/A (*see* Figure 5). This is consistent with the surprising finding that mrBoNT/AB specifically targeted Aδ- and C-type fibres (*see* Example 1).

Conclusions

The utilisation of the rat aDRG CGRP release model allowed a functional comparison of the different clostridial neurotoxins in an *in vitro* pain model. Consistent with the fiber subtype binding specificities of the toxin, mrBoNT/AB was clearly more potent at inhibiting CGRP release from aDRGs than rBoNT/A. Thus, chimeric clostridial neurotoxins having a botulinum neurotoxin A (BoNT/A) light-chain and translocation domain (H_N domain), and a BoNT/B receptor binding domain (H_C domain) are improved analgesics.

EXAMPLE 3

Treatment of a Subject with Chronic Migraine Pain

Joe, aged 43, is diagnosed by his GP with chronic migraine and is treated with a chimeric clostridial neurotoxin of the invention comprising SEQ ID NO: 1 (converted into a di-chain form). The chimeric clostridial neurotoxin is administered by way of a unit dose of 5,000 pg, where a single unit dose is administered via intramuscular injection to each of the procerus, both corrugator supercilia muscles, both masseter muscles, both temporalis muscles, both occipitalis muscles, and both trapezius muscles (i.e. 11x unit doses are administered total). Joe's pain is significantly reduced with no significant pain 9 months later when he receives his next treatment.

EXAMPLE 4

Pre-Clinical Testing of Chimeric Clostridial Neurotoxin BoNT/AB (SEQ ID NO: 1)

BoNT/AB chimera SEQ ID NO: 1 (converted into a di-chain form) was tested in a mouse LD_{50} assay yielding a result of 1.202 ng/kg. 1 Unit of SEQ ID NO: 1 (converted into a di-chain form) therefore corresponds to 24.04 pg in this assay.

EXAMPLE 5

Calculation of a Unit Dose of Chimeric Clostridial Neurotoxin BoNT/AB (SEQ ID NO: 1) for Treating Migraine

In view of pre-clinical pharmacology data, a suitable unit dose range (UD) for administration of chimeric clostridial neurotoxin BoNT/AB in humans has been calculated.

A DAS ED_{50} of 13 pg/kg was calculated for SEQ ID NO: 1 (converted into a di-chain form). ED_{50} is considered as a minimal pharmacologically active dose, which is approximately 300-fold lower than the no observed adverse effect level (NOAEL) of 4 ng/kg in the same animal species. An ED_{50} of 13 pg/kg of SEQ ID NO: 1 (converted into a di-chain form) in rats corresponds to a 0.8 ng dose for a human of 60 kg body weight.

Thus, the lower limit of a unit dose of 1,000 pg was selected. An upper limit of the unit dose of 5,000 pg was selected, which is lower than the NOAEL of 4 ng/kg from both nonclinical safety species (rat and monkey) converted into human dose for 60 kg body weight.

In view of the improved safety profile the maximum total dose for the treatment of migraine was set at 175,000 pg, which is derived from the NOAEL of 4 ng/kg from both nonclinical safety species (rat and monkey) converted into human dose for 60 kg body weight.

Advantageously, chimeric clostridial neurotoxin BoNT/AB (SEQ ID NO: 1 [converted into a di-chain form]) can be injected to a greater number of muscles in the treatment migraine before reaching the maximum dose. This is a significant and advantageous finding leading to improved treatment of migraine while providing clinicians with a greater range of treatment options.

EXAMPLE 6

Safety & Efficacy of Chimeric Clostridial Neurotoxin BoNT/AB (SEQ ID NO: 1) in Humans

SEQ ID NO: 1 (converted into a di-chain form) was administered to human subjects by way of a single unit dose. 5 cohorts were administered different (increasing) amounts of mrBoNT/AB (SEQ ID NO: 1 [converted into a di-chain form]). Cohort 1 was administered 2x 1,000 pg unit doses of mrBoNT/AB (i.e. 2,000 pg maximum), while cohort 5 was administered 2x 16,000 pg unit doses of mrBoNT/AB (i.e. 32,000 pg maximum).

Results showed that all unit doses of mrBoNT/AB tested, (i.e. up to 16,000 pg unit doses), were effective at muscle paralysis, safely tolerated, and no adverse effects were observed, despite the exceptionally high dosage per muscle. This shows that mrBoNT/AB does not diffuse away from the injection site and highlights the exceptional safety profile of mrBoNT/AB (SEQ ID NO: 1 [converted into a di-chain form]).

EXAMPLE 7

Safety & Efficacy of Chimeric Clostridial Neurotoxin BoNT/AB (SEQ ID NO: 1) in Humans

SEQ ID NO: 1 (converted into a di-chain form) was administered to human subjects by way of a single unit dose. 7 cohorts were administered different (increasing) amounts of mrBoNT/AB (SEQ ID NO: 1 [converted into a di-chain form]) into facial muscles. Cohort 1 was administered 5x 20 pg unit doses of mrBoNT/AB (i.e. 100 pg maximum), while cohort 7 was administered 5x 1,500 pg unit doses of mrBoNT/AB (i.e. 7,500 pg maximum).

Results showed that all unit doses of mrBoNT/AB tested, (i.e. up to 1,500 pg unit doses), were effective at muscle paralysis, safely tolerated, and no adverse effects were observed, despite the high dosage per muscle. This shows that mrBoNT/AB does not diffuse away from the injection site and highlights the exceptional safety profile of mrBoNT/AB (SEQ ID NO: 1 [converted into a di-chain form]).

EXAMPLE 8

Treatment of a Subject with Chronic Migraine Pain via Intramuscular Injection

Derek, aged 25, is diagnosed by his GP with chronic migraine and is treated with a chimeric clostridial neurotoxin of the invention comprising SEQ ID NO: 1 (converted into a di-chain form). The chimeric clostridial neurotoxin comprising SEQ ID NO: 1 (converted into a di-

chain form) is administered by way of a unit dose of 2,500 pg and is administered via intramuscular injection as follows:

- 2 unit doses to a frontalis muscle on the left side of Derek's face and 2 unit doses to a frontalis muscle on the right side of Derek's face;
- 1 unit dose to a procerus muscle;
- 1 unit dose to a corrugator muscle on the left side of Derek's face and 1 unit dose to a corrugator muscle on the right side of Derek's face;
- 4 unit doses to a temporalis muscle on the left side of Derek's head and 4 unit doses to a temporalis muscle on the right side of Derek's head;
- 3 unit doses to an occipitalis muscle on the left side of Derek's neck/head and 3 unit doses to an occipitalis muscle on the right side of Derek's neck/head;
- 3 unit doses to a trapezius muscle at on the left side of Derek's neck and 3 unit doses to a trapezius muscle on the right side of Derek's neck; and
- 4 unit doses to a cervical paraspinal group muscle on the left side of Derek's neck and 4 unit doses to a cervical paraspinal group muscle on the right side of Derek's neck.

In total 35 unit doses (i.e. 87,500 pg) of chimeric clostridial neurotoxin comprising SEQ ID NO: 1 (converted into a di-chain form) are administered. Derek's pain is significantly reduced with no significant pain 9 months later when he receives his next treatment. The treatment is safely tolerated and no adverse events are observed.

EXAMPLE 9

Treatment of a Subject with Episodic Migraine Pain via Intradermal Injection

Tessa, aged 51, is diagnosed by her GP with episodic migraine and is treated with a chimeric clostridial neurotoxin of the invention comprising SEQ ID NO: 1 (converted into a di-chain form). The chimeric clostridial neurotoxin comprising SEQ ID NO: 1 (converted into a di-chain form) is administered by way of a unit dose of 5,000 pg and is administered via intradermal injection as follows:

- 1 unit dose in the region of a supraorbital nerve at a first side of Tessa's face and/or 1 unit dose in the region of a supraorbital nerve at a second side of Tessa's face;
- 1 unit dose in the region of a supratrochlear nerve at a first side of Tessa's face and/or 1 unit dose in the region of a supratrochlear nerve at a second side of Tessa's face;

- 1 unit dose in the region of an intratrochlear nerve at a first side of Tessa's face and/or 1 unit dose in the region of an intratrochlear nerve at a second side of Tessa's face;
- 1 unit dose in the region of a zygomaticotemporal nerve at a first side of Tessa's face and/or 1 unit dose in the region of a zygomaticotemporal nerve at a second side of Tessa's face;
- 1 unit dose in the region of a zygomaticofacial nerve at a first side of Tessa's face and/or 1 unit dose in the region of a zygomaticofacial nerve at a second side of Tessa's face;
- 2 unit doses in the region of an auriculotemporal nerve at a first side of Tessa's face and/or 2 unit doses in the region of an auriculotemporal nerve at a second side of Tessa's face;
- 2 unit doses in the region of a greater occipital nerve at a first side of Tessa's neck and/or 2 unit doses in the region of a greater occipital nerve at a second side of Tessa's neck; and/or
- 1 unit dose in the region of a lesser occipital nerve at a first side of Tessa's neck and/or 1 unit dose in the region of a lesser occipital nerve at a second side of Tessa's neck.

In total 20 unit doses (i.e. 100,000 pg) of chimeric clostridial neurotoxin comprising SEQ ID NO: 1 (converted into a di-chain form) are administered. Tessa's pain is significantly reduced with no significant pain 9 months later when she receives her next treatment. The treatment is safely tolerated and no adverse events are observed.

EXAMPLE 10

Chimeric Clostridial Neurotoxin BoNT/AB is More Effective at Inhibiting Calcitonin Gene Related Peptide (CGRP) Release from Neurons of the Trigeminal Ganglion than BoNT/A

The effect and potency of mrBoNT/AB was assessed in rat primary neurons prepared from the trigeminal ganglion, the structure from where the three sensory branches of the trigeminal nerve emanate. The trigeminal ganglion is a pivotal region enriched in neurons (TGNs) functionally involved in the pathophysiology of migraine. Briefly, primary rat TGN cultures were generated from 5 to 8 weeks old rats (*see* Sidders *et al* (2018), J Mol Biol., 14;430(18 Pt A):3005-3015, for example). After incubating the cells with toxin concentrations (either rBoNT/A [SEQ ID NO: 6 converted into a di-chain form] or mrBoNT/AB [SEQ ID NO: 1 converted into a di-chain form]) from 1pM to 100nM for 24 hours, cultures were stimulated

with 65mM KCl for 10 min to induce the release of CGRP, the main neurotransmitter believed to be responsible for the initiation and propagation of migraine. Following measurement of CGRP release by ELISA (measured as described in Example 2; n=3, in quadriplicates), TGNs were lysed for western blot analysis of SNAP25.

As depicted in Figure 7, mrBoNT/AB (Figure 7B) was more potent than rBoNT/A at reducing CGRP release (Figure 7A) and also at cleaving SNAP25 (Figure 8).

These data are further evidence of the improved efficacy of mrBoNT/AB (when compared to rBoNT/A) in targeting and inhibiting release of pain mediators (e.g. CGRP) from neurons relevant in the pathophysiology of migraine. Thus, it is credible that administration of chimeric clostridial neurotoxins having a botulinum neurotoxin A (BoNT/A) light-chain and translocation domain (H_N domain), and a BoNT/B receptor binding domain (H_C domain) constitute an improved treatment for pain generally, and migraine in particular.

EXAMPLE 11

Chimeric Clostridial Neurotoxin BoNT/AB Demonstrates Higher Potency in Human Sensory Neurons Compared to BoNT/A

mrBoNT/AB was compared to rBoNT/A in a pain-related human setting, i.e. sensory neurons derived from human induced pluripotent stem cells (hiPSCs) (methodology associated with the cell culture was as per the manufacturer's instructions: https://www.anatomic.tech/, *see* also Walsh *et al* (2020), Stem Cells, 38, 11, 1400–1408, for example). Briefly, sensory neurons derived from hiPSCs were cultured for 14 days and subsequently incubated with 3fM to 1nM rBoNT/A (SEQ ID NO: 6 converted into a di-chain form) or mrBoNT/AB (SEQ ID NO: 1 converted into a di-chain form) for 24h, before they were lysed for SNAP25 cleavage assessment by western blot (n=3, in triplicates). Figure 9 illustrates the higher potency of mrBoNT/AB compared to rBoNT/A in cleaving SNAP25 in such human cells. Again, this further supports that administration of chimeric clostridial neurotoxins having a botulinum neurotoxin A (BoNT/A) light-chain and translocation domain (H_N domain), and a BoNT/B receptor binding domain (H_C domain) constitutes an improved treatment for human pain generally, and human migraine in particular.

EXAMPLE 12

Chimeric Clostridial Neurotoxin BoNT/AB Cleaves SNAP25 in Neurons Relevant for Pain Transmission *in vivo*

Materials & Methods

Naïve female Sprague Dawley rats were used for the study (180-220g at treatment initiation, Janvier Labs, France). Animals were kept on a reversed 12-h light/dark cycle (lights on from 18:00 to 06:00) and maintained in an enriched environment under a constant temperature (22 ± 2°C) and humidity (55 ± 5%) with food and water available *ad libitum*. Animals were acclimatized for at least 7 days prior to experimentation. The study was performed in full compliance with the ARRIVE guidelines, European Communities Council Directive 2010/63/EU and French National Committee decree 87/848.

Animals were administered vehicle (saline, 2 rats/group) or mrBoNT/AB (SEQ ID NO: 1 converted into a di-chain form) (300pg/kg, 6 rats/group) via intramuscular (IM) or intradermal (ID) injection or Botox (6 rats/group) using IM injection (for the trigeminal ganglia analysis). IM administrations were performed by dividing the total dose into 4 muscles of the head and neck (right and left temporalis, right and left occipitalis, 10µL of injection volume each). ID administrations were performed by dividing the total dose into the dermis of the skin located above the 4 aforementioned muscles (10µL of injection volume each). 10 days after treatment administration, animals were euthanized and the following tissues were harvested: the trigeminal ganglia, the brainstem comprising spinal trigeminal nuclei and the cervical spinal cord. Tissues were then fixed in isotonic buffered formalin 10% solution (VWR, France) for 48h, embedded in paraffin blocks and histologic slides were prepared.

To evaluate the biological effect of both toxins in the tissues, an immunohistochemical staining of the cleaved form of SNAP25 (c-SNAP25) was performed. After a heat-induced epitope retrieval step, endogenous peroxidases were blocked for 10 min in a 3% H_2O_2 solution in a TBS buffer. The sections were incubated with a non-commercial primary rabbit polyclonal antibody (EF14007, Ipsen Innovation, France) which is specific for the cleaved form of SNAP25 by BoNT/A only. Sections were then incubated with a biotinylated secondary antibody for 30 min (anti-rabbit IgG, Vector Laboratories, USA), followed by a 30-min incubation with an amplification system (avidin-biotin) coupled to horseradish peroxidase (Vector Laboratories, USA). Finally, sections were incubated for 5 min with a solution of 0.02% diaminobenzidine (DAKO, USA), counterstaining was done using haematoxylin (DAKO, USA), and the slides were visualized under the light microscope. For trigeminal ganglia samples, the quantification of the amount of c-SNAP25 was determined using the

following 5-point scale scoring system: 0 (no staining), 1 (minimal staining intensity and density), 2 (moderate staining intensity and density), 3 (strong staining intensity and density) and 4 (very strong staining intensity and density). In the spinal cord, the intensity and density of c-SNAP25 positive nerve endings was graded as follow: 0 (no staining), 1 (minimal), 2 (mild), 3 (moderate), 4 (marked) on the 5 most intensely stained spinal cord sections, a cumulative score (0 to 20) was then calculated for each animal. For brainstem samples, SNAP25 cleavage staining was quantified using a dedicated image analysis method that measures the proportion of nerve fibers stained for c-SNAP25.

Results

As expected, no specific c-SNAP25 staining was observed in tissues from any vehicle-treated animal. Figure 10 shows that administration of mrBoNT/AB via the IM or ID route both resulted in SNAP25 cleavage at the spinal trigeminal sensory nuclei of the brainstem, while cleavage in the trigeminal motor nuclei of the brainstem was lower when administered via the ID route. A lower amount of SNAP25 cleavage in the motor nuclei may result in reduced off-target effects, such as motor effects associated with treatment.

Figure 11 shows that administration of mrBoNT/AB via the IM or ID routes resulted in SNAP25 cleavage in the cervical spinal cord, specifically in the dorsal horn and ventral horn.

Figure 12 shows that administration of mrBoNT/AB via the IM or ID routes resulted in SNAP25 cleavage in axons of the trigeminal ganglia. Surprisingly, Botox did not cleave SNAP25 in the axons of the trigeminal ganglia, thereby supporting a role for an improved effect of mrBoNT/AB in the treatment of pain, such as in the treatment of migraine.

EXAMPLE 13

SNAP25 and Chimeric Clostridial Neurotoxin BoNT/AB Receptors are Present in Various Human Tissues Relevant for Pain Transmission

Materials and Methods

Human tissues were purchased from ProteoGenex (USA), Cureline (USA) and Clinisciences (France) and assessed for the presence of SNAP25, SytII and SytI. The following tissues were assessed, with n = 3 to 5 donors per tissue:

- Pons and medulla oblongata (includes parts of the brainstem and contains central trigeminal motor and sensory nuclei); and
- Cervical spinal cord (includes the dorsal horn).

For quantification of protein expression, the same immunohistochemical technique as described in Example 12 was used. Tissues were stained with antibodies against SNAP25 (using antibody 111 008), SytII (using antibody 105 123) and SytI (using antibody ab126253). Tissues were also stained with an antibody against beta-3-tubulin (using antibody G7121), a pan-neuronal marker, as a control.

Staining was quantified under a light microscope and intensity of staining was graded using a score of 0 to 4, with a score of 0 = no staining; 1 = very weak / very rare / not frequent staining; 2 = moderate staining; 3 = strong / frequent staining; and 4 = very strong / intense / extremely frequent staining.

Results

The results are shown in Table 3. Beta-3-tubulin control staining, as well as SNAP25 staining, was graded as maximal (4) in the pons, medulla oblongata, and cervical spinal cord. Meanwhile, SytII and SytI were found to be strongly expressed in the pons, medulla oblongata and dorsal horn layers 1, 3 and 4. SytI was strongly expressed in the dorsal horn layer 2 while SytII exhibited moderate staining in this structure.

Together, these results show that SNAP25, SytII and SytI are highly expressed in several human tissues relevant for pain transmission. Thus, the appropriate SytII and SytI receptors are present for mrBoNT/AB to bind, be internalised, and cleave SNAP25 in neurons present in these tissues, for example following neuronal (e.g. retrograde) transport of mrBoNT/AB from distal sites of administration, thereby inhibiting pain transmission.

Table 3: Intensity of staining of SNAP25, SytII, and SytI in various human tissues relevant for pain transmission

Tissue	Structures	Beta-3-tubulin	SNAP25	SytII	SytI
Pons	Includes the trigeminal motor and main sensory nuclei	4	4	3	3
Medulla oblongata	Spinal trigeminal sensory nuclei	4	4	3	4
Cervical spinal cord	Dorsal horn – layers 1, 3, 4	4	4	3	3
	Dorsal horn – layer 2	4	4	2	4

EXAMPLE 14

Treatment of a Patient with Migraine

Timothy 33 is diagnosed by his GP with migraine. He is treated by way of a unit dose (UD) of 2,500 pg of SEQ ID NO: 1 (converted into a di-chain form) administered as follows:

Muscle Injected	Total no. injection sites	Dose per injection site	Dose per session
Frontalis	4 (2 per side)	2.5 ng	10 ng
Corrugator	2 (1 per side)	2.5 ng	5 ng
Nasalis	2 (1 per side)	2.5 ng	5 ng
Orbicularis oculi	2 (1 per side)	2.5 ng	5 ng
Temporalis	8 (4 per side)	2.5 ng	20 ng
Occipitalis	6 (3 per side)	2.5 ng	15 ng
Trapezius	4 (2 per side)	2.5 ng	10 ng
Total	**28**		**70 ng**

He receives a total dose of 70,000 pg of SEQ ID NO: 1 (converted into a di-chain form). The treatment is successful and his symptoms are alleviated. He does not require treatment for greater than 9 months.

EXAMPLE 15

Treatment of a Patient with Migraine

Joseph 31 is diagnosed by his GP with migraine. He is treated by way of a unit dose (UD) of 4,000 pg of SEQ ID NO: 1 (converted into a di-chain form) administered as follows:

Muscle Injected	Total no. injection sites	Dose per injection site	Dose per session
Frontalis	4 (2 per side)	4ng	16 ng
Corrugator	2 (1 per side)	4ng	8 ng
Nasalis	2 (1 per side)	4ng	8 ng
Orbicularis oculi	2 (1 per side)	4ng	8 ng
Temporalis	8 (4 per side)	4ng	32 ng
Occipitalis	6 (3 per side)	4ng	24 ng
Trapezius	4 (2 per side)	4ng	16 ng
Total	**28**		**112 ng**

He receives a total dose of 112,000 pg of SEQ ID NO: 1 (converted into a di-chain form). The treatment is successful and his symptoms are alleviated. He does not require treatment for greater than 9 months.

All publications mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the described methods and system of the present invention will be apparent to those skilled in the art without departing from the scope and spirit of the present invention. Although the present invention has been described in

connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in biochemistry and biotechnology or related fields are intended to be within the scope of the following claims.

CLAIMS

1. A chimeric clostridial neurotoxin for use in treating pain, wherein the chimeric clostridial neurotoxin comprises a botulinum neurotoxin A (BoNT/A) light-chain and translocation domain (H_N domain), and a BoNT/B receptor binding domain (H_C domain).

2. A method for treating pain, the method comprising administering to a subject a chimeric clostridial neurotoxin, wherein the chimeric clostridial neurotoxin comprises a botulinum neurotoxin A (BoNT/A) light-chain and translocation domain (H_N domain), and a BoNT/B receptor binding domain (H_C domain).

3. Use of a chimeric clostridial neurotoxin in the manufacture of a medicament for treating pain, wherein the chimeric clostridial neurotoxin comprises a botulinum neurotoxin A (BoNT/A) light-chain and translocation domain (H_N domain), and a BoNT/B receptor binding domain (H_C domain).

4. The chimeric clostridial neurotoxin for use, method, or use according to any one of claims 1-3, wherein the chimeric clostridial neurotoxin treats pain by inhibiting release of a pain mediator from a neuron comprising an Aδ nerve fiber or a C nerve fiber, wherein the chimeric clostridial neurotoxin binds to the neuron comprising the Aδ nerve fiber or the C nerve fiber, respectively.

5. The chimeric clostridial neurotoxin for use, method, or use according to any one of the preceding claims, wherein the chimeric clostridial neurotoxin treats pain by inhibiting secretion from a neuron of the central nervous system, preferably by inhibiting secretion of a mediator, more preferably a pain mediator from a neuron of the central nervous system.

6. A chimeric clostridial neurotoxin for use in treating migraine (preferably migraine pain), wherein the chimeric clostridial neurotoxin comprises a botulinum neurotoxin A (BoNT/A) light-chain and translocation domain (H_N domain), and a BoNT/B receptor binding domain (H_C domain).

7. A method for treating migraine (preferably migraine pain), the method comprising administering to a subject a chimeric clostridial neurotoxin, wherein the chimeric clostridial neurotoxin comprises a botulinum neurotoxin A (BoNT/A) light-chain and translocation domain (H_N domain), and a BoNT/B receptor binding domain (H_C domain).

8. Use of a chimeric clostridial neurotoxin in the manufacture of a medicament for treating migraine (preferably migraine pain), wherein the chimeric clostridial neurotoxin comprises a botulinum neurotoxin A (BoNT/A) light-chain and translocation domain (H_N domain), and a BoNT/B receptor binding domain (H_C domain).

9. The chimeric clostridial neurotoxin for use, method, or use according to any one of claims 6-8, wherein the chimeric clostridial neurotoxin treats migraine by inhibiting release of a pain mediator from a neuron comprising an Aδ nerve fiber or a C nerve fiber, wherein the chimeric clostridial neurotoxin binds to the neuron comprising the Aδ nerve fiber or the C nerve fiber, respectively.

10. The chimeric clostridial neurotoxin for use, method, or use according to any one of claims 6-9, wherein the chimeric clostridial neurotoxin treats migraine by inhibiting secretion from a neuron of the central nervous system, preferably by inhibiting secretion of a mediator, more preferably a pain mediator from a neuron of the central nervous system.

11. A chimeric clostridial neurotoxin for use in a method for treating a disorder of a subject for a longer duration and/or with a greater efficacy than that of a subject treated with BoNT/A, the method comprising administering a chimeric clostridial neurotoxin to the subject, wherein the chimeric clostridial neurotoxin comprises a botulinum neurotoxin A (BoNT/A) light-chain and translocation domain (H_N domain), and a BoNT/B receptor binding domain (H_C domain).

12. A method for treating a disorder of a subject for a longer duration and/or with a greater efficacy than that of a subject treated with BoNT/A, the method comprising administering a chimeric clostridial neurotoxin to the subject, wherein the chimeric clostridial neurotoxin comprises a botulinum neurotoxin A (BoNT/A) light-chain and translocation domain (H_N domain), and a BoNT/B receptor binding domain (H_C domain).

13. Use of a chimeric clostridial neurotoxin in the manufacture of a medicament for treating a disorder of a subject for a longer duration and/or with a greater efficacy than that of a subject treated with BoNT/A, wherein the chimeric clostridial neurotoxin comprises a botulinum neurotoxin A (BoNT/A) light-chain and translocation domain (H_N domain), and a BoNT/B receptor binding domain (H_C domain).

14. A chimeric clostridial neurotoxin for use in a method for reducing pain of a subject by a greater amount than that of a subject treated with BoNT/A, the method comprising administering a chimeric clostridial neurotoxin to the subject, wherein the chimeric clostridial neurotoxin comprises a botulinum neurotoxin A (BoNT/A) light-chain and translocation domain (H_N domain), and a BoNT/B receptor binding domain (H_C domain).

15. A method for reducing pain of a subject by a greater amount than that of a subject treated with BoNT/A, the method comprising administering a chimeric clostridial neurotoxin to the subject, wherein the chimeric clostridial neurotoxin comprises a botulinum neurotoxin A (BoNT/A) light-chain and translocation domain (H_N domain), and a BoNT/B receptor binding domain (H_C domain).

16. Use of a chimeric clostridial neurotoxin in the manufacture of a medicament for reducing pain of a subject by a greater amount than that of a subject treated with BoNT/A, wherein the chimeric clostridial neurotoxin comprises a botulinum neurotoxin A (BoNT/A) light-chain and translocation domain (H_N domain), and a BoNT/B receptor binding domain (H_C domain).

17. A chimeric clostridial neurotoxin for use in a method for reducing an amount of a pain mediator in a biofluid and/or in a brain of a subject by a greater amount than the amount of the same pain mediator in the same biofluid and/or in the brain of a subject treated with BoNT/A, the method comprising administering a chimeric clostridial neurotoxin to the subject, wherein the chimeric clostridial neurotoxin comprises a botulinum neurotoxin A (BoNT/A) light-chain and translocation domain (H_N domain), and a BoNT/B receptor binding domain (H_C domain).

18. A method for reducing an amount of a pain mediator in a biofluid and/or in a brain of a subject by a greater amount than the amount of the same pain mediator in the same biofluid and/or in the brain of a subject treated with BoNT/A, the method comprising administering a chimeric clostridial neurotoxin to the subject, wherein the chimeric clostridial neurotoxin comprises a botulinum neurotoxin A (BoNT/A) light-chain and translocation domain (H_N domain), and a BoNT/B receptor binding domain (H_C domain).

19. Use of a chimeric clostridial neurotoxin in the manufacture of a medicament for reducing an amount of a pain mediator in a biofluid and/or in a brain of a subject by a greater amount than the amount of the same pain mediator in the same biofluid and/or in a brain of a subject treated with BoNT/A, wherein the chimeric clostridial neurotoxin

comprises a botulinum neurotoxin A (BoNT/A) light-chain and translocation domain (H_N domain), and a BoNT/B receptor binding domain (H_C domain).

20. The chimeric clostridial neurotoxin for use, method or use according to any one of claims 11-19, wherein the chimeric clostridial neurotoxin inhibits release of a mediator (preferably a pain mediator) from a neuron comprising an Aδ nerve fiber or a C nerve fiber, wherein the chimeric clostridial neurotoxin binds to the neuron comprising the Aδ nerve fiber or the C nerve fiber, respectively.

21. The chimeric clostridial neurotoxin for use, method, or use according to any one of claims 11-20, wherein the chimeric clostridial neurotoxin inhibits secretion from a neuron of the central nervous system, preferably inhibits secretion of a mediator, more preferably a pain mediator from a neuron of the central nervous system.

22. The chimeric clostridial neurotoxin for use, method, or use according to any one of the preceding claims, wherein the chimeric clostridial neurotoxin travels by neuronal (e.g. retrograde) transport to a neuron of the central nervous system and cleaves a SNARE protein (e.g. SNAP25) of said neuron.

23. A chimeric clostridial neurotoxin for use in a method for treating a sensory disorder by inhibiting release of a mediator from a neuron comprising an Aδ nerve fiber or a C nerve fiber, wherein the chimeric clostridial neurotoxin binds to the neuron comprising the Aδ nerve fiber or the C nerve fiber, respectively, and wherein the chimeric clostridial neurotoxin comprises a botulinum neurotoxin A (BoNT/A) light-chain and translocation domain (H_N domain), and a BoNT/B receptor binding domain (H_C domain).

24. A method for treating a sensory disorder by inhibiting release of a mediator from a neuron comprising an Aδ nerve fiber or a C nerve fiber, the method comprising administering to a subject a chimeric clostridial neurotoxin, wherein the chimeric clostridial neurotoxin binds to the neuron comprising the Aδ nerve fiber or the C nerve fiber, respectively, and wherein the chimeric clostridial neurotoxin comprises a botulinum neurotoxin A (BoNT/A) light-chain and translocation domain (H_N domain), and a BoNT/B receptor binding domain (H_C domain).

25. Use of a chimeric clostridial neurotoxin in the manufacture of a medicament for treating a sensory disorder by inhibiting release of a mediator from a neuron comprising an Aδ nerve fiber or a C nerve fiber, wherein the chimeric clostridial neurotoxin binds to

the neuron comprising the Aδ nerve fiber or the C nerve fiber, respectively, and wherein the chimeric clostridial neurotoxin comprises a botulinum neurotoxin A (BoNT/A) light-chain and translocation domain (H_N domain), and a BoNT/B receptor binding domain (H_C domain).

26. The chimeric clostridial neurotoxin for use, method, or use according to any one of claims 1-5 or 11-25, wherein the pain or disorder is headache pain, such as migraine pain or cluster headache pain, or bladder pain.

27. The chimeric clostridial neurotoxin for use, method, or use according to any one of claims 1-5 or 11-26, wherein the pain or disorder is migraine pain.

28. The chimeric clostridial neurotoxin for use, method, or use according to any one of claims 1-3, 5-8, 10-19, 21-22 or 26-27, wherein the chimeric clostridial neurotoxin binds to a neuron comprising an Aδ nerve fiber or a C nerve fiber.

29. The chimeric clostridial neurotoxin for use, method, or use according to any one of claims 1-3, 5-8, 10-19, 21-22 or 26-27, wherein the chimeric clostridial neurotoxin inhibits release of a mediator from a neuron comprising an Aδ nerve fiber or a C nerve fiber.

30. The chimeric clostridial neurotoxin for use, method, or use according to any one of the preceding claims, wherein the chimeric clostridial neurotoxin treats the pain, migraine, or disorder by inhibiting release of a mediator (e.g. pain mediator) from a neuron comprising an Aδ nerve fiber or a C nerve fiber, wherein the chimeric clostridial neurotoxin binds to the neuron comprising the Aδ nerve fiber or the C nerve fiber, respectively, and by inhibiting secretion (e.g. of a mediator, preferably a pain mediator) from a neuron of the central nervous system.

31. The chimeric clostridial neurotoxin for use, method, or use according to any one of the preceding claims, wherein the mediator (preferably pain mediator) is a neurotransmitter (preferably a pain neurotransmitter).

32. The chimeric clostridial neurotoxin for use, method, or use according to any one of the preceding claims, wherein the (pain) mediator is one or more selected from: calcitonin gene-related peptide (CGRP); substance P; and a neurokinin.

33. The chimeric clostridial neurotoxin for use, method, or use according to any one of the preceding claims, wherein the (pain) mediator is CGRP and the pain is CGRP-associated pain or the disorder is CGRP-associated pain or wherein when treating migraine, CGRP-associated migraine pain is treated.

34. The chimeric clostridial neurotoxin for use, method, or use according to claim 33, wherein the CGRP-associated pain is CGRP-associated headache pain.

35. The chimeric clostridial neurotoxin for use, method, or use according to claim 33 or 34, wherein the CGRP-associated pain is CGRP-associated migraine pain.

36. The chimeric clostridial neurotoxin for use, method, or use according to any one of claims 33-35, wherein the CGRP-associated pain is:

(a) CGRP-associated somatic pain selected from: headache pain (e.g. post traumatic headache, head injury headache or post-traumatic brain injury headache), arthritic pain (e.g. osteo arthritis pain and/or rheumatoid arthritis pain), exercise pain, degenerative disc disease pain, carpal tunnel compression pain, soft tissue injury pain, temporomandibular joint pain, musculoskeletal pain, CGRP-associated somatic pain caused by or associated with a vascular disorder (e.g. Raynaud's syndrome, Buerger's disease, peripheral venous disease, peripheral arterial disease, varicose veins, blood clots in the veins, blood clotting disorders or lymphedema), facial pain, CGRP-associated somatic pain caused by or associated with trigeminal autonomic cephalalgia, CGRP-associated somatic pain caused by or associated with trigeminal neuralgia, and CGRP-associated cancer-induced pain (e.g. CGRP-associated cancer-induced bone pain);

(b) CGRP-associated visceral pain selected from: endometriosis pain, pancreatitis pain, gastrointestinal pain, and CGRP-associated visceral pain caused by or associated with a vascular disorder;

(c) CGRP-associated inflammatory pain selected from: chronic pain, wound healing pain, pruritus pain, and burn pain; and/or

(d) CGRP-associated neuropathic pain selected from: post herpetic neuralgia pain, diabetes pain, chronic neuropathic pain, and Morton's neuroma pain.

37. The chimeric clostridial neurotoxin for use, method, or use according to any one of the preceding claims, wherein the neuron is a neuron of the trigeminal ganglion.

38. The chimeric clostridial neurotoxin for use, method, or use according to any one of the preceding claims, wherein the chimeric clostridial neurotoxin is administered to the face, neck, and/or skull.

39. The chimeric clostridial neurotoxin for use, method, or use according to any one of the preceding claims, wherein the chimeric clostridial neurotoxin is administered intradermally.

40. The chimeric clostridial neurotoxin for use, method, or use according to any one of the preceding claims, wherein the chimeric clostridial neurotoxin is administered by intradermal injection at up to 10 injection sites per treatment session.

41. The chimeric clostridial neurotoxin for use, method, or use according to any one of claims 1-39, wherein the chimeric clostridial neurotoxin is administered by intradermal injection at 10-40 injection sites per treatment session.

42. The chimeric clostridial neurotoxin for use, method, or use according to any one of claims 1-39 or 41, wherein the chimeric clostridial neurotoxin is administered by intradermal injection at 25-35 injection sites per treatment session.

43. The chimeric clostridial neurotoxin for use, method, or use according to any one of claims 1-38, wherein the chimeric clostridial neurotoxin is administered intramuscularly.

44. The chimeric clostridial neurotoxin for use, method, or use according to any one of claims 1-38 or 43, wherein the chimeric clostridial neurotoxin is administered by intramuscular injection at up to 10 injection sites per treatment session.

45. The chimeric clostridial neurotoxin for use, method, or use according to any one of claims 1-38 or 43, wherein the chimeric clostridial neurotoxin is administered by intramuscular injection at 10-40 injection sites per treatment session.

46. The chimeric clostridial neurotoxin for use, method, or use according to any one of claims 1-38, 43 or 45, wherein the chimeric clostridial neurotoxin is administered by intramuscular injection at 25-35 injection sites per treatment session.

47. The chimeric clostridial neurotoxin for use, method, or use according to any one of the preceding claims, comprising administering the chimeric clostridial neurotoxin to at least one of a: frontalis muscle, corrugator (e.g. corrugator supercilii) muscle, procerus (e.g. procerus nasalis), occipitalis (e.g. upper or lower occipitalis) muscle, temporalis muscle, trapezius (e.g. upper, mid or lower trapezius) muscle, masseter muscle, nasalis muscle, orbicularis oculi muscle, cervical paraspinal muscle, temporal fascia muscle, auricularis superior muscle, auricularis anterior muscle, auricularis posterior muscle, sternocleidomastoid muscle, platysma muscle, dilatator naris anterior muscle, dilatator naris posterior muscle, depressor septi muscle, mentalis muscle, orbicularis oris muscle, zygomaticus muscle, risorius muscle, buccinator muscle, occipitofrontalis muscle, levator labii superioris muscle, depressor labii inferioris muscle, depressor anguli oris muscle, thyrohyoid muscle, omohyoid muscle, sternohyoid muscle, splenius cervicis muscle, splenius capitis muscle, semispinalis cervicis muscle, semispinalis capitis muscle, levator scapulae muscle, digastric muscle, or scalene muscle.

48. The chimeric clostridial neurotoxin for use, method, or use according to any one of the preceding claims, comprising administering the chimeric clostridial neurotoxin to at least one of a: frontalis muscle, corrugator (e.g. corrugator supercilii) muscle, nasalis muscle, orbicularis oculi muscle, temporalis muscle, occipitalis muscle, or trapezius muscle.

49. The chimeric clostridial neurotoxin for use, method, or use according to any one of the preceding claims, comprising administering the chimeric clostridial neurotoxin to a: frontalis muscle, corrugator (e.g. corrugator supercilii) muscle, nasalis muscle, orbicularis oculi muscle, temporalis muscle, occipitalis muscle, and trapezius muscle.

50. The chimeric clostridial neurotoxin for use, method, or use according to any one of the preceding claims, wherein administration of the chimeric clostridial neurotoxin comprises:

(a) 2 injections to a frontalis muscle (preferably 2 injections per frontalis muscle);

(b) 1 injection to a corrugator muscle (preferably 1 injection per corrugator muscle);

(c) 1 injection to a nasalis muscle (preferably 1 injection per nasalis muscle);

(d) 1 injection to an orbicularis oculi muscle (preferably 1 injection per orbicularis oculi muscle);

(e) 4 injections to a temporalis muscle (preferably 4 injections per temporalis muscle);

(f) 3 injections to an occipitalis muscle (preferably 3 injections per occipitalis muscle); and

(g) 2 injections to a trapezius muscle (preferably 2 injections per trapezius muscle).

51. The chimeric clostridial neurotoxin for use, method, or use according to any one of the preceding claims, wherein administration of the chimeric clostridial neurotoxin comprises:

(a) 4 injections to the frontalis muscles (preferably 2 injections to a frontalis muscle at a first side of the face and 2 injections to a frontalis muscle at a second side of the face);

(b) 2 injections to the corrugator muscles (preferably 1 injection to a corrugator muscle at a first side of the face and 1 injection to a corrugator muscle at a second side of the face);

(c) 2 injections to the nasalis muscles (preferably 1 injection to a nasalis muscle at a first side of the face and 1 injection to a nasalis muscle at a second side of the face);

(d) 2 injections to the orbicularis oculi muscles (preferably 1 injection to an orbicularis oculi muscle at a first side of the face and 1 injection to an orbicularis oculi muscle at a second side of the face);

(e) 8 injections to the temporalis muscles (preferably 4 injections to a temporalis muscle at a first side of the head and 4 injections to a temporalis muscle at a second side of the head);

(f) 6 injections to the occipitalis muscles (preferably 3 injections to an occipitalis muscle at a first side of the head and 3 injections to an occipitalis muscle at a second side of the head); and

(g) 4 injections to the trapezius muscles (preferably 2 injections to a trapezius muscle at a first side of the neck and 2 injections to a trapezius muscle at a second side of the neck).

52. The chimeric clostridial neurotoxin for use, method, or use according to any one of the preceding claims, wherein the chimeric clostridial neurotoxin is administered by way of a unit dose per injection (e.g. per injection site).

53. The chimeric clostridial neurotoxin for use, method, or use according to any one of the preceding claims, wherein administration of the chimeric clostridial neurotoxin comprises administering:

(a) 2 unit doses to a frontalis muscle (preferably 2 unit doses per frontalis muscle);

(b) 1 unit dose to a corrugator muscle (preferably 1 unit dose per corrugator muscle);

(c) 1 unit dose to a nasalis muscle (preferably 1 unit dose per nasalis muscle);

(d) 1 unit dose to an orbicularis oculi muscle (preferably 1 unit dose per orbicularis oculi muscle);

(e) 4 unit doses to a temporalis muscle (preferably 4 unit doses per temporalis muscle);

(f) 3 unit doses to an occipitalis muscle (preferably 3 unit doses per occipitalis muscle); and

(g) 2 unit doses to a trapezius muscle (preferably 2 unit doses per trapezius muscle).

54. The chimeric clostridial neurotoxin for use, method, or use according to any one of the preceding claims, wherein administration of the chimeric clostridial neurotoxin comprises:

(a) 4 unit doses to the frontalis muscles (preferably 2 unit doses to a frontalis muscle at a first side of the face and 2 unit doses to a frontalis muscle at a second side of the face);

(b) 2 unit doses to the corrugator muscles (preferably 1 unit dose to a corrugator muscle at a first side of the face and 1 unit dose to a corrugator muscle at a second side of the face);

(c) 2 unit doses to the nasalis muscles (preferably 1 unit dose to a nasalis muscle at a first side of the face and 1 injection to a nasalis muscle at a second side of the face);

(d) 2 unit doses to the orbicularis oculi muscles (preferably 1 unit dose to an orbicularis oculi muscle at a first side of the face and 1 unit dose to an orbicularis oculi muscle at a second side of the face);

(e) 8 unit doses to the temporalis muscles (preferably 4 unit doses to a temporalis muscle at a first side of the head and 4 unit doses to a temporalis muscle at a second side of the head);

(f) 6 unit doses to the occipitalis muscles (preferably 3 unit doses to an occipitalis muscle at a first side of the head and 3 unit doses to an occipitalis muscle at a second side of the head); and

(g) 4 unit doses to the trapezius muscles (preferably 2 unit doses to a trapezius muscle at a first side of the neck and 2 unit doses to a trapezius muscle at a second side of the neck).

55. The chimeric clostridial neurotoxin for use, method, or use according to any one of the preceding claims, wherein the chimeric clostridial neurotoxin is administered by way of a unit dose of 5 pg to 17,000 pg of the chimeric clostridial neurotoxin.

56. The chimeric clostridial neurotoxin for use, method, or use according to any one of the preceding claims, wherein the chimeric clostridial neurotoxin is administered by way of a unit dose of 500 pg to 17,000 pg.

57. The chimeric clostridial neurotoxin for use, method, or use according to any one of the preceding claims, wherein the chimeric clostridial neurotoxin is administered by way of a unit dose of 1,000 pg to 17,000 pg.

58. The chimeric clostridial neurotoxin for use, method, or use according to any one of the preceding claims, wherein the total dose administered per treatment session is up to 255,000 pg of the chimeric clostridial neurotoxin, e.g. 3,640-255,000 pg, or up to 160,000 pg (preferably up to 155,000 pg).

59. The chimeric clostridial neurotoxin for use, method, or use according to any one of the preceding claims, wherein the total dose administered per treatment session is up to 120,000 pg, preferably up to 112,000 pg.

60. The chimeric clostridial neurotoxin for use, method, or use according to any one of the preceding claims, wherein the total dose administered per treatment session is up to 100,000 pg, preferably up to 70,000 pg.

61. The chimeric clostridial neurotoxin for use, method, or use according to any one of the preceding claims, wherein the chimeric clostridial neurotoxin is administered by way of a unit dose of 3,640 pg to 17,000 pg.

62. The chimeric clostridial neurotoxin for use, method, or use according to any one of the preceding claims, wherein the chimeric clostridial neurotoxin is administered by way of a unit dose of 1,000 to 5,500 pg.

63. The chimeric clostridial neurotoxin for use, method, or use according to any one of the preceding claims, wherein the chimeric clostridial neurotoxin is administered by way of a unit dose of 2,000 to 4,500 pg.

64. The chimeric clostridial neurotoxin for use, method, or use according to any one of the preceding claims, wherein the chimeric clostridial neurotoxin is administered by way of a unit dose of 3,500 to 4,500 pg or 2,000 to 3,000 pg (e.g. 2,500 pg).

65. The chimeric clostridial neurotoxin for use, method, or use according to any one of the preceding claims, wherein the chimeric clostridial neurotoxin is administered by way of a unit dose of 4,000 pg.

66. The chimeric clostridial neurotoxin for use, method or use according to any one of claims 47-65, wherein the administration to (e.g. injection to) the muscle is: via intramuscular injection or via intradermal injection in the region of the muscle; preferably via intramuscular injection.

67. The chimeric clostridial neurotoxin for use, method, or use according to any one of claims 1-38 or 47-65, wherein the chimeric clostridial neurotoxin is administered intraneurally, perineurally or by periganglial administration.

68. The chimeric clostridial neurotoxin for use, method, or use according to any one of claims 1-38, 47-65, or 67, wherein the chimeric clostridial neurotoxin is administered to the trigeminal nerve, Gasserian ganglion, nervus intermedius, glossopharyngeal, vagus nerve, and/or to the upper cervical roots via the occipital nerves.

69. The chimeric clostridial neurotoxin for use, method, or use according to any one of claims 1-38 or 47-65, wherein the chimeric clostridial neurotoxin is administered by perivascular administration.

70. The chimeric clostridial neurotoxin for use, method, or use according to any one of the preceding claims, wherein the treatment is prophylactic treatment, preferably the prophylactic treatment of migraine.

71. A unit dosage form (e.g. for treating pain), the unit dosage form comprising:
    a. 5 pg to 17,000 pg of a chimeric clostridial neurotoxin; or
    b. 0.2 Units up to 707 Units of a chimeric clostridial neurotoxin, wherein 1 Unit is an amount of the chimeric clostridial neurotoxin that corresponds to the calculated median lethal dose (LD_{50}) in mice; and
    c. optionally a pharmaceutically acceptable carrier, excipient, adjuvant, and/or salt;
wherein the chimeric clostridial neurotoxin comprises a botulinum neurotoxin A (BoNT/A) light-chain and translocation domain (H_N domain), and a BoNT/B receptor binding domain (H_C domain).

72. The unit dosage form according to claim 71, wherein the unit dosage form comprises:
    a. 1,000 pg to 5,500 pg of the chimeric clostridial neurotoxin; or
    b. 42 Units up to 229 Units of the chimeric clostridial neurotoxin, wherein 1 Unit is an amount of the chimeric clostridial neurotoxin that corresponds to the calculated median lethal dose (LD_{50}) in mice.

73. The unit dosage form according to claim 71 or 72, wherein the unit dosage form comprises 2,000 to 4,500 pg of the chimeric clostridial neurotoxin.

74. The unit dosage form according to any one of claims 71-73, wherein the unit dosage form comprises 3,500 to 4,500 pg or 2,000 to 3,000 pg (e.g. 2,500 pg) of the chimeric clostridial neurotoxin.

75. The unit dosage form according to any one of claims 71-74, wherein the unit dosage form comprises 4,000 pg of the chimeric clostridial neurotoxin.

76. The chimeric clostridial neurotoxin for use, method, use, or unit dosage form according to any one of the preceding claims, wherein the chimeric clostridial neurotoxin has a Safety Ratio of greater than 7 (preferably a Safety Ratio of at least 10), wherein

the Safety Ratio is calculated as: dose of toxin required for -10% bodyweight change measured as pg/mouse divided by DAS ED_{50} measured as pg/mouse, wherein ED_{50} = dose required to produce a DAS score of 2.

77. The chimeric clostridial neurotoxin for use, method, use, or unit dosage form according to any one of the preceding claims, wherein the C-terminal amino acid residue of said H_N domain corresponds to the first amino acid residue of the 3_{10} helix separating the H_N and H_C domains in BoNT/A, and wherein the N-terminal amino acid residue of said H_C domain corresponds to the second amino acid residue of the 3_{10} helix separating the H_N and H_C domains in BoNT/B.

78. The chimeric clostridial neurotoxin for use, method, use, or unit dosage form according to any one of the preceding claims, wherein the chimeric clostridial neurotoxin comprises a polypeptide sequence having at least 70% sequence identity to SEQ ID NO: 1.

79. The chimeric clostridial neurotoxin for use, method, use, or unit dosage form according to any one of the preceding claims, wherein the chimeric clostridial neurotoxin is a di-chain chimeric clostridial neurotoxin in which the light-chain (L-chain) is linked to the heavy-chain (H-chain) via a di-sulphide bond obtainable by a method comprising contacting a single-chain chimeric clostridial neurotoxin comprising SEQ ID NO: 1 with a protease that hydrolyses a peptide bond in the activation loop thereof, thereby converting the single-chain chimeric clostridial neurotoxin into the corresponding di-chain chimeric clostridial neurotoxin.

80. The chimeric clostridial neurotoxin for use, method, use, or unit dosage form according to any one of the previous claims, wherein the chimeric clostridial neurotoxin is a di-chain chimeric clostridial neurotoxin in which the L-chain is linked to the H-chain via a di-sulphide bond obtainable by a method comprising contacting a single-chain chimeric clostridial neurotoxin consisting of SEQ ID NO: 1 with a protease that hydrolyses a peptide bond in the activation loop thereof, thereby converting the single-chain chimeric clostridial neurotoxin into the corresponding di-chain chimeric clostridial neurotoxin.

81. The chimeric clostridial neurotoxin for use, method, use, or unit dosage form according to any one of the preceding claims, wherein the BoNT/B H_C domain comprises one or more substitution mutation(s) selected from the group consisting of: E1191M;

S1199Y; V1118M; Y1183M; E1191I; E1191Q; E1191T; S1199F; S1199L; S1201V; and combinations thereof.

82. The chimeric clostridial neurotoxin for use, method, use, or unit dosage form according to any one of the preceding claims, wherein the BoNT/B H_C domain comprises substitution mutations at E1191M and S1199Y.

83. The chimeric clostridial neurotoxin for use, method, use, or unit dosage form according to any one of the preceding claims, wherein an initial methionine amino acid residue of a polypeptide sequence of the chimeric clostridial neurotoxin is optional.

84. The chimeric clostridial neurotoxin for use, method, use, or unit dosage form according to any one of the preceding claims, wherein an initial methionine amino acid residue of a polypeptide sequence of the chimeric clostridial neurotoxin is absent.

85. The chimeric clostridial neurotoxin for use, method, use, or unit dosage form according to any one of the preceding claims, wherein the chimeric clostridial neurotoxin is a di-chain chimeric clostridial neurotoxin comprising (or consisting of) a light-chain comprising SEQ ID NO: 17 or 18 (preferably SEQ ID NO: 17) and a heavy-chain comprising SEQ ID NO: 19, wherein the light-chain and heavy-chain are joined together by a di-sulphide bond.

86. A kit comprising:

(a) the unit dosage form according to any one of claims 71-85; and

(b) instructions for use of the same, e.g. in treating pain; and

(c) optionally a diluent.

87. A method for determining whether or not a clostridial neurotoxin is suitable for treating pain, the method comprising:

(a) comparing a level of calcitonin gene-related peptide (CGRP) comprised in a first sample with the level of CGRP comprised in a second sample, wherein the first sample has been obtained from a subject prior to administration of the clostridial neurotoxin, and wherein the second sample has been obtained from the same subject after administration of the clostridial neurotoxin; and

(b) determining that the clostridial neurotoxin is suitable for treating pain when the level of CGRP in the second sample is lower than the level of CGRP in the first sample; or

(c) determining that the clostridial neurotoxin is unsuitable for treating pain when the level of CGRP in the second sample is not lower (e.g. is higher or the same) than the level of CGRP in the first sample.

FIGURE 1

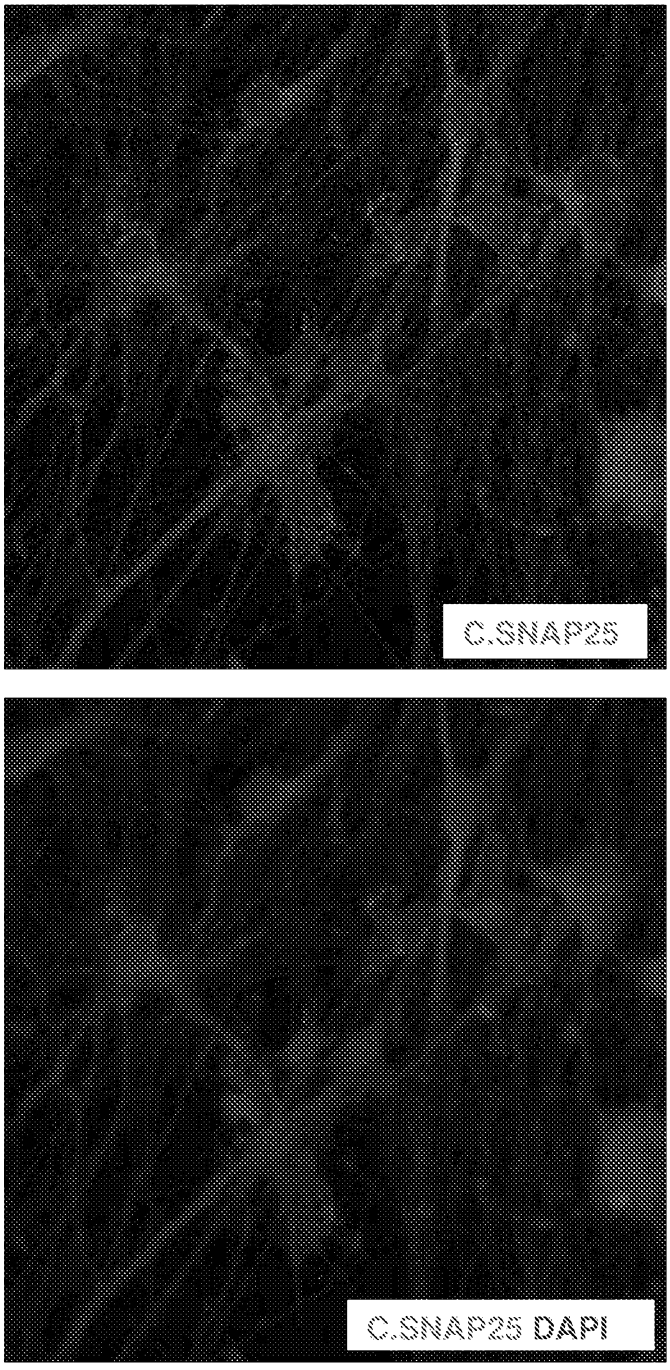


FIGURE 2

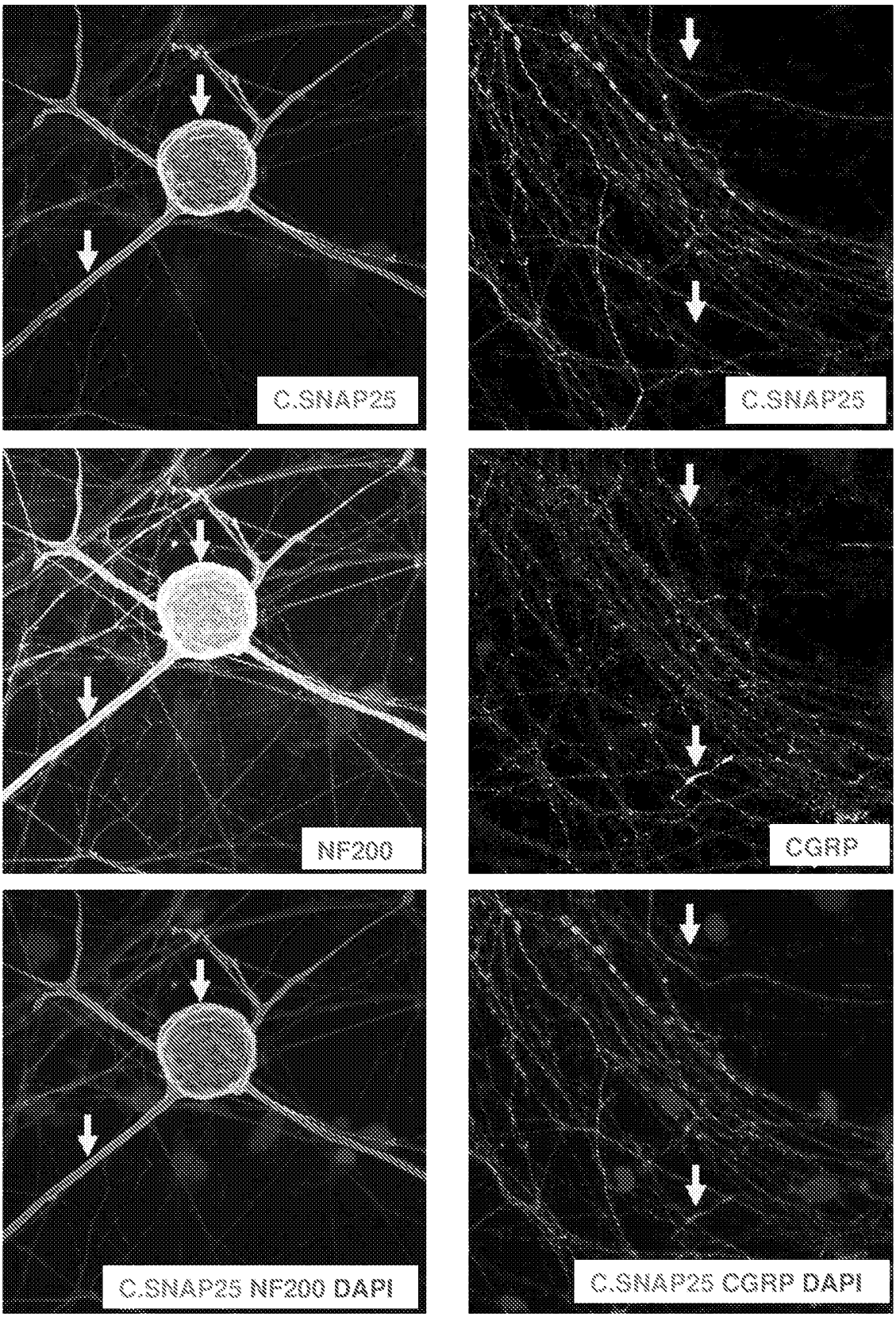

C.SNAP25
C.SNAP25
NF200
CGRP
C.SNAP25 NF200 DAPI
C.SNAP25 CGRP DAPI

FIGURE 2 CONTINUED

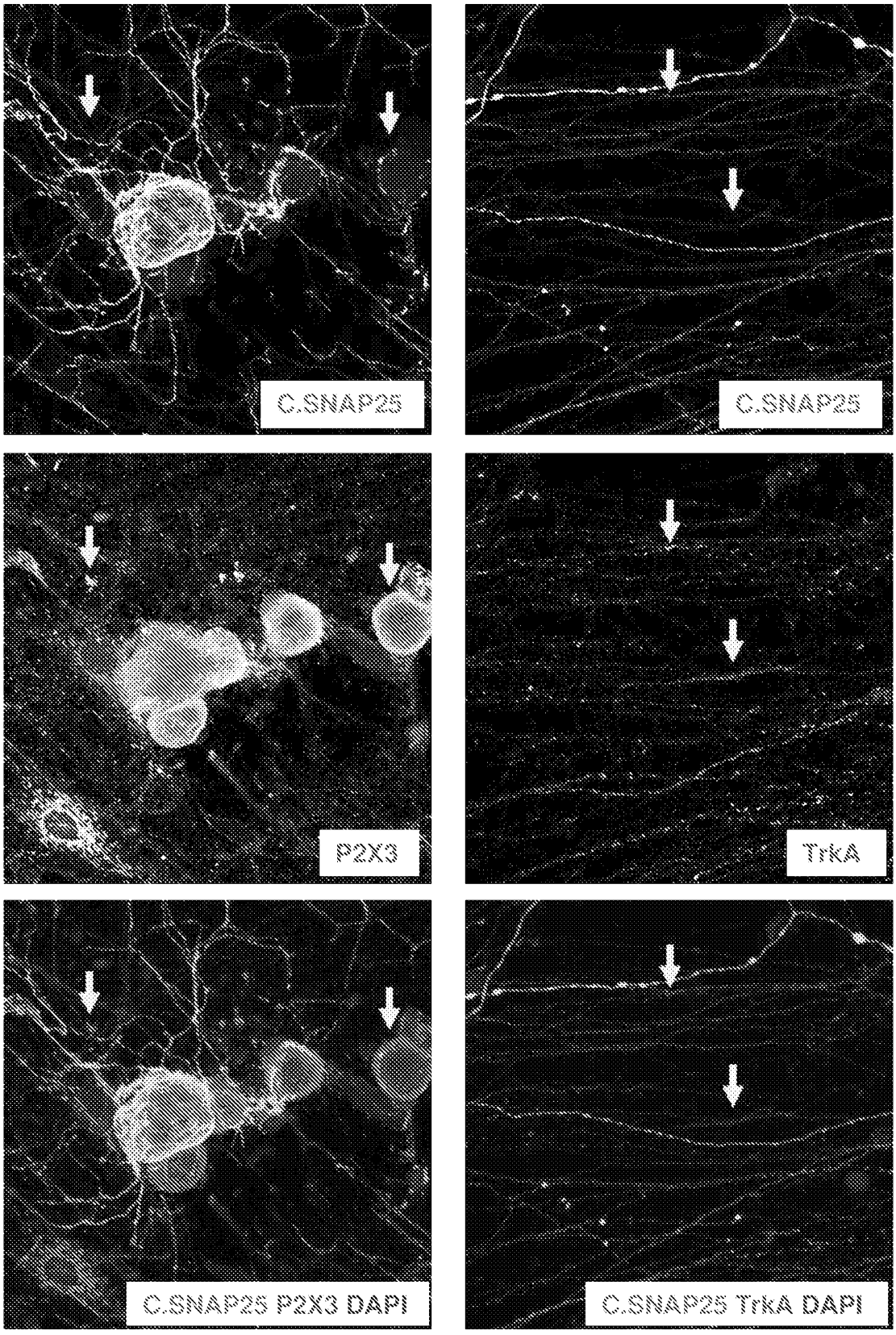


FIGURE 3

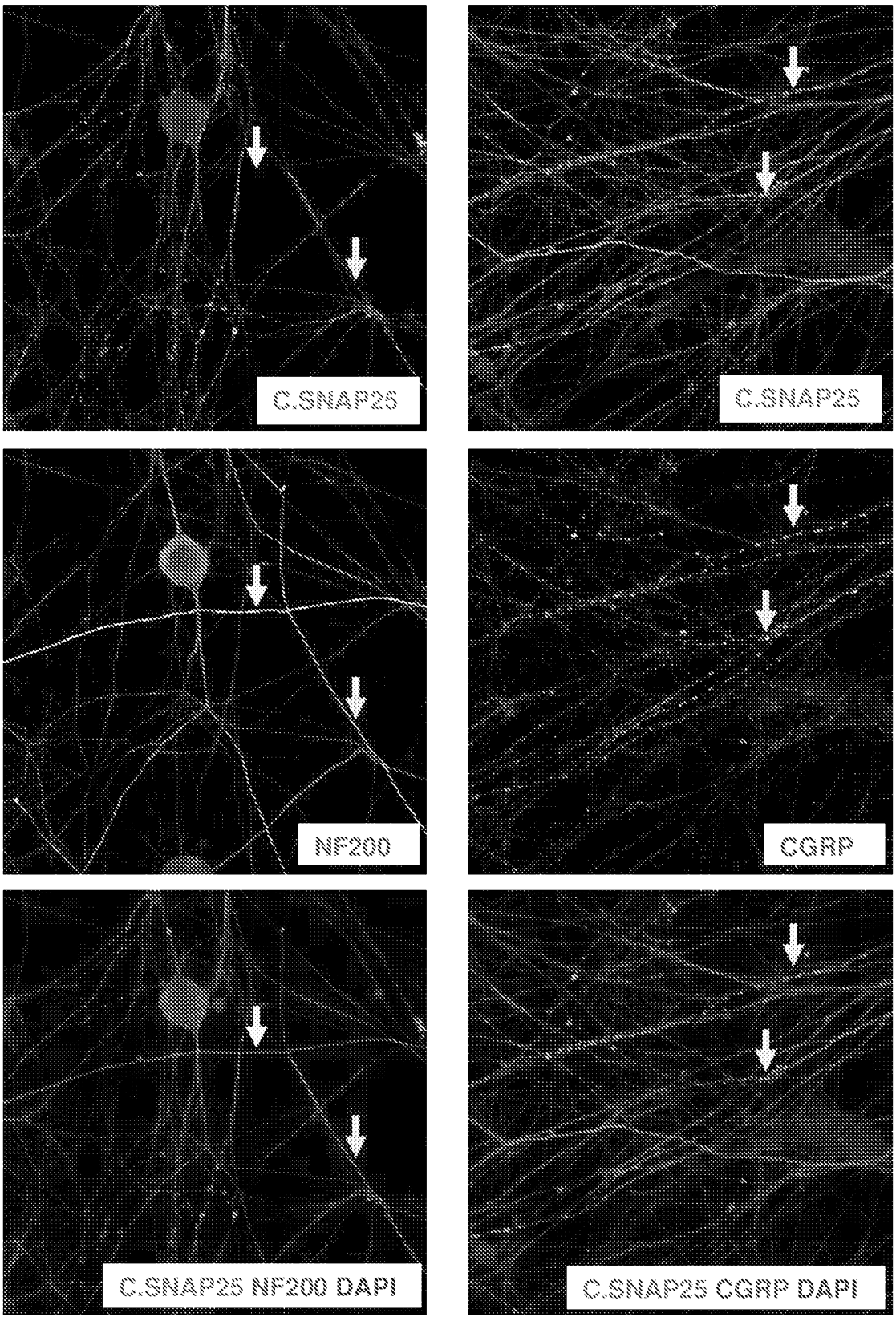

C.SNAP25
C.SNAP25
NF200
CGRP
C.SNAP25 NF200 DAPI
C.SNAP25 CGRP DAPI

FIGURE 3 CONTINUED

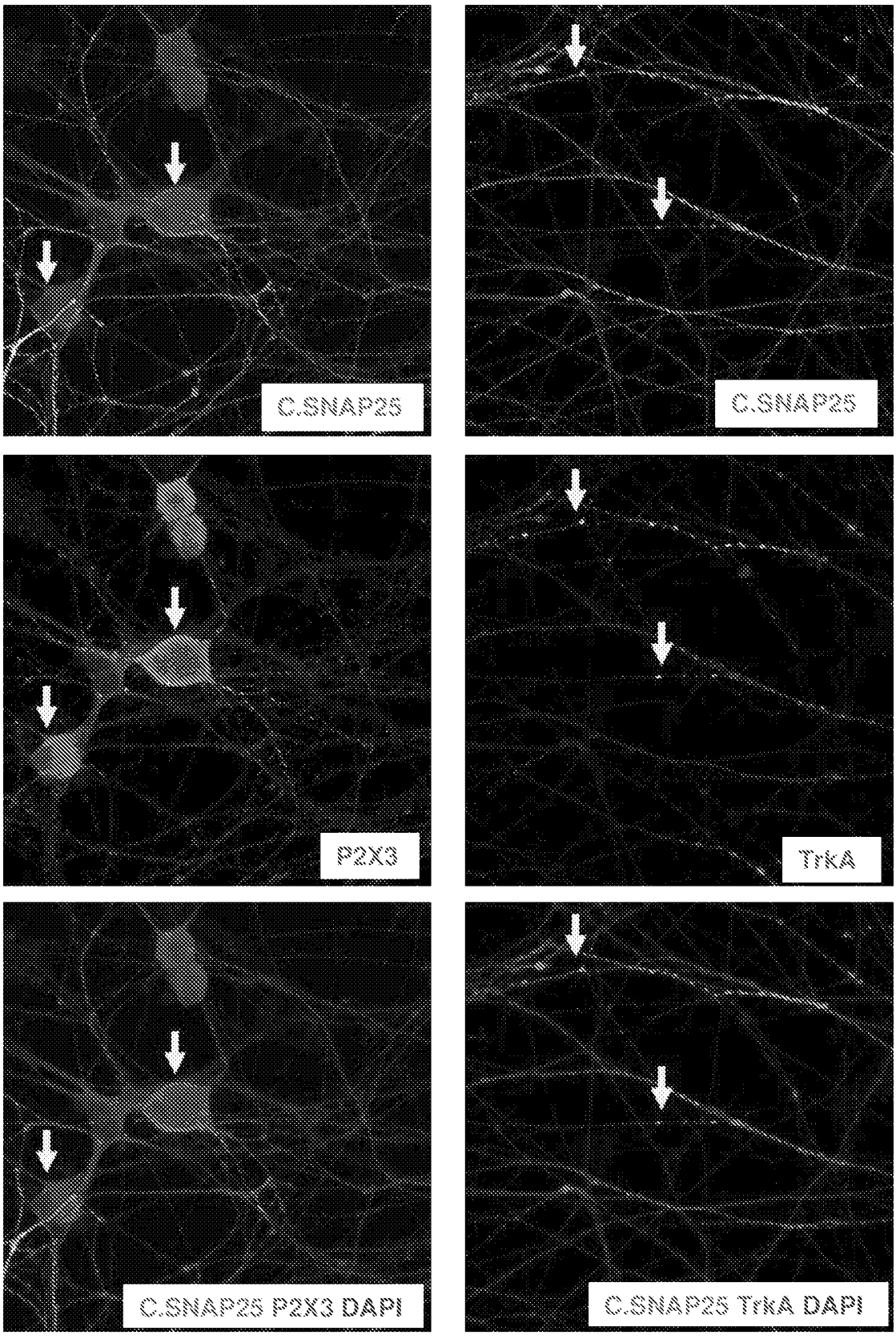


FIGURE 4

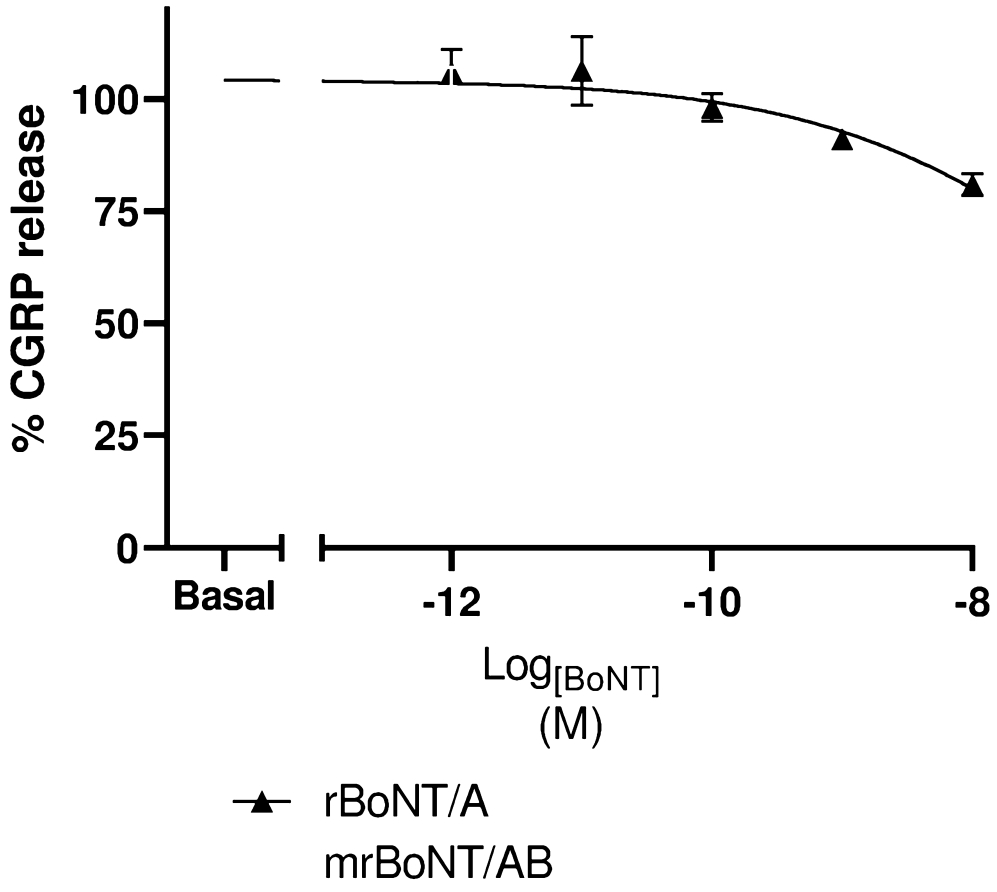

% CGRP release
100
75
50
25
0
Basal
-12
-10
-8
Log[BoNT]
(M)
rBoNT/A
mrBoNT/AB

FIGURE 5

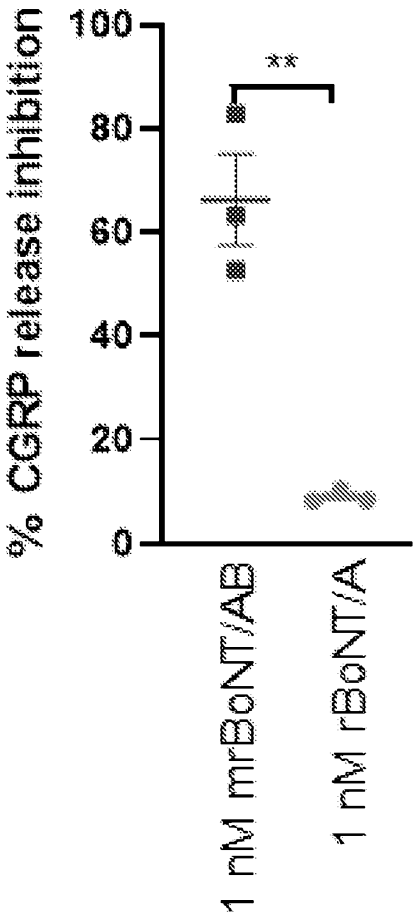
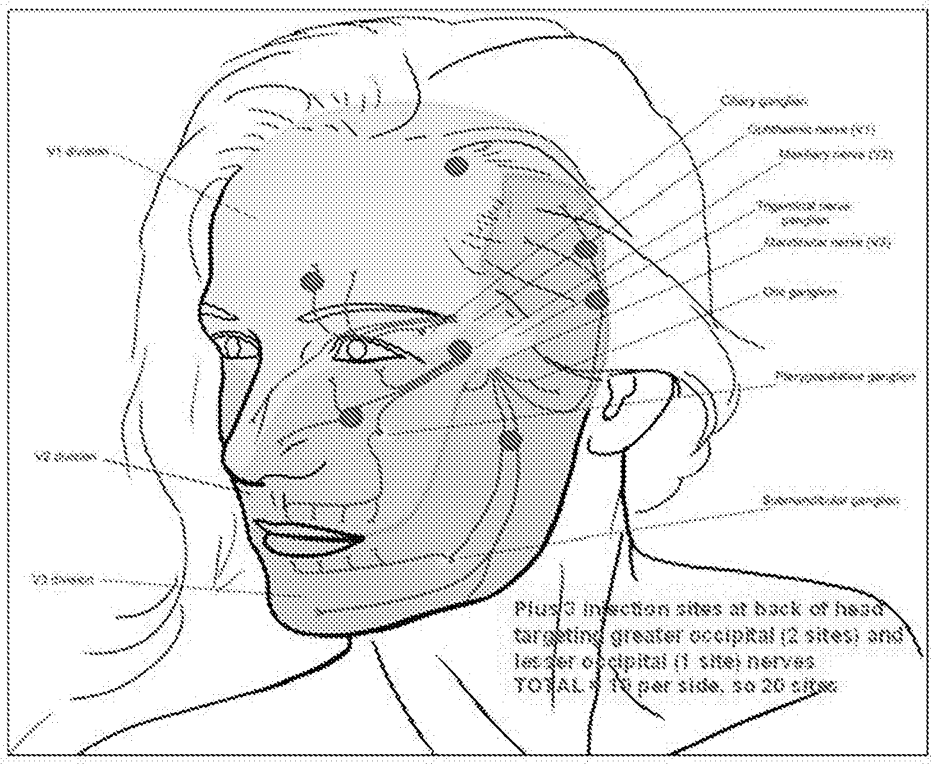

% CGRP release inhibition
100
80
60
40
20
0
**
1 nM mrBoNT/AB
1 nM rBoNT/A

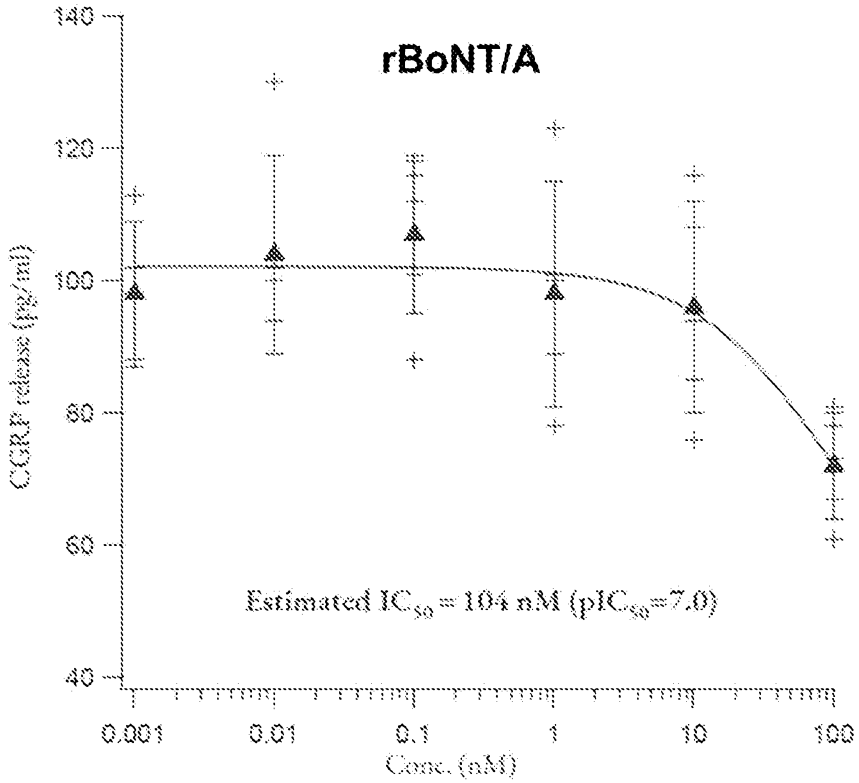
FIGURE 6



Region	Target nerve terminals	No. injections per side/total
Trigeminal, opthalmic	Supraorbital	1/2
	Supratrochlear	1/2
	Intratrochlear	1/2
Trigeminal, maxillary	Zygomaticotemporal	1/2
	Zygomaticofacial	1/2
Trigeminal, mandibula	Auriculotemporal	2/4
Back of head	Greater occipital	2/4
	Lesser occipital	1/2
	TOTAL	10/20

FIGURE 7

A


rBoNT/A
CGRP release (pg/ml)
140
120
100
80
60
40
Estimated IC50 = 104 nM (pIC50=7.0)
0.001
0.01
0.1
1
10
100
Conc. (nM)

B

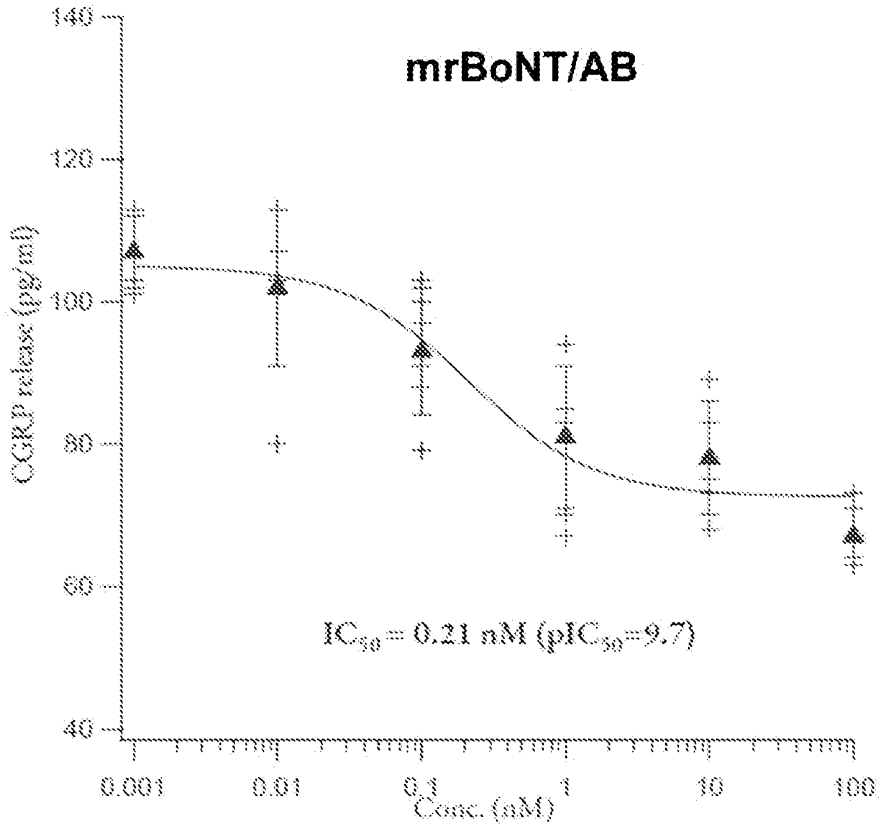

mrBoNT/AB
CGRP release (pg/ml)
140
120
100
80
60
40
IC50 = 0.21 nM (pIC50=9.7)
0.001
0.01
0.1
1
10
100
Conc. (nM)

FIGURE 8

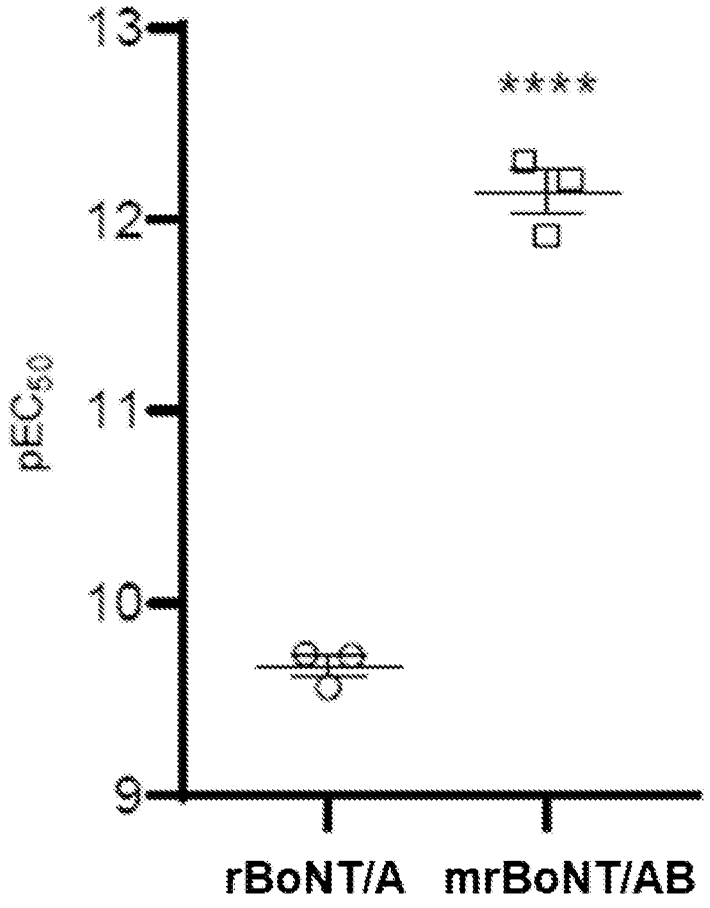


FIGURE 9

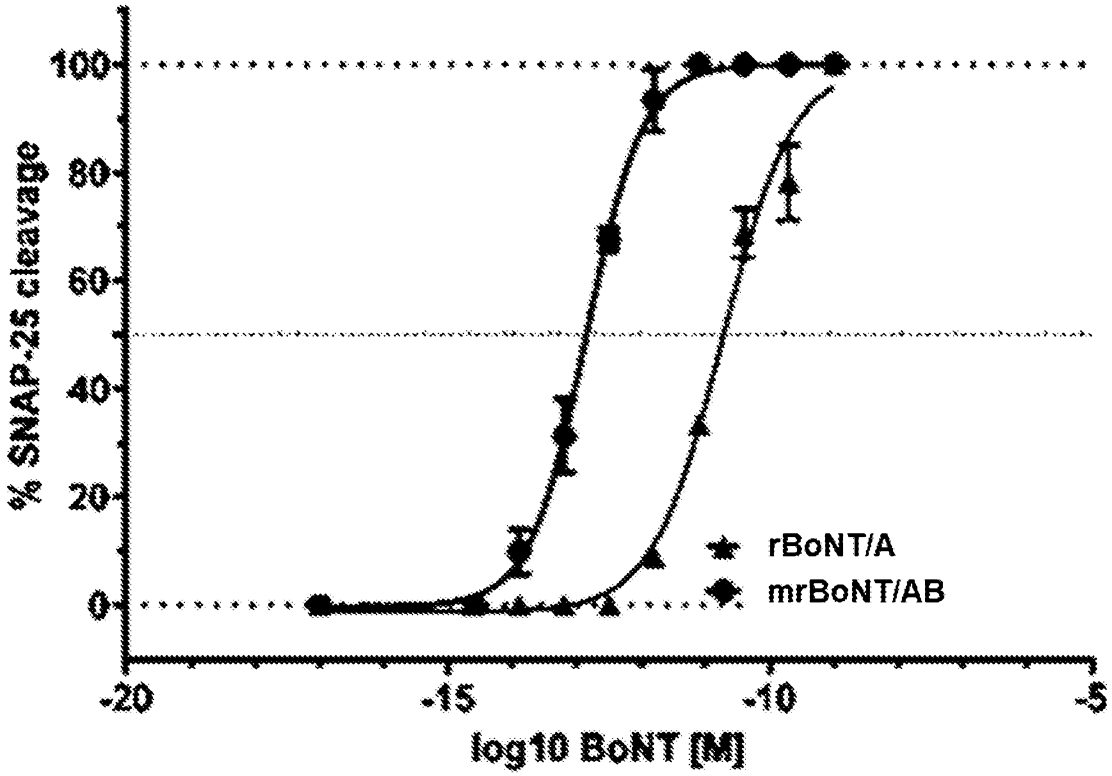

100
80
60
40
20
0
% SNAP-25 cleavage
-20
-15
-10
-5
log10 BoNT [M]
rBoNT/A
mrBoNT/AB

FIGURE 10

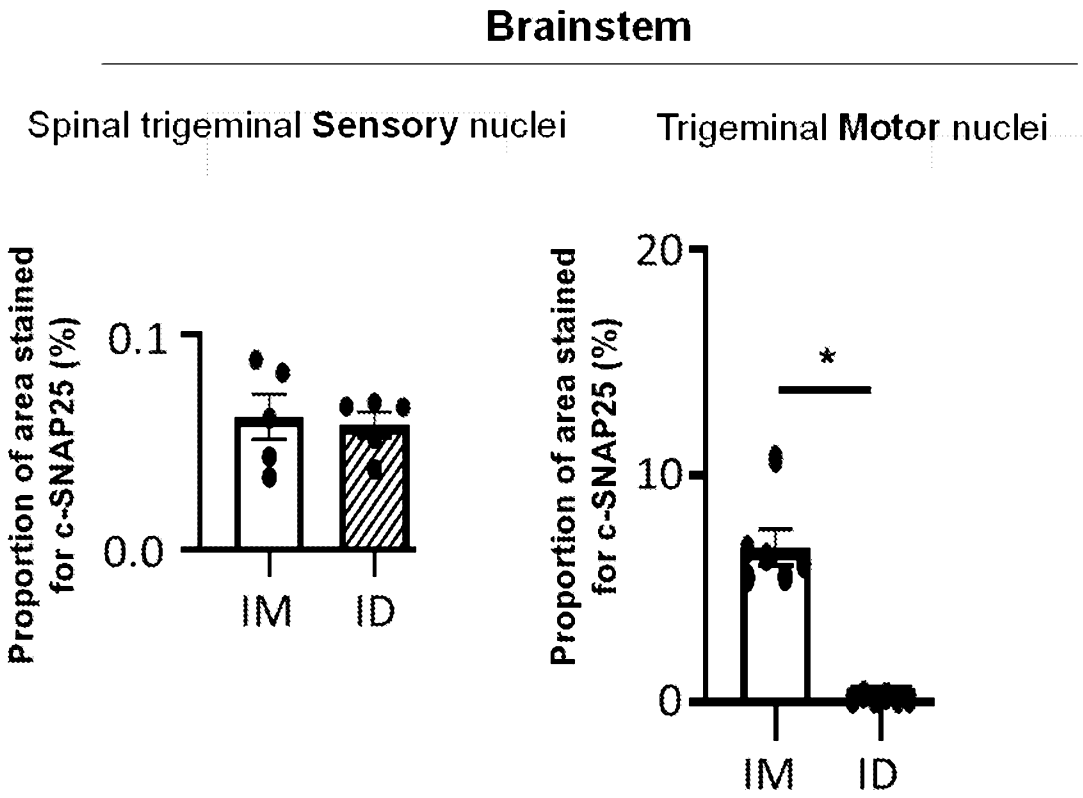


FIGURE 11

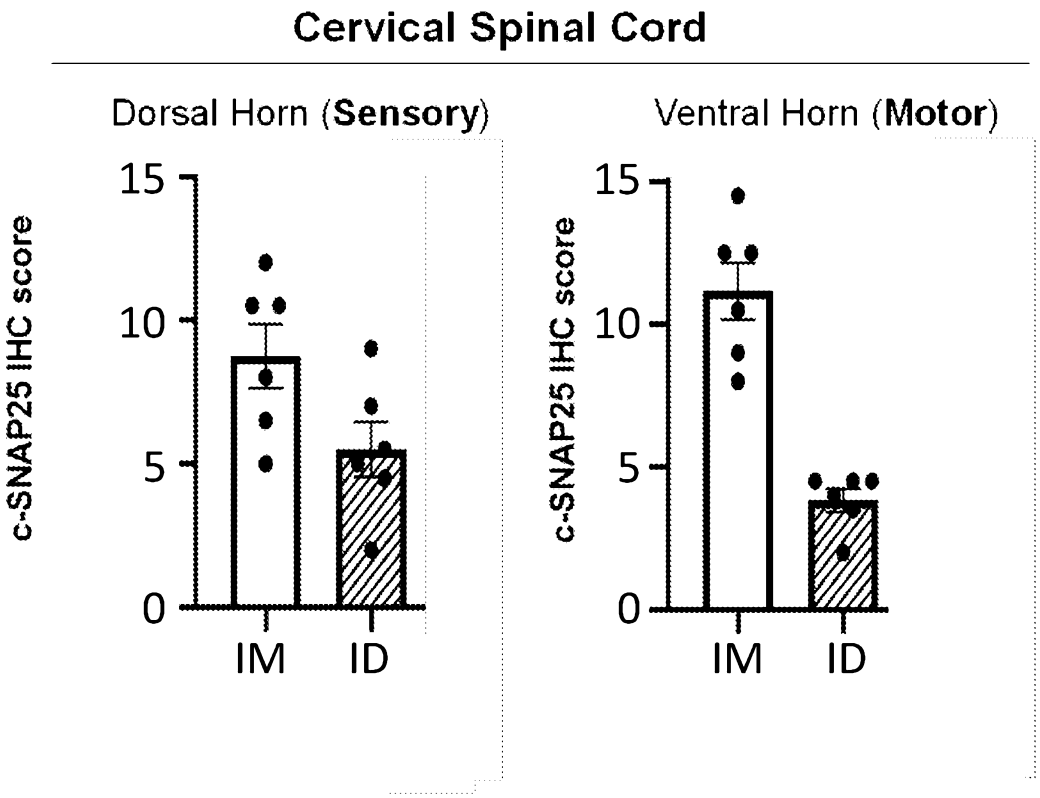
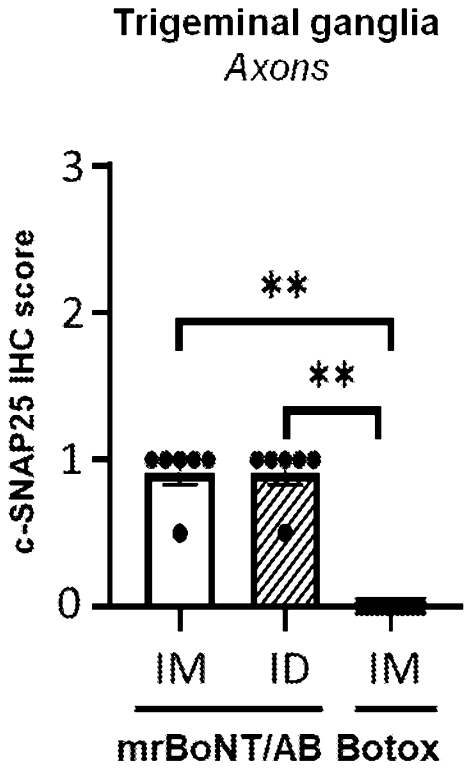


FIGURE 12


Trigeminal ganglia
Axons
c-SNAP25 IHC score
3
2
1
0
**
**
IM
ID
IM
mrBoNT/AB
Botox

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2022/052957

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K38/48 A61P29/02
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2021/064369 A1 (IPSEN BIOPHARM LTD [GB]) 8 April 2021 (2021-04-08) page 19, line 6 - line 8 page 26, line 7 - line 16	1-5, 14-22, 26-70, 76-86
X	US 2019/300869 A1 (DONG MIN [US] ET AL) 3 October 2019 (2019-10-03) paragraphs [0044], [0112], [0113]	1-5, 14-22, 26-70, 76-86
X	US 2019/127427 A1 (LIU SAI MAN [GB]) 2 May 2019 (2019-05-02) paragraphs [0119], [0145]	1-5, 14-22, 26-70, 76-86

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance;; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance;; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 January 2023	27/03/2023

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Pilling, Stephen

1

Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2022/052957

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2021/186160 A2 (IPSEN BIOPHARM LTD [GB]) 23 September 2021 (2021-09-23) page 1, line 5 - line 19 page 64, line 31 - line 35 page 68, line 6 - line 30 page 69, line 23 - line 26 page 72, line 26 - line 29	1-5, 14-22, 26-70, 76-86
X,P	WO 2022/200809 A1 (IPSEN BIOPHARM LTD [GB]) 29 September 2022 (2022-09-29) page 5, line 10 - page 6, line 16; sequences 69,70	1-5, 14-22, 26-70, 76-86
X,P	WO 2022/208091 A1 (IPSEN BIOPHARM LTD [GB]) 6 October 2022 (2022-10-06) page 1, line 5 - line 6 page 52, line 31 - page 53, line 30 page 57, line 6 - line 29	1-5, 14-22, 26-70, 76-86
X,P	WO 2022/208039 A1 (IPSEN BIOPHARM LTD [GB]) 6 October 2022 (2022-10-06) page 1, line 5 - line 6 page 35, line 6 - line 13 page 38, line 6 - page 39, line 14	1-5, 14-22, 26-70, 76-86
A	JIAFU WANG ET AL: "Longer-acting and highly potent chimaeric inhibitors of excessive exocytosis created with domains from botulinum neurotoxin A and B", BIOCHEMICAL JOURNAL, PUBLISHED BY PORTLAND PRESS ON BEHALF OF THE BIOCHEMICAL SOCIETY, GB, vol. 444, no. Part 1, 15 May 2012 (2012-05-15), pages 59-67, XP002690949, ISSN: 0264-6021, DOI: 10.1042/BJ20120100 abstract	1-5, 14-22, 26-70, 76-86

1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB2022/052957

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. [X] forming part of the international application as filed.

   b. [ ] furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)).

      [ ] accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

2. [ ] With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB2022/052957

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims;; it is covered by claims Nos.:
1-5, 14-19(completely); 20-22, 26-70, 76-86(partially)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-5, 14-19(completely); 20-22, 26-70, 76-86(partially)

A chimeric clostridial neurotoxin for use in treating pain, wherein the chimeric clostridial neurotoxin comprises a botulinum neurotoxin A (BoNT/A) light-chain and translocation domain (HN domain), and a BoNT/B receptor binding domain (Hc domain).

---

2. claims: 6-10(completely); 20-22, 26-70, 76-86(partially)

A chimeric clostridial neurotoxin for use in treating migraine (preferably migraine pain), wherein the chimeric clostridial neurotoxin comprises a botulinum neurotoxin A (BoNT/A) light-chain and translocation domain (HN domain), and a BoNT/B receptor binding domain (Hc domain).

---

3. claims: 11-13(completely); 20-22, 26-70, 76-86(partially)

A chimeric clostridial neurotoxin for use in a method for treating a disorder of a subject for a longer duration and/or with a greater efficacy than that of a subject treated with BoNT/A, the method comprising administering a chimeric clostridial neurotoxin to the subject, wherein the chimeric clostridial neurotoxin comprises a botulinum neurotoxin A (BoNT/A) light-chain and translocation domain (HN domain), and a BoNT/B receptor binding domain (Hc domain).

---

4. claims: 23-25(completely); 26-70, 76-86(partially)

A chimeric clostridial neurotoxin for use in a method for treating a sensory disorder by inhibiting release of a mediator from a neuron comprising an A? nerve fiber or a C nerve fiber, wherein the chimeric clostridial neurotoxin binds to the neuron comprising the A? nerve fiber or the C nerve fiber, respectively, and wherein the chimeric clostridial neurotoxin comprises a botulinum neurotoxin A (BoNT/A) light-chain and translocation domain (HN domain), and a BoNT/B receptor binding domain (Hc domain).

---

5. claims: 71-75(completely); 76-86(partially)

A unit dosage form (e.g. for treating pain), the unit dosage form comprising:a. 5 pg to 17,000 pg of a chimeric clostridial neurotoxin; orb. 0.2 Units up to 707 Units of a chimeric clostridial neurotoxin, wherein 1 Unit is an amount of the chimeric clostridial neurotoxin that corresponds to the calculated median lethal dose (LD50) in mice; andc.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

optionally a pharmaceutically acceptable carrier, excipient, adjuvant, and/or salt;wherein the chimeric clostridial neurotoxin comprises a botulinum neurotoxin A (BoNT/A) light-chain and translocation domain (HN domain), and a BoNT/B receptor binding domain (Hc domain).

---

6. claim: 87

A method for determining whether or not a clostridial neurotoxin is suitable for treating pain, the method comprising:(a) comparing a level of calcitonin gene-related peptide (CGRP) comprised in a first sample with the level of CGRP comprised in a second sample, wherein the first sample has been obtained from a subject prior to administration of the clostridial neurotoxin, and wherein the second sample has been obtained from the same subject after administration of the clostridial neurotoxin; and(b) determining that the clostridial neurotoxin is suitable for treating pain when the level of CGRP in the second sample is lower than the level of CGRP in the first sample; or(c) determining that the clostridial neurotoxin is unsuitable for treating pain when the level of CGRP in the second sample is not lower (e.g. is higher or the same) than the level of CGRP in the first sample.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/GB2022/052957

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2021064369 A1	08-04-2021	AU 2020357905 A1	24-03-2022
		CA 3153670 A1	08-04-2021
		CN 114502574 A	13-05-2022
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		KR 20220070284 A	30-05-2022
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		ES 2866899 T3	20-10-2021
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		BR 112022018456 A2	01-11-2022
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		TW 202140523 A	01-11-2021
		WO 2021186160 A2	23-09-2021

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/GB2022/052957

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2022200809 A1	29-09-2022	NONE	
WO 2022208091 A1	06-10-2022	NONE	
WO 2022208039 A1	06-10-2022	NONE	

(19)国家知识产权局

(12)发明专利申请

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(43)申请公布日 2024.07.02

(21)申请号 202280077178.9
(22)申请日 2022.11.22
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2206359.8 2022.04.29 GB
PCT/GB2022/052947 2022.11.21 GB
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2024.05.21
(86)PCT国际申请的申请数据
PCT/GB2022/052957 2022.11.22
(87)PCT国际申请的公布数据
WO2023/089343 EN 2023.05.25
(71)申请人 益普生生物制药有限公司
地址 英国
(72)发明人 E·方弗里亚苏比罗斯 J·克虏伯 J·C·梅盖尔 L·庞斯 V·马丁
(74)专利代理机构 北京市中咨律师事务所 11247
专利代理师 黄革生 隋晓平
(51)Int.Cl.
A61K 38/48(2006.01)
A61P 29/02(2006.01)

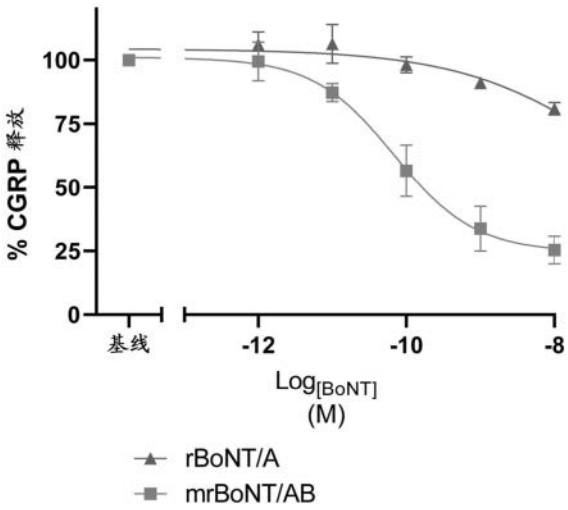
权利要求书9页 说明书107页
序列表（电子公布） 附图12页

(54)发明名称

疼痛的治疗

(57)摘要

本发明尤其涉及疼痛的治疗。例如，提供了用于通过抑制疼痛介质从包含Aδ神经纤维或C神经纤维的神经元释放来治疗疼痛的嵌合梭菌神经毒素，其中所述嵌合梭菌神经毒素分别结合至包含Aδ神经纤维或C神经纤维的神经元，并且其中所述嵌合梭菌神经毒素包含肉毒杆菌神经毒素A(BoNT/A)轻链和转位结构域(H_N结构域)以及BoNT/B受体结合结构域(H_C结构域)。还提供了方法、用途、试剂盒和单位剂型。



CN 118284427 A

1.一种用于治疗疼痛的嵌合梭菌神经毒素,其中所述嵌合梭菌神经毒素包含肉毒杆菌神经毒素A(BoNT/A)轻链和转位结构域(H_N结构域)以及BoNT/B受体结合结构域(H_C结构域)。

2.一种治疗疼痛的方法,所述方法包括向受试者施用嵌合梭菌神经毒素,其中所述嵌合梭菌神经毒素包含肉毒杆菌神经毒素A(BoNT/A)轻链和转位结构域(H_N结构域)以及BoNT/B受体结合结构域(H_C结构域)。

3.嵌合梭菌神经毒素在制备用于治疗疼痛的药物中的用途,其中所述嵌合梭菌神经毒素包含肉毒杆菌神经毒素A(BoNT/A)轻链和转位结构域(H_N结构域)以及BoNT/B受体结合结构域(H_N结构域)。

4.根据权利要求1-3中任一项所述用于所述用途的嵌合梭菌神经毒素、方法或用途,其中所述嵌合梭菌神经毒素通过抑制疼痛介质从包含Aδ神经纤维或C神经纤维的神经元的释放来治疗疼痛,其中所述嵌合梭菌神经毒素分别结合至包含Aδ神经纤维或C神经纤维的神经元。

5.根据前述权利要求中任一项所述用于所述用途的嵌合梭菌神经毒素、方法或用途,其中所述嵌合梭菌神经毒素通过抑制中枢神经系统神经元的分泌、优选通过抑制介质、更优选疼痛介质从中枢神经系统神经元的分泌来治疗疼痛。

6.一种用于治疗偏头痛(优选偏头痛疼痛)的嵌合梭菌神经毒素,其中所述嵌合梭菌神经毒素包含肉毒杆菌神经毒素A(BoNT/A)轻链和转位结构域(H_N结构域)以及BoNT/B受体结合结构域(H_C结构域)。

7.一种治疗偏头痛(优选偏头痛疼痛)的方法,所述方法包括向受试者施用嵌合梭菌神经毒素,其中所述嵌合梭菌神经毒素包含肉毒杆菌神经毒素A(BoNT/A)轻链和转位结构域(H_N结构域)以及BoNT/B受体结合结构域(H_C结构域)。

8.嵌合梭菌神经毒素在制备用于治疗偏头痛(优选偏头痛疼痛)的药物中的用途,其中所述嵌合梭菌神经毒素包含肉毒杆菌神经毒素A(BoNT/A)轻链和转位结构域(H_N结构域)以及BoNT/B受体结合结构域(H_C结构域)。

9.根据权利要求6至8中任一项所述用于所述用途的嵌合梭菌神经毒素、方法或用途,其中所述嵌合梭菌神经毒素通过抑制疼痛介质从包含Aδ神经纤维或C神经纤维的神经元的释放来治疗偏头痛,其中所述嵌合梭菌神经毒素分别结合至包含Aδ神经纤维或C神经纤维的神经元。

10.根据权利要求6至9中任一项所述用于所述用途的嵌合梭菌神经毒素、方法或用途,其中所述嵌合梭菌神经毒素通过抑制中枢神经系统神经元的分泌、优选通过抑制介质、更优选疼痛介质从中枢神经系统神经元的分泌来治疗偏头痛。

11.一种嵌合梭菌神经毒素,其用于治疗受试者的病症的方法,与用BoNT/A治疗受试者相比,所述方法持续时间更长和/或具有更好的疗效,所述方法包括向受试者施用嵌合梭菌神经毒素,其中所述嵌合梭菌神经毒素包含肉毒杆菌神经毒素A(BoNT/A)轻链和转位结构域(H_N结构域)以及BoNT/B受体结合结构域(H_C结构域)。

12.一种用于治疗受试者的病症的方法,与用BoNT/A治疗的受试者相比,所述方法治疗受试者的病症的持续时间更长和/或具有更好的疗效,所述方法包括向受试者施用嵌合梭菌神经毒素,其中所述嵌合梭菌神经毒素包含肉毒杆菌神经毒素A(BoNT/A)轻链和转位结构域(H_N结构域)以及BoNT/B受体结合结构域(H_C结构域)。

13.嵌合梭菌神经毒素在制备用于治疗受试者的病症的药物中的用途,与用BoNT/A治疗的受试者相比,所述药物治疗受试者的病症的持续时间更长和/或具有更好的疗效,其中嵌合梭菌神经毒素包含肉毒杆菌神经毒素A(BoNT/A)轻链和转位结构域(H_N结构域)以及BoNT/B受体结合结构域(H_C结构域)。

14.一种嵌合梭菌神经毒素,其用于减轻受试者疼痛的方法,与用BoNT/A治疗受试者相比,所述方法更大程度地减轻受试者的疼痛,所述方法包括向受试者施用嵌合梭菌神经毒素,其中所述嵌合梭菌神经毒素包含肉毒杆菌神经毒素A(BoNT/A)轻链和转位结构域(H_N结构域)以及BoNT/B受体结合结构域(H_C结构域)。

15.一种减轻受试者疼痛的方法,与用BoNT/A治疗受试者相比,所述方法更大程度地减轻受试者的疼痛,所述方法包括向受试者施用嵌合梭菌神经毒素,其中嵌合梭菌神经毒素包含肉毒杆菌神经毒素A(BoNT/A)轻链和转位结构域(H_N结构域)以及BoNT/B受体结合结构域(H_C结构域)。

16.嵌合梭菌神经毒素在制备用于减轻受试者的疼痛的药物中的用途,与用BoNT/A治疗的受试者相比,所述药物更大程度地减轻受试者的疼痛,其中所述嵌合梭菌神经毒素包含肉毒杆菌神经毒素A(BoNT/A)轻链和转位结构域(H_N结构域)以及BoNT/B受体结合结构域(H_C结构域)。

17.一种嵌合梭菌神经毒素,其用于减少受试者的生物流体和/或脑中疼痛介质的量的方法,与BoNT/A治疗的受试者的相同生物流体和/或脑中相同疼痛介质减少的量相比,所述方法更大程度地减少受试者的生物流体和/或脑中疼痛介质的量,所述方法包括向受试者施用嵌合梭菌神经毒素,其中嵌合梭菌神经毒素包含肉毒杆菌神经毒素A(BoNT/A)轻链和转位结构域(H_N结构域)以及BoNT/B受体结合结构域(H_C结构域)。

18.一种用于减少受试者的生物流体和/或脑中疼痛介质的量的方法,与用BoNT/A治疗的受试者的相同生物流体和/或脑中相同疼痛介质减少的量相比,所述方法更大程度地减少受试者的生物流体和/或脑中疼痛介质的量,所述方法包括向受试者施用嵌合梭菌神经毒素,其中嵌合梭菌神经毒素包含肉毒杆菌神经毒素A(BoNT/A)轻链和转位结构域(H_N结构域)以及BoNT/B受体结合结构域(H_C结构域)。

19.嵌合梭菌神经毒素在制备用于减少受试者的生物流体和/或脑中疼痛介质的量的药物中的用途,与用BoNT/A治疗的受试者的相同生物流体和/或脑中相同疼痛介质减少的量相比,所述药物更大程度地减少受试者的生物流体和/或脑中疼痛介质的量,其中所述嵌合梭菌神经毒素包含肉毒杆菌神经毒素A(BoNT/A)轻链和转位结构域(H_N结构域)以及BoNT/B受体结合结构域(H_C结构域)。

20.根据权利要求11至19中任一项所述用于所述用途的嵌合梭菌神经毒素、方法或用途,其中所述嵌合梭菌神经毒素抑制介质(优选疼痛介质)从包含Aδ神经纤维或C神经纤维的神经元的释放,其中所述嵌合梭菌神经毒素分别结合至包含Aδ神经纤维或C神经纤维的神经元。

21.根据权利要求11至20中任一项所述用于所述用途的嵌合梭菌神经毒素、方法或用途,其中所述嵌合梭菌神经毒素抑制中枢神经系统神经元的分泌、优选抑制介质、更优选疼痛介质从中枢神经系统神经元的分泌。

22.根据前述权利要求中任一项所述用于所述用途的嵌合梭菌神经毒素、方法或用途,

其中所述嵌合梭菌神经毒素通过神经元(例如逆向)运输进入中枢神经系统的神经元并切割所述神经元的SNARE蛋白(例如SNAP25)。

23.一种嵌合梭菌神经毒素,其用于通过抑制介质从包含Aδ神经纤维或C神经纤维的神经元释放来治疗感觉障碍的方法,其中所述嵌合梭菌神经毒素分别结合至包含Aδ神经纤维或C神经纤维的神经元,并且其中所述嵌合梭菌神经毒素包含肉毒杆菌神经毒素A(BoNT/A)轻链和转位结构域(H_N结构域)以及BoNT/B受体结合结构域(H_C结构域)。

24.一种通过抑制介质从包含Aδ神经纤维或C神经纤维的神经元释放来治疗感觉障碍的方法,所述方法包括向受试者施用所述嵌合梭菌神经毒素,其中所述嵌合梭菌神经毒素分别结合至包含Aδ神经纤维或C神经纤维的神经元,并且其中所述嵌合梭菌神经毒素包含肉毒杆菌神经毒素A(BoNT/A)轻链和转位结构域(H_N结构域)以及BoNT/B受体结合结构域(H_C结构域)。

25.嵌合梭菌神经毒素在制备用于通过抑制介质从包含Aδ神经纤维或C神经纤维的神经元中释放来治疗感觉障碍的药物中的用途,其中所述嵌合梭菌神经毒素分别结合至包含Aδ神经纤维或C神经纤维的神经元,并且其中所述嵌合梭菌神经毒素包含肉毒杆菌神经毒素A(BoNT/A)轻链和转位结构域(H_N结构域)以及BoNT/B受体结合结构域(H_C结构域)。

26.根据权利要求1-5或11-25中任一项所述用于所述用途的嵌合梭菌神经毒素、方法或用途,其中所述疼痛或病症是头痛疼痛如偏头痛疼痛或丛集性头痛,或膀胱疼痛。

27.根据权利要求1-5或11-26中任一项所述用于所述用途的嵌合梭菌神经毒素、方法或用途,其中所述疼痛或病症是偏头痛疼痛。

28.根据权利要求1-3、5-8、10-19、21-22或26-27中任一项所述用于所述用途的嵌合梭菌神经毒素、方法或用途,其中所述嵌合梭菌神经毒素结合至包含Aδ神经纤维或C神经纤维的神经元。

29.根据权利要求1-3、5-8、10-19、21-22或26-27中任一项所述用于所述用途的嵌合梭菌神经毒素、方法或用途,其中所述嵌合梭菌神经毒素抑制介质从包含Aδ神经纤维或C神经纤维的神经元的释放。

30.根据前述权利要求中任一项所述用于所述用途的嵌合梭菌神经毒素、方法或用途,其中所述嵌合梭菌神经毒素通过抑制介质(例如疼痛介质)从包含Aδ神经纤维或C神经纤维的神经元的释放和通过抑制(例如介质,优选疼痛介质)从中枢神经系统神经元的分泌来治疗疼痛、偏头痛或病症,其中所述嵌合梭菌神经毒素分别结合至包含Aδ神经纤维或C神经纤维的神经元。

31.根据前述权利要求中任一项所述用于所述用途的嵌合梭菌神经毒素、方法或用途,其中所述介质(优选疼痛介质)是神经递质(优选疼痛神经递质)。

32.根据前述权利要求中任一项所述用于所述用途的嵌合梭菌神经毒素、方法或用途,其中所述(疼痛)介质是选自以下的一种或多种:降钙素基因相关肽(CGRP);P物质;和神经激肽。

33.根据前述权利要求中任一项所述用于所述用途的嵌合梭菌神经毒素、方法或用途,其中所述(疼痛)介质是CGRP并且所述疼痛是CGRP相关疼痛或所述病症是CGRP相关疼痛,或其中当治疗偏头痛时,治疗CGRP相关偏头痛疼痛。

34.根据权利要求33所述用于所述用途的嵌合梭菌神经毒素、方法或用途,其中所述

CGRP相关疼痛是CGRP相关头痛疼痛。

35.根据权利要求33或34所述用于所述用途的嵌合梭菌神经毒素、方法或用途,其中所述CGRP相关疼痛是CGRP相关偏头痛疼痛。

36.根据权利要求33至35中任一项所述用于所述用途的嵌合梭菌神经毒素、方法或用途,其中所述CGRP相关疼痛是:

(a)CGRP相关躯体疼痛选自:头痛疼痛(例如外伤后头痛、头部受伤头痛或外伤性脑损伤后头痛)、关节炎疼痛(例如骨关节炎疼痛和/或类风湿性关节炎疼痛)、运动疼痛、退行性椎间盘疾病疼痛、腕管压迫疼痛、软组织损伤疼痛、颞下颌关节疼痛、肌肉骨骼疼痛、由血管病症(例如雷诺氏综合征、血栓闭塞性脉管炎、外周静脉疾病、外周动脉疾病、静脉曲张、静脉血栓、凝血障碍或淋巴水肿)引起或与之相关的CGRP相关躯体疼痛、面部疼痛、由三叉神经自主神经性头痛引起或与之相关的CGRP相关躯体疼痛;由三叉神经痛引起或与之相关的CGRP相关躯体疼痛;和CGRP相关的癌症引起的疼痛(例如CGRP相关的癌症引起的骨痛);

(b)CGRP相关内脏疼痛选自:子宫内膜异位症疼痛、胰腺炎疼痛、胃肠道疼痛和由血管病症引起或与之相关的CGRP相关内脏疼痛;

(c)CGRP相关炎性疼痛选自:慢性疼痛、伤口愈合疼痛、瘙痒疼痛和烧伤疼痛;和/或

(d)CGRP相关神经性疼痛选自:带状疱疹后神经痛、糖尿病疼痛、慢性神经性疼痛和莫顿神经瘤疼痛。

37.根据前述权利要求中任一项所述用于所述用途的嵌合梭菌神经毒素、方法或用途,其中所述神经元是三叉神经节的神经元。

38.根据前述权利要求中任一项所述用于所述用途的嵌合梭菌神经毒素、方法或用途,其中将所述嵌合梭菌神经毒素施用于面部、颈部和/或颅骨。

39.根据前述权利要求中任一项所述用于所述用途的嵌合梭菌神经毒素、方法或用途,其中所述嵌合梭菌神经毒素皮内施用。

40.根据前述权利要求中任一项所述用于所述用途的嵌合梭菌神经毒素、方法或用途,其中所述嵌合梭菌神经毒素通过在每次治疗期间至多10个注射部位皮内注射施用。

41.根据权利要求1至39中任一项所述用于所述用途的嵌合梭菌神经毒素、方法或用途,其中所述嵌合梭菌神经毒素通过在每次治疗期间的10-40个注射部位皮内注射施用。

42.根据权利要求1至39或41中任一项所述用于所述用途的嵌合梭菌神经毒素、方法或用途,其中所述嵌合梭菌神经毒素通过在每次治疗期间的25-35个注射部位皮内注射施用。

43.根据权利要求1至38中任一项所述用于所述用途的嵌合梭菌神经毒素、方法或用途,其中所述嵌合梭菌神经毒素肌内施用。

44.根据权利要求1至38或43中任一项所述用于所述用途的嵌合梭菌神经毒素、方法或用途,其中嵌合梭菌神经毒素通过在每次治疗期间最多10个注射部位肌内注射施用。

45.根据权利要求1至38或43中任一项所述用于所述用途的嵌合梭菌神经毒素、方法或用途,其中所述嵌合梭菌神经毒素通过在每次治疗期间的10-40个注射部位肌内注射施用。

46.根据权利要求1至38、43或45中任一项所述用于所述用途的嵌合梭菌神经毒素、方法或用途,其中所述嵌合梭菌神经毒素通过在每次治疗期间的25-35个注射部位肌内注射施用。

47.根据前述权利要求中任一项所述用于所述用途的嵌合梭菌神经毒素、方法或用途,

其包括将嵌合梭菌神经毒素施用至以下的至少一块:额肌肌肉、皱眉肌(例如皱眉肌(corrugator supercilii))肌肉、降眉间肌(例如降眉间肌-鼻肌(procerus nasalis))、枕肌(例如上或下枕肌)肌肉、颞肌肌肉、斜方肌(例如上、中或下斜方肌)肌肉、咬肌肌肉、鼻肌肌肉、眼轮匝肌肌肉、颈椎旁肌肌肉、颞筋膜肌肉、耳上肌肌肉、耳前肌肌肉、耳后肌肌肉、胸锁乳突肌肌肉、颈阔肌肌肉、前鼻孔扩张肌肌肉、后鼻孔扩张肌肌肉、降鼻中隔肌肌肉、颏肌肌肉、口轮匝肌肌肉、颧肌肌肉、笑肌肌肉、颊肌肌肉、枕额肌肌肉、提上唇肌肌肉、降下唇肌肌肉、降口角肌肌肉、甲状舌骨肌肌肉、肩胛舌骨肌肌肉、胸骨舌骨肌肌肉、颈夹肌肌肉、头夹肌肌肉、颈半棘肌肌肉、头半棘肌肌肉、肩胛提肌肌肉、二腹肌肌肉或斜角肌肌肉。

48.根据前述权利要求中任一项所述用于所述用途的嵌合梭菌神经毒素、方法或用途,其包括将嵌合梭菌神经毒素施用至以下至少一块:额肌肌肉、皱眉肌(例如皱眉肌(corrugator supercilii))肌肉、鼻肌肌肉、眼轮匝肌肌肉、颞肌肌肉、枕肌肌肉或斜方肌肌肉。

49.根据前述权利要求中任一项所述用于所述用途的嵌合梭菌神经毒素、方法或用途,其包括将嵌合梭菌神经毒素施用至:额肌肌肉、皱眉肌(例如皱眉肌(corrugator supercilii))肌肉、鼻肌肌肉、眼轮匝肌肌肉、颞肌肌肉、枕肌肌肉和斜方肌肌肉。

50.根据前述权利要求中任一项所述用于所述用途的嵌合梭菌神经毒素、方法或用途,其中所述嵌合梭菌神经毒素的施用包括:

(a)注射2次至额肌肌肉(优选每块额肌肌内注射2次);

(b)注射1次至皱眉肌肌肉(优选每块皱眉肌肌内注射1次);

(c)注射1次至鼻肌肌肉(优选每块鼻肌肌内注射1次);

(d)注射1次至眼轮匝肌肌肉(优选每块眼轮匝肌肌内注射1次);

(e)注射4次至颞肌肌肉(优选每块颞肌肌内注射4次);

(f)注射3次至枕肌肌肉(优选每块枕肌肌内注射3次);和

(g)注射2次至斜方肌肌肉(优选每块斜方肌肌内注射2次)。

51.根据前述权利要求中任一项所述用于所述用途的嵌合梭菌神经毒素、方法或用途,其中所述嵌合梭菌神经毒素的施用包括:

(a)注射4次至额肌肌肉(优选注射2次至面部第一侧的额肌肌肉,以及注射2次至面部第二侧的额肌肌肉);

(b)注射2次至皱眉肌肌肉(优选注射1次至面部第一侧的皱眉肌肌肉,以及注射1次至面部第二侧的皱眉肌肌肉);

(c)注射2次至鼻肌肌肉(优选注射1次至面部第一侧的鼻肌肌肉,以及注射1次至面部第二侧的鼻肌肌肉));

(d)注射2次至眼轮匝肌肌肉(优选注射1次至面部第一侧的眼轮匝肌肌肉,以及注射1次至面部第二侧的眼轮匝肌肌肉);

(e)注射8次至颞肌肌肉(优选注射4次至头部第一侧的颞肌肌肉,以及注射4次至头部第二侧的颞肌肌肉);

(f)注射6次至枕肌肌肉(优选注射3次至头部第一侧的枕肌肌肉,以及注射3次至头部第二侧的枕肌肌肉);和

(g)注射4次至斜方肌肌肉(优选注射2次至颈部第一侧的斜方肌肌肉,以及注射2次至

颈部第二侧的斜方肌肌肉)。

52.根据前述权利要求中任一项所述用于所述用途的嵌合梭菌神经毒素、方法或用途,其中所述嵌合梭菌神经毒素通过(例如每个注射部位)每次注射单位剂量的方式施用。

53.根据前述权利要求中任一项所述用于所述用途的嵌合梭菌神经毒素、方法或用途,其中所述嵌合梭菌神经毒素的施用包括施用:

(a)2个单位剂量至额肌肌肉(优选每块额肌肌肉2个单位剂量);

(b)1个单位剂量至皱眉肌肌肉(优选每块皱眉肌肌肉1个单位剂量);

(c)1个单位剂量至鼻肌肌肉(优选每块鼻肌肌肉1个单位剂量);

(d)1个单位剂量至眼轮匝肌肌肉(优选每块眼轮匝肌肌肉1个单位剂量);

(e)4个单位剂量至颞肌肌肉(优选每块颞肌肌肉4个单位剂量);

(f)3个单位剂量至枕肌肌肉(优选每块枕肌肌肉3个单位剂量);和

(g)2个单位剂量至斜方肌肌肉(优选每块斜方肌肌肉2个单位剂量)。

54.根据前述权利要求中任一项所述用于所述用途的嵌合梭菌神经毒素、方法或用途,其中所述嵌合梭菌神经毒素的施用包括:

(a)4个单位剂量至额肌肌肉(优选2个单位剂量至面部第一侧额肌肌肉,以及2个单位剂量至面部第二侧额肌肌肉);

(b)2个单位剂量至皱眉肌肌肉(优选1个单位剂量至面部第一侧的皱眉肌肌肉,以及1个单位剂量至面部第二侧的皱眉肌肌肉);

(c)2个单位剂量至鼻肌肌肉(优选1个单位剂量至面部第一侧的鼻肌肌肉,以及1个单位剂量至面部第二侧的鼻肌肌肉);

(d)2个单位剂量至眼轮匝肌肌肉(优选1个单位剂量至面部第一侧的眼轮匝肌肌肉,以及1个单位剂量至面部第二侧的眼轮匝肌肌肉);

(e)8个单位剂量至颞肌肌肉(优选4个单位剂量至头部第一侧的颞肌肌肉,以及4个单位剂量至头部第二侧的颞肌肌肉);

(f)6个单位剂量至枕肌肌肉(优选3个单位剂量至头部第一侧的枕肌肌肉,以及3个单位剂量至头部第二侧的枕肌肌肉);和

(g)4个单位剂量至斜方肌肌肉(优选2个单位剂量至颈部第一侧的斜方肌肌肉,以及2个单位剂量至颈部第二侧的斜方肌肌肉)。

55.根据前述权利要求中任一项所述用于所述用途的嵌合梭菌神经毒素、方法或用途,其中所述嵌合梭菌神经毒素以5pg至17,000pg嵌合梭菌神经毒素的单位剂量施用。

56.根据前述权利要求中任一项所述用于所述用途的嵌合梭菌神经毒素、方法或用途,其中所述嵌合梭菌神经毒素以500pg至17,000pg的单位剂量施用。

57.根据前述权利要求中任一项所述用于所述用途的嵌合梭菌神经毒素、方法或用途,其中所述嵌合梭菌神经毒素以1,000pg至17,000pg的单位剂量施用。

58.根据前述权利要求中任一项所述用于所述用途的嵌合梭菌神经毒素、方法或用途,其中每次治疗期间施用的总剂量为最多255,000pg的嵌合梭菌神经毒素,例如3,640-255,000pg或最多160,000pg(优选最多155,000pg)。

59.根据前述权利要求中任一项所述用于所述用途的嵌合梭菌神经毒素、方法或用途,其中每次治疗期间施用的总剂量为最多120,000pg,优选最多112,000pg。

60.根据前述权利要求中任一项所述用于所述用途的嵌合梭菌神经毒素、方法或用途，其中每次治疗期间施用的总剂量为最多100,000pg,优选最多70,000pg。

61.根据前述权利要求中任一项所述用于所述用途的嵌合梭菌神经毒素、方法或用途，其中所述嵌合梭菌神经毒素以3,640pg至17,000pg的单位剂量施用。

62.根据前述权利要求中任一项所述用于所述用途的嵌合梭菌神经毒素、方法或用途，其中所述嵌合梭菌神经毒素以1,000至5,500pg的单位剂量施用。

63.根据前述权利要求中任一项所述用于所述用途的嵌合梭菌神经毒素、方法或用途，其中所述嵌合梭菌神经毒素以2,000至4,500pg的单位剂量施用。

64.根据前述权利要求中任一项所述用于所述用途的嵌合梭菌神经毒素、方法或用途，其中所述嵌合梭菌神经毒素以3,500至4,500pg或2,000至3,000pg(例如2,500pg)的单位剂量施用。

65.根据前述权利要求中任一项所述用于所述用途的嵌合梭菌神经毒素、方法或用途，其中所述嵌合梭菌神经毒素以4,000pg的单位剂量施用。

66.根据权利要求47至65中任一项所述用于所述用途的嵌合梭菌神经毒素、方法或用途,其中向肌肉的施用(例如向其注射)是:通过肌内注射或通过在肌肉区域皮内注射;优选通过肌内注射。

67.根据权利要求1至38或47至65中任一项所述用于所述用途的嵌合梭菌神经毒素、方法或用途,其中所述嵌合梭菌神经毒素通过神经内、神经周围或通过神经节周围施用来施用。

68.根据权利要求1至38、47至65或67中任一项所述用于所述用途的嵌合梭菌神经毒素、方法或用途,其中所述嵌合梭菌神经毒素被施用至三叉神经、半月神经节、中间神经、舌咽神经、迷走神经和/或经由枕神经施用至上颈根。

69.根据权利要求1至38或47至65中任一项所述用于所述用途的嵌合梭菌神经毒素、方法或用途,其中所述嵌合梭菌神经毒素通过血管周围施用来施用。

70.根据前述权利要求中任一项所述用于所述用途的嵌合梭菌神经毒素、方法或用途，其中所述治疗是预防性治疗,优选偏头痛的预防性治疗。

71.一种(例如用于治疗疼痛的)单位剂型,所述单位剂型包含:

a.5pg至17,000pg的嵌合梭菌神经毒素;或

b.0.2单位至最多707单位的嵌合梭菌神经毒素,其中1单位是对应于计算的小鼠半数致死剂量(LD_{50})的嵌合梭菌神经毒素的量;和

c.任选地药学上可接受的载体、赋形剂、佐剂和/或盐;

其中所述嵌合梭菌神经毒素包含肉毒杆菌神经毒素A(BoNT/A)轻链和转位结构域(H_N结构域)以及BoNT/B受体结合结构域(H_C结构域)。

72.根据权利要求71所述的单位剂型,其中所述单位剂型包含:

a.1,000pg至5,500pg的嵌合梭菌神经毒素;或

b.42单位至最多229单位的嵌合梭菌神经毒素,其中1单位是对应于计算的小鼠半数致死剂量(LD_{50})的嵌合梭菌神经毒素的量。

73.根据权利要求71或72所述的单位剂型,其中所述单位剂型包含2,000至4,500pg的嵌合梭菌神经毒素。

74.根据权利要求71至73中任一项所述的单位剂型,其中所述单位剂型包含3,500至4,500pg或2,000至3,000pg(例如2,500pg)的嵌合梭菌神经毒素。

75.根据权利要求71至74中任一项所述的单位剂型,其中所述单位剂型包含4,000pg的嵌合梭菌神经毒素。

76.根据前述权利要求中任一项所述用于所述用途的嵌合梭菌神经毒素、方法、用途或单位剂型,其中嵌合梭菌神经毒素具有大于7的安全比(优选至少10的安全比),其中所述安全比计算为:以pg/小鼠测量的-10%体重变化所需的毒素剂量除以以pg/小鼠测量的DAS ED_{50},其中ED_{50}=产生DAS得分为2所需的剂量。

77.根据前述权利要求中任一项所述用于所述用途的嵌合梭菌神经毒素、方法、用途或单位剂型,其中所述H_N结构域的C-末端氨基酸残基对应于分隔BoNT/A的H_N和H_C结构域的3_{10}螺旋的第一个氨基酸残基,并且其中所述H_C结构域的N-末端氨基酸残基对应于分隔BoNT/B的H_N和H_C结构域的3_{10}螺旋的第二个氨基酸残基。

78.根据前述权利要求中任一项所述用于所述用途的嵌合梭菌神经毒素、方法、用途或单位剂型,其中所述嵌合梭菌神经毒素包含与SEQ ID NO:1具有至少70%序列同一性的多肽序列。

79.根据前述权利要求中任一项所述用于所述用途的嵌合梭菌神经毒素、方法、用途或单位剂型,其中所述嵌合梭菌神经毒素是双链嵌合梭菌神经毒素,其中轻链(L-链)通过可由以下方法获得的二硫键连接至重链(H-链),所述方法包括将包含SEQ ID NO:1的单链嵌合梭菌神经毒素与水解其活化环中的肽键的蛋白酶接触,从而将单链嵌合梭菌神经毒素转化为相应的双链嵌合梭菌神经毒素。

80.根据前述权利要求中任一项所述用于所述用途的嵌合梭菌神经毒素、方法、用途或单位剂型,其中所述嵌合梭菌神经毒素是双链嵌合梭菌神经毒素,其中L-链通过可由以下方法获得的二硫键连接至H-链,所述方法包括将由SEQ ID NO:1组成的单链嵌合梭菌神经毒素与水解其活化环中的肽键的蛋白酶接触,从而将单链嵌合梭菌神经毒素转化为相应的双链嵌合梭菌神经毒素。

81.根据前述权利要求中任一项所述用于所述用途的嵌合梭菌神经毒素、方法、用途或单位剂型,其中所述BoNT/B H_C结构域包含一个或多个选自以下的取代突变:E1191M;S1199Y;V1118M;Y1183M;E1191I;E1191Q;E1191T;S1199F;S1199L;S1201V;及其组合。

82.根据前述权利要求中任一项所述用于所述用途的嵌合梭菌神经毒素、方法、用途或单位剂型,其中所述BoNT/B H_C结构域包含在E1191M和S1199Y处的取代突变。

83.根据前述权利要求中任一项所述用于所述用途的嵌合梭菌神经毒素、方法、用途或单位剂型,其中所述嵌合梭菌神经毒素的多肽序列的起始甲硫氨酸氨基酸残基是任选的。

84.根据前述权利要求中任一项所述用于所述用途的嵌合梭菌神经毒素、方法、用途或单位剂型,其中所述嵌合梭菌神经毒素的多肽序列的起始甲硫氨酸氨基酸残基是不存在的。

85.根据前述权利要求中任一项所述用于所述用途的嵌合梭菌神经毒素、方法、用途或单位剂型,其中所述嵌合梭菌神经毒素是包含轻链和重链(或由轻链和重链组成)的双链嵌合梭菌神经毒素,所述轻链包含SEQ ID NO:17或18(优选SEQ ID NO:17),所述重链包含SEQ ID NO:19,其中所述轻链和重链通过二硫键连接在一起。

86.一种试剂盒,其包含:

(a)根据权利要求71-85中任一项所述的单位剂型;和

(b)使用其例如治疗疼痛的说明书;以及

(c)任选的稀释剂。

87.一种确定梭菌神经毒素是否适于治疗疼痛的方法,所述方法包括:

(a)比较第一样品中包含的降钙素基因相关肽(CGRP)水平与第二样品中包含的CGRP水平,其中第一样品在施用梭菌神经毒素之前从受试者获得,并且其中第二样品在施用梭菌神经毒素之后从相同受试者获得;和

(b)当第二样品中的CGRP水平低于第一样品中的CGRP水平时,确定所述梭菌神经毒素适合治疗疼痛;或者

(c)当第二样品中的CGRP水平不低于(例如,高于或等于)第一样品中的CGRP水平时,确定所述梭菌神经毒素不适合治疗疼痛。

疼痛的治疗

技术领域

[0001] 疼痛是与实际或潜在的组织损伤相关或类似的令人不愉快的感觉和情绪体验。疼痛还被描述为神经系统病况(neurologic condition),其特点是神经系统的病理变化,或更确切地,内源性伤害性感受系统的功能障碍(Raffaeli&Arnaudo(2017),J Pain Res,10,2003-2008)。

[0002] 伤害性感受是将关于实际组织损伤(或如果继续施加有害刺激,则可能造成这种损伤)的信息传递给脑部的过程。参与伤害性感受的感觉神经元分为三个主要组:A组;B组;和C组(Yam等人(2018),Int J Mol Sci,19,8,2164)。

[0003] A组神经纤维被分类为有髓纤维,可进一步细分为Aα、Aβ、Aγ和Aδ,各自具有不同的特征集合。这些纤维通常终止于脊髓背角的I、III、IV和V层,具有一些II层内突出物。来自肌肉纺锤体末端和高尔基体腱的Ia型和Ib型感觉纤维都是Aα型。Aβ型纤维通常是低阈值的、皮肤的、缓慢或快速适应的机械性感受器,并且包括来自牵张感受器的II型传入纤维。Aβ-纤维通常属于III层和IV层。Aγ型纤维可包括来自牵张感受器的II型传入纤维。Aδ型纤维可以包括终止于rexed分层的I层和V层的热和机械伤害性感受器,以及III型传入纤维。Aδ-纤维通常也是最小的有髓神经,并且可以具有~30m/s的相对快的传导速度。Aδ-纤维的直径通常为约2-5μm,并且通常对短暂疼痛和刺痛敏感。

[0004] B组神经纤维通常中度髓鞘化,具有3-14m/s的传导速度。自主神经系统(ANS)的节前神经纤维和一般内脏传入纤维属于该组。

[0005] C组神经纤维是无髓鞘的,直径通常小于2μm,传导速度相对较慢,通常至多约2m/s。ANS中背根的神经纤维(IV型传入纤维)和节后纤维可以归类于该组中。所有这些纤维在功能上主要是伤害性感受性的,携带感觉信息并聚集大约70%的传入伤害性感受性信息,然后传入伤害性感受性信息进入脊髓。C-纤维可终止于脊髓灰质中的I层和II层。就伤害性感受而言,C-纤维伤害性感受器可以是多模态的,因为它们被热、机械和/或化学刺激激活。例如,C-纤维可通过不良局部刺激而被激活。就神经化学而言,C-纤维可分类为肽能或非肽能的,并且这些纤维中约50%表达神经肽,包括降钙素基因相关肽(CGRP)、神经激肽和P物质(SP)。

[0006] 疼痛中涉及多种神经递质,包括所有主要类型的神经递质,例如炎性介质:前列腺素E2(PGE2)、前列环素(PGI2)、白三烯B4(LTB4)、神经生长因子(NGF)、质子、缓激肽(BK)、ATP、腺苷、SP、神经激肽A(NKA)、神经激肽B(NKB)、5-羟色胺(5-HT)、组胺、谷氨酸盐、去甲肾上腺素(NE)和一氧化氮(NO);和非炎性介质:CGRP、γ-氨基丁酸(GABA)、阿片样肽、甘氨酸和大麻素(Yam等人(2018),Int J Mol Sci,19,8,2164)。

[0007] 特别有治疗意义的是CGRP,其在中枢和外周神经系统中广泛产生;然而,它主要位于初级传入神经中。作为背根神经节(DRG)的直接衍生物,CGRP可以在脊髓的背角中发现并且与有害刺激的传导相关。CGRP与SP的兴奋作用有关,其导致Ca^{2+}释放。CGRP的受体(降钙素受体样受体(CALCRL))通常位于伏隔核中,表明CNS可以控制CGRP介导的疼痛传递。CGRP广泛分布于外周和中枢神经系统,其受体在疼痛通路中表达。CGRP样免疫反应性(CGRP-LI)通

常在40-50％的DRG神经元中发现。此外,CGRP通常与其他神经肽共定位,包括DRG神经元中的P物质和神经激肽。外周CGRP-LI纤维可终止于脊髓的I、III和V层,并且含有CGRP的DRG神经元支配关节。因此,CGRP及其受体可广泛分布于外周和中枢疼痛通路中(Schou等人(2017),The Journal of Headache and Pain,18,34,1-17)。在动物中,CGRP可在有害的疼痛和/或皮肤的机械刺激时从外周和中枢神经末梢释放。在大鼠中,循环CGRP的主要部分可从血管周围神经末梢释放。急性和慢性伤害性感受可导致CGRP从感觉神经末梢和中枢末梢释放到脊髓背角中的改变。已知CGRP是最有效的血管扩张剂之一。已经表征了两种亚型:α-CGRP和β-CGRP(Russell等人(2014),Physiol Rev,94,4,1099-1142)。α亚型主要在初级感觉神经元中表达,而β亚型主要发现于内源性肠神经元中。该神经肽的成熟形式由37个氨基酸组成,且其表达在DRG和三叉神经节的感觉神经元中尤其引人注目。成熟形式储存在定位于中枢和外周神经末梢的末端区域的囊泡中,从那里它可以分泌到脊髓背侧或各种外周组织中,尤其是分泌到可以调节血管紧张度的周围血管中。此外,已经观察到啮齿动物和人脑膜血管中存在CGRP阳性的伤害性感受器网络,并且已经发现约40-50％的三叉神经节神经元对CGRP呈阳性。此外,已在CNS区域中观察到CGRP表达,例如下丘脑、丘脑、中脑导水管周围灰质、上丘和下丘、扁桃体、三叉神经复合体和小脑。考虑到CGRP改变三叉颈核复合体处的突触和神经元活性的能力,以及伤害性感受信号向丘脑和皮层区域的传递,这些提到的脑部区域可能与偏头痛病理生理学有关(Tardiolo等人(2019),Int J Mol Sci,20(12),2932)。

[0008] 疼痛(例如CGRP相关疼痛)的常规治疗包括单克隆抗体和小分子拮抗剂,一旦疼痛介质(例如CGRP)已经由突触前神经元释放,则靶向所述疼痛介质。作为替代方法,某些常规治疗剂靶向疼痛介质的受体。这些方法有许多缺点,包括:对化学介质(例如,CGRP)的系统性影响;恶心;呕吐;消化不良;腹泻;心动过缓;低血压;支气管痉挛;呼吸困难;疲劳;失眠;头晕;口干燥;脸红(flushing);热或冷感觉;胸痛;便秘;瘙痒;倦睡;耳鸣;躁动;肌肉痉挛;注射部位疼痛;上呼吸道感染;疲劳;鼻咽炎;注射部位红斑;注射部位硬结;焦虑;抑郁;注射部位瘙痒;流感;尿路感染;嗜睡;感觉异常;心率增加;中风;和/或心脏病发作(Woo(2020),Nature,586,S4-S6和Tardiolo等人(2019),Int J Mol Sci,20(12),2932)。因此,需要改进的疼痛治疗剂,其副作用较少和/或在释放点阻断疼痛介质。

[0009] 本发明克服了上述问题中的一个或多个。

发明内容

[0010] 本发明人已经发现,与BoNT/A不同,包含肉毒杆菌神经毒素A(BoNT/A)轻链和转位结构域(H_N结构域)以及BoNT/B受体结合结构域(H_C结构域)的嵌合梭菌神经毒素可结合至包含分泌疼痛介质的Aδ或C神经纤维的神经元,并且可有效抑制所述介质从所述神经元释放。因此,通过这种抑制,本发明的嵌合梭菌神经毒素可用作能够治疗疼痛的镇痛剂。特别地,本发明人已经表明,所要求保护的嵌合梭菌神经毒素可有效抑制CGRP从所述神经元释放。因此,通过所述CGRP释放抑制,本发明的嵌合梭菌神经毒素可用作能够治疗CGRP相关疼痛的镇痛剂。

[0011] 不希望受理论束缚,据信通过在分泌点阻断化学介质,本发明的嵌合梭菌神经毒素可防止疼痛介质(例如CGRP)到达邻近和远端细胞。有利地,这可提供:选择性阻断疼痛相

关的异常介质释放,从而在别处保持介质释放;具有较长作用持续时间(与非嵌合梭菌神经毒素相比具有较少的副作用和/或增加的安全窗口)的治疗剂;和/或与常规治疗剂相比更少的副作用。特别地,一旦释放后CGRP作用和/或CGRP受体被常规治疗剂阻断,可导致恶心、疲劳和心率增加、中风和/或心脏病发作。本发明可以最小化/避免所述副作用。

[0012] 本发明人还发现嵌合梭菌神经毒素能够在与偏头痛病理生理学相关的中枢神经系统结构中切割SNAP25。有利地,嵌合梭菌神经毒素在治疗偏头痛(例如偏头痛疼痛)中特别有效。

具体实施方式

[0013] 在一个方面,本发明提供了用于治疗疼痛的嵌合梭菌神经毒素,其中该嵌合梭菌神经毒素包含肉毒杆菌神经毒素A(BoNT/A)轻链和转位结构域(H_N结构域)以及BoNT/B受体结合结构域(H_C结构域)。

[0014] 在一个方面,本发明提供了治疗疼痛的方法,该方法包括向受试者施用嵌合梭菌神经毒素,其中嵌合梭菌神经毒素包含肉毒杆菌神经毒素A(BoNT/A)轻链和转位结构域(H_N结构域)以及BoNT/B受体结合结构域(H_C结构域)。

[0015] 在一个方面,本发明提供了嵌合梭菌神经毒素在制备用于治疗疼痛的药物中的用途,其中嵌合梭菌神经毒素包含肉毒杆菌神经毒素A(BoNT/A)轻链和转位结构域(H_N结构域)以及BoNT/B受体结合结构域(H_C结构域)。

[0016] 在一个方面,本发明提供了用于治疗偏头痛(优选偏头痛疼痛)的嵌合梭菌神经毒素,其中嵌合梭菌神经毒素包含肉毒杆菌神经毒素A(BoNT/A)轻链和转位结构域(H_N结构域)以及BoNT/B受体结合结构域(H_C结构域)。

[0017] 在一个方面,本发明提供了治疗偏头痛(优选偏头痛疼痛)的方法,该方法包括向受试者施用嵌合梭菌神经毒素,其中嵌合梭菌神经毒素包含肉毒杆菌神经毒素A(BoNT/A)轻链和转位结构域(H_N结构域)以及BoNT/B受体结合结构域(H_C结构域)。

[0018] 在一个方面,本发明提供了嵌合梭菌神经毒素在制备用于治疗偏头痛(优选偏头痛疼痛)的药物中的用途,其中嵌合梭菌神经毒素包含肉毒杆菌神经毒素A(BoNT/A)轻链和转位结构域(H_N结构域)以及BoNT/B受体结合结构域(H_C结构域)。

[0019] 偏头痛可以是阵发性偏头痛或慢性偏头痛(优选慢性偏头痛)。如果受试者每月经历头痛(例如偏头痛)少于15天(例如每月至少1天但少于15天),优选地如果受试者每月经历头痛(例如偏头痛)至少4天但少于15天,则受试者可能患有阵发性偏头痛。换言之,阵发性偏头痛可定义为每月少于15天(例如每月至少1天但少于15天)的头痛(例如偏头痛),优选为每月至少4天但少于15天的头痛(例如偏头痛)。如果受试者每月经历头痛(例如偏头痛)至少15天,则受试者可能患有慢性偏头痛。如果受试者每月经历头痛(例如偏头痛)至少15天并持续至少3个月,并且具有每月至少8天偏头痛的特征,则受试者可能患有慢性偏头痛。换言之,慢性偏头痛可定义为每月至少15天的头痛(例如偏头痛)。换言之,慢性偏头痛可定义为每月至少15天并持续至少3个月的头痛(例如偏头痛),并且具有每月至少8天的偏头痛的特征。在一个实施方案中,慢性偏头痛可持续一天4小时或更长。除了头痛之外,偏头痛还可能与一种或多种其他症状有关,包括光敏感性增加、恶心和/或呕吐。

[0020] 优选在治疗偏头痛时,用嵌合梭菌神经毒素治疗偏头痛疼痛。

[0021] 在一个方面,本发明提供了用于通过抑制疼痛介质从包含Aδ神经纤维或C神经纤维的神经元释放来治疗疼痛的嵌合梭菌神经毒素,其中所述嵌合梭菌神经毒素分别结合至包含Aδ神经纤维或C神经纤维的神经元,并且其中所述嵌合梭菌神经毒素包含肉毒杆菌神经毒素A(BoNT/A)轻链和转位结构域(H_N结构域)以及BoNT/B受体结合结构域(H_C结构域)。

[0022] 在一个相关方面,本发明提供了通过抑制疼痛介质从包含Aδ神经纤维或C神经纤维的神经元释放来治疗疼痛的方法,所述方法包含向受试者施用所述嵌合梭菌神经毒素,其中所述嵌合梭菌神经毒素分别结合至包含Aδ神经纤维或C神经纤维的神经元,并且其中所述嵌合梭菌神经毒素包含肉毒杆菌神经毒素A(BoNT/A)轻链和转位结构域(H_N结构域)以及BoNT/B受体结合结构域(H_C结构域)。

[0023] 在另一相关方面,本发明提供了嵌合梭菌神经毒素在制备用于通过抑制疼痛介质从包含Aδ神经纤维或C神经纤维的神经元中释放来治疗疼痛的药物中的用途,其中所述嵌合梭菌神经毒素分别结合至包含Aδ神经纤维或C神经纤维的神经元,并且其中所述嵌合梭菌神经毒素包含肉毒杆菌神经毒素A(BoNT/A)轻链和转位结构域(H_N结构域)以及BoNT/B受体结合结构域(H_C结构域)。

[0024] 在一个实施方案中,本发明提供了用于通过抑制CGRP从包含Aδ神经纤维或C神经纤维的神经元释放来治疗CGRP相关疼痛的嵌合梭菌神经毒素,其中所述嵌合梭菌神经毒素分别结合至包含Aδ神经纤维或C神经纤维的神经元,并且其中所述嵌合梭菌神经毒素包含肉毒杆菌神经毒素A(BoNT/A)轻链和转位结构域(H_N结构域)以及BoNT/B受体结合结构域(H_C结构域)。还提供了相应的治疗方法和用途。

[0025] 介质可以是从神经元释放的在病症(如疼痛)中起作用的任何分子。根据本发明的嵌合梭菌神经毒素对所述介质释放的抑制可以治疗所述病症(例如可以治疗疼痛)。

[0026] 介质可以是神经递质。

[0027] 抑制介质从神经元的释放可以是部分或完全抑制,优选完全抑制。例如,嵌合梭菌神经毒素可以抑制从神经元释放的至少40%、50%、60%、70%、80%、90%、95%或99%的介质。优选地,嵌合梭菌神经毒素抑制从神经元释放的100%的介质。

[0028] 疼痛介质可以是神经递质。

[0029] 抑制疼痛介质从所述神经元的释放可以是部分或完全抑制,优选完全抑制。例如,嵌合梭菌神经毒素可以抑制从神经元释放的至少40%、50%、60%、70%、80%、90%、95%或99%的疼痛介质。优选地,嵌合梭菌神经毒素抑制从神经元释放的100%的疼痛介质。

[0030] 所述抑制优选是SNARE相关(例如SNAP25相关)释放的抑制。

[0031] 与BoNT/A(优选如SEQ ID NO:6所示的天然BoNT/A[例如SEQ ID NO:6的双链形式])抑制介质从神经元释放相比,本发明的嵌合梭菌神经毒素优选更大程度地抑制介质从神经元的释放。在给定剂量(例如1nM)下,本发明的嵌合梭菌神经毒素可比相同剂量(例如1nM)下的BoNT/A多抑制至少10%或20%(优选至少30%)的来自神经元的介质。在给定剂量(例如1nM)下,本发明的嵌合梭菌神经毒素可比相同剂量(例如1nM)下的BoNT/A多抑制10-90%或20-90%(优选30-85%)的来自神经元的介质。因此,与BoNT/A相比,可能需要低得多的嵌合梭菌神经毒素剂量来抑制相同量的介质从神经元的释放。例如,嵌合梭菌神经毒素的剂量可以比抑制相同量的介质从神经元释放所需的BoNT/A的剂量低至少100倍、低200倍或低500倍,优选低1000倍。嵌合梭菌神经毒素的剂量可以比抑制相同量的介质从神经元释

放所需的BoNT/A的剂量低至少500-2000倍、或750-1750倍,优选低1000-1500倍。

[0032] 与BoNT/A(优选如SEQ ID NO:6所示的天然BoNT/A[例如SEQ ID NO:6的双链形式])抑制介质从神经元释放相比,本发明的嵌合梭菌神经毒素优选更大程度地抑制疼痛介质从神经元的释放。在给定剂量(例如1nM)下,本发明的嵌合梭菌神经毒素可比相同剂量(例如1nM)下的BoNT/A多抑制至少10%或20%(优选至少30%)的来自神经元的疼痛介质。在给定剂量(例如1nM)下,本发明的嵌合梭菌神经毒素可比相同剂量(例如1nM)下的BoNT/A多抑制10-90%或20-90%(优选30-85%)的来自神经元的疼痛介质。因此,与BoNT/A相比,可能需要低得多的嵌合梭菌神经毒素剂量来抑制相同量的疼痛介质从神经元的释放。例如,嵌合梭菌神经毒素的剂量可以比抑制相同量的疼痛介质从神经元释放所需的BoNT/A的剂量低至少100倍、低200倍或低500倍,优选低1000倍。嵌合梭菌神经毒素的剂量可比抑制相同量的疼痛介质从神经元释放所需的BoNT/A的剂量低至少500-2000倍、或750-1750倍,优选低1000-1500倍。

[0033] 嵌合梭菌神经毒素可抑制多种介质从神经元的释放。

[0034] 嵌合梭菌神经毒素可抑制多种疼痛介质从神经元的释放。

[0035] 本发明的嵌合梭菌神经毒素优选具有镇痛特性。换言之,本发明的嵌合梭菌神经毒素优选是镇痛的嵌合梭菌神经毒素。

[0036] 优选地,本发明的嵌合梭菌神经毒素既不促进神经元生长也不促进神经元修复以治疗疼痛。换言之,优选地,嵌合梭菌神经毒素不通过以下任何方式治疗疼痛:通过促进神经元生长、通过促进神经元修复或通过促进神经元生长和修复。

[0037] 优选地,本发明的嵌合梭菌神经毒素既不促进神经元生长也不促进神经元修复以治疗本文所述的病症。换言之,优选地,嵌合梭菌神经毒素不通过以下任何方式治疗本文所述的病症:通过促进神经元生长、通过促进神经元修复或通过促进神经元生长和修复。

[0038] 术语“促进神经元生长和/或神经元修复”涵盖增加神经元生长和/或神经元修复的速率。术语“神经元生长和/或神经元修复”涵盖重建受损的神经元回路,从而恢复神经元网络或群体中的活动和/或神经元通信。因此,本文所用的术语“神经元修复”涵盖特定神经元的修复以及神经元回路的修复。该术语还涵盖神经元可塑性。本文所用的术语“神经元可塑性”涵盖轴突出芽、树突出芽、神经发生(例如新神经元的产生)、成熟、分化和/或突触可塑性(例如包括突触强度、活性、解剖学和/或连接性的改变)。术语“促进神经元生长和/或神经元修复”还涵盖(例如在损伤部位或损伤部位附近)促进功能性突触的建立。本文所用的术语“神经元生长”涵盖神经元的任何部分的生长,包括轴突和/或树突的生长。所述术语涵盖神经突长度、神经突数目(例如每个细胞的神经突数目)的增加和/或来自神经元的细胞体或细胞膜的突出的长度和/或数目的增加,例如神经元的轴突生长和/或轴突出芽,例如受试者中的神经元。所述轴突生长可以促进神经元之间的连接和/或化学通信。

[0039] 优选地,本发明的嵌合梭菌神经毒素不促进神经免疫应答以治疗疼痛。优选地,本发明的嵌合梭菌神经毒素不促进神经免疫应答以治疗本文所述的病症。本文中的神经免疫应答涵盖小胶质细胞应答。因此,在一个实施方案中,本发明的嵌合梭菌神经毒素不促进小胶质细胞应答以治疗疼痛。因此,在一个实施方案中,本发明的嵌合梭菌神经毒素不促进小胶质细胞应答以治疗本文所述的病症。

[0040] 在优选的实施方案中,疼痛不是与脑部病症相关或由脑部病症引起的疼痛。在优

选的实施方案中,本文所述的病症不是与脑部病症相关或由脑部病症引起的病症。本文中使用的术语“脑部病症”可与“脑部疾病”互换。本文所用的“脑部病症”涵盖源自脑内或脑外的病症,并且包括与导致脑组织损伤的身体损伤相关的病症。本文中涵盖的脑部病症的实例包括以下中的任一种(或多种):创伤性脑损伤、癌症(例如脑肿瘤)、感染性疾病(例如脑炎、脑膜炎、脑脓肿和脑炎)、中风、神经退行性病症(例如阿尔茨海默病、帕金森病、帕金森病相关病症、运动神经元疾病(例如肌萎缩性侧索硬化)、朊病毒病、亨廷顿病、脊髓小脑共济失调、共济失调、哈勒沃登-施帕茨(Hallervorden-Spatz)病和额颞叶变性)、脑动脉瘤、多发性硬化、缺氧损伤、毒性损伤和代谢损伤。脑部病症可由创伤性脑损伤、癌症、感染性疾病(例如脑炎,脑膜炎,脑脓肿和脑炎)、中风、神经退行性病症(例如阿尔茨海默病、帕金森病、帕金森病相关病症、运动神经元疾病(例如肌萎缩性侧索硬化)、朊病毒病、亨廷顿病、脊髓小脑共济失调、共济失调、哈勒沃登-施帕茨(Hallervorden-Spatz)病和额颞叶变性)、脑动脉瘤、多发性硬化、缺氧损伤、毒性损伤和/或代谢损伤引起。

[0041] 嵌合梭菌神经毒素优选与包含Aδ纤维或C纤维的神经元结合。所述结合可由嵌合梭菌神经毒素的BoNT/B H_C结构域(例如其H_{CC}部分)介导。在与神经元结合后,嵌合梭菌神经毒素可通过核内体内化,并且BoNT/A轻链可通过BoNT/A转位结构域从核内体转位到神经元的胞质溶胶中。一旦在胞质溶胶中,轻链可以切割SNARE蛋白(例如SNAP25),从而抑制神经元释放/分泌(包括从神经元释放/分泌疼痛介质)。

[0042] 包含Aδ纤维或C纤维的神经元描述于Pichon&Chesler(2014),Frontiers in Neuroanatomy(https://doi.org/10.3389/fnana.2014.00021)和Yam等人(2018),Int J Mol Sci,19,8,2164。术语“纤维”(例如在Aδ纤维或C纤维的上下文中)优选是指神经元的轴突。通常,多根纤维(例如分别为多根Aδ纤维或多根C纤维)一起可以将受试者体内较大的神经/神经元结构限定为例如纤维束。例如,一束Aδ纤维或一束C纤维。在一些实施方案中,所述多根纤维可以包括除Aδ纤维或C纤维之外的纤维。例如,神经可以包括多个神经元,包括包含Aδ纤维的神经元和/或包含C纤维的神经元。

[0043] 嵌合梭菌神经毒素可结合至包含Aδ纤维的神经元。Aδ纤维(或包含其的神经元)可以表征为肽能的、快速传导的、轻度髓鞘化的、涉及锐痛/快痛、涉及伤害性感受和/或涉及温度感觉。优选地,Aδ纤维(或包含其的神经元)可以具有5-75m/s(例如5-35m/s)的传导速度和/或约1-5μm(例如2-5μm)的直径。包含由本发明的嵌合梭菌神经毒素结合的Aδ纤维的神经元是能够释放疼痛介质的神经元。特别地,所述神经元能够释放CGRP并因此在CGRP相关疼痛中发挥效应。通过与包含Aδ纤维的神经元结合,嵌合梭菌神经毒素通过切割其SNARE蛋白(例如SNAP25)抑制疼痛介质从所述神经元的释放,从而抑制疼痛介质从所述神经元的释放/分泌。

[0044] 嵌合梭菌神经毒素可与包含C纤维的神经元结合。C纤维(或包含其的神经元)可以表征为肽能的、低(例如缓慢)传导的、无髓鞘的、涉及钝痛/慢痛、涉及神经性疼痛、涉及热感觉和/或涉及瘙痒感觉。包含C纤维的神经元可以是多模态的(polymodal)。优选地,C纤维(或包含其的神经元)可具有0.5-2m/s的传导速度和/或约0.2-1.5μm(例如0.2-0.5μm)的直径。包含由本发明的嵌合梭菌神经毒素结合的C纤维的神经元是能够释放疼痛介质的神经元。特别地,所述神经元能够释放CGRP并因此在CGRP相关疼痛中发挥效应。通过与包含C纤维的神经元结合,嵌合梭菌神经毒素通过切割其SNARE蛋白(例如SNAP25)可抑制疼痛介质

从所述神经元的释放,从而抑制疼痛介质从所述神经元的释放/分泌。

[0045] 优选地,嵌合梭菌神经毒素结合的神经元是包含C纤维的神经元。

[0046] 原肌球蛋白受体激酶A(TrkA)的表达可以是用于区分包含Aδ神经纤维或C神经纤维的神经元(例如,来自包含Aβ纤维的神经元)的标志物。换言之,包含本发明的Aδ神经纤维或C神经纤维的神经元可以是表达TrkA的神经元。

[0047] 在使用中,嵌合梭菌神经毒素可结合至多个神经元,所述神经元至少包括包含Aδ纤维的神经元和包含C纤维的神经元。所述多个神经元可以是受试者中较大的神经/神经元结构的一部分,例如包括纤维束。

[0048] 包含Aδ神经纤维或C神经纤维的神经元可以是中枢神经系统(例如下丘脑、丘脑、中脑导水管周围灰质、上丘、下丘、杏仁核、三叉颈核复合体和/或小脑)或外周神经系统的神经元。当治疗某些病况如头痛、优选偏头痛时,嵌合梭菌神经毒素可以抑制介质从中枢神经系统的神经元的释放。当治疗某些疼痛病况如头痛、优选偏头痛时,嵌合梭菌神经毒素可以抑制疼痛介质从中枢神经系统的神经元的释放。

[0049] 包含根据本发明的Aδ神经纤维或C神经纤维的神经元优选为感觉神经元。感觉神经元可以是初级感觉神经元,例如初级传入神经元。例如,嵌合梭菌神经毒素结合的神经元可以是背根神经节和/或三叉神经节的感觉神经元。附加地或者可替代地,神经元可以是内源性肠神经元。

[0050] 本发明的嵌合梭菌神经毒素可以与包含Aδ纤维或C纤维的神经元结合,其亲和力大于BoNT/A(优选如SEQ ID NO:6所示的天然BoNT/A[例如SEQ ID NO:6的双链形式])与神经元结合的亲和力。特别地,本发明的嵌合梭菌神经毒素可以结合至包含Aδ纤维或C纤维的神经元,其亲和力比BoNT/A结合至神经元的亲和力大至少2×、5×、10×、50×、100×、1,000×或10,000×。

[0051] 本发明的嵌合梭菌神经毒素可以与包含Aδ纤维或C纤维的神经元结合,其亲和力大于嵌合梭菌神经毒素与不包含Aδ纤维或C纤维的神经元(优选感觉神经元)(例如包含Aβ纤维的神经元)结合的亲和力。例如,本发明的嵌合梭菌神经毒素可以结合至包含Aδ纤维或C纤维的神经元,其亲和力比嵌合梭菌神经毒素结合至不包含Aδ纤维或C纤维的神经元(优选感觉神经元)(例如包含Aβ纤维的神经元)的亲和力大至少2×、5×、10×、50×、100×、1,000×或10,000×。

[0052] Aβ纤维(或包含其的神经元)通常可表征为有髓鞘的、快速传导的、参与触摸的和/或对其他非有害刺激响应的。优选地,Aβ纤维(或包含其的神经元)可具有80-120m/s的传导速度和/或约6-20μm的直径。神经丝200(NF200)的表达可以是用于区分包含Aβ神经纤维的神经元(例如,来自包含Aδ纤维或C纤维的神经元)的标志物。换言之,包含Aβ纤维的神经元可以是表达NF200的神经元。

[0053] 在其他实施方案中,嵌合梭菌神经毒素可能能够在施用部位(例如注射)远端的部位发挥效应。例如,在施用嵌合梭菌神经毒素后,SNARE蛋白切割(例如SNAP25切割)可发生在施用(例如注射)部位远端的部位。优选地,这种作用通过嵌合梭菌神经毒素从其施用部位到远端部位的神经元运输而发生。当治疗疼痛(优选头痛,最优选偏头痛疼痛)或偏头痛时,嵌合梭菌神经毒素优选在施用部位(例如注射)远端的部位发挥效应。在一个实施方案中,该效应可以附加于外周效应。因此,优选地,当治疗疼痛(优选头痛,最优选偏头痛疼痛)

或偏头痛时,可以通过神经元运输来运输嵌合梭菌神经毒素。

[0054] 神经元运输可以是逆向运输或顺向运输,优选逆向运输。运输可以是轴突运输。

[0055] “逆向运输(retrograde transport)”可能是轴突运输的一种形式(又名轴浆运输或轴浆流动);一种细胞过程,通常负责线粒体、脂质、突触囊泡、蛋白质和其他细胞器通过称为轴浆的轴突细胞质进出神经元细胞体。轴突长约为几米,因此神经元不能依靠扩散将细胞核和细胞器的产物运送到它们轴突的末端,因此使用轴突运输。轴突运输还可以负责将注定降解的分子从轴突移动回到细胞体,在细胞体中它们被溶酶体分解。

[0056] “逆向运输”可指朝向神经元的细胞体的移动,而“顺向运输(anterograde transport)”可指朝向神经元的突触的移动。

[0057] 在一个实施方案中,神经元向中枢神经系统的神经元的(例如逆向)运输可以指嵌合梭菌神经毒素向位于中枢神经系统附近的神经元细胞体的运输(例如轴突运输)。

[0058] 现在更详细地描述神经元(例如逆向)运输。在一个实施方案中,嵌合梭菌神经毒素可以在施用部位结合第一神经元(例如初级感觉传入)。嵌合梭菌神经毒素可以被第一神经元内化,在第一神经元内运输,然后从第一神经元释放。优选地,梭菌神经毒素在肌内或皮内施用(例如肌内或皮内注射)的部位结合第一神经元。这样的神经元可以是外周神经元,优选包含Aδ纤维或C纤维的神经元。一旦释放,嵌合梭菌神经毒素可以结合第二神经元、被内化,并在所述第二神经元内切割SNARE蛋白(例如SNAP25)。可替代地,嵌合梭菌神经毒素可与第二神经元结合、被第二神经元内化、在第二神经元内运输,然后从第二神经元释放。可以重复该过程,直到嵌合梭菌神经毒素与神经元(例如第三神经元)结合、被内化,并在所述神经元内切割SNARE蛋白(例如SNAP25)。第二神经元可以是次级感觉传入。优选地,第二神经元是中枢神经系统的神经元,例如存在于脑部、脑干或脊髓中的神经元。第二神经元可以是存在于三叉神经节中的神经元(例如,SNARE切割可以发生在其轴突中)。

[0059] 在一些实施方案中,当肌内施用时,嵌合梭菌神经毒素可以通过运动神经元在神经元上(例如逆向地)运输,从运动神经元释放,并进入第二神经元,优选中枢神经系统的神经元。所述神经元可以是感觉神经元。

[0060] 在一个实施方案中,当肌内施用时,嵌合梭菌神经毒素可扩散至存在于骨膜或皮肤中的感觉神经元并与其结合(例如终止于骨膜或皮肤)。

[0061] 不希望受理论束缚,据信通过上述神经元(例如逆向)运输机制,本发明的嵌合梭菌神经毒素可抑制中枢神经系统的一个或多个神经元的分泌。因此,嵌合梭菌神经毒素可以通过神经元(例如逆向)运输到达中枢神经系统的神经元,并切割所述神经元的SNARE蛋白(例如SNAP25)。

[0062] 在一个优选的实施方案中,通过抑制来自中枢神经系统的一个或多个神经元的分泌(例如抑制介质(例如疼痛介质)的释放),嵌合梭菌神经毒素可治疗本文所述的疼痛或病症。这在治疗疼痛或偏头痛、优选治疗偏头痛疼痛中可能特别相关。所述嵌合梭菌神经毒素可以通过神经元(例如逆向)运输到达所述中枢神经系统的神经元,并切割所述神经元的SNARE蛋白(例如SNAP25)。因此,嵌合梭菌神经毒素可以通过抑制中枢神经系统神经元的分泌、优选通过抑制介质、更优选疼痛介质从中枢神经系统神经元的分泌来治疗疼痛(例如头痛或偏头痛疼痛)或偏头痛。

[0063] 中枢神经系统的神经元可以是脑干、脊髓和/或脑部的神经元。例如,中枢神经系

统的神经元可以是以下部位的神经元:三叉神经核(例如脊髓三叉神经核(spinal trigeminal nucleus),如脊髓三叉神经感觉核(spinal trigeminal sensory nucleus))、脊髓(优选脊髓背角的神经元)、下丘脑、丘脑、中脑导水管周围灰质、上丘、下丘、杏仁核、三叉颈核复合体、皮质和/或小脑。三叉神经核的神经元可以是三叉神经脊束核尾(trigeminal nucleus caudalis)(例如尾侧部)的神经元。

[0064] 在一个优选的实施方案中,嵌合梭菌神经毒素在脑干的神经元、更优选三叉神经核的神经元(甚至更优选脊髓三叉神经(感觉)核)中切割SNARE蛋白(例如SNAP25)。嵌合梭菌神经毒素可抑制所述神经元中(例如介质,优选疼痛介质)的分泌。所述SNARE蛋白的切割可以通过嵌合梭菌神经毒素从施用部位的神经元(例如逆向)运输而发生。这样的神经元可以通过将嵌合梭菌神经毒素施用于受感觉三叉神经神经元(sensory trigeminal neurons)支配的肌肉、骨膜和/或皮肤(例如位于受试者面部和/或头皮的肌肉、骨膜和/或皮肤)来靶向。可替代地,神经元可包含Aδ神经纤维或C神经纤维,嵌合梭菌神经毒素可与其结合,然后切割其SNARE蛋白(例如通过神经元细胞质的运输/扩散后)。所述切割可以在存在于脊髓三叉神经感觉核中的神经元末端。最优选地,所述SNARE切割和分泌抑制可治疗偏头痛或偏头痛疼痛。

[0065] 在一个实施方案中,嵌合梭菌神经毒素切割三叉神经运动核神经元的SNARE蛋白(例如SNAP25)。嵌合梭菌神经毒素可抑制所述神经元的分泌。所述SNARE蛋白的切割可以通过嵌合梭菌神经毒素从施用部位的神经元(例如逆向)运输而发生。

[0066] 在另一个优选的实施方案中,嵌合梭菌神经毒素在脊髓,例如颈脊髓的神经元中切割SNARE蛋白(例如SNAP25)。更优选地,所述神经元是存在于脊髓的背角中(例如与感觉神经元相关)的神经元。嵌合梭菌神经毒素可抑制所述神经元中(例如介质,优选疼痛介质)的分泌。所述SNARE蛋白的切割可以通过嵌合梭菌神经毒素从施用部位的神经元(例如逆向)运输而发生。这样的神经元可以通过将嵌合梭菌神经毒素施用于受感觉脊髓神经元支配的肌肉、骨膜和/或皮肤(例如,位于受试者头部后部和/或颈部的肌肉、骨膜和/或皮肤)来靶向。可替代地,神经元可包含Aδ神经纤维或C神经纤维,嵌合梭菌神经毒素可与其结合,然后切割其SNARE蛋白(例如通过神经元细胞质的运输/扩散后)。所述切割可以在存在于脊髓中的神经元末端。最优选地,所述SNARE切割和分泌抑制可治疗偏头痛或偏头痛疼痛。

[0067] 在一个实施方案中,嵌合梭菌神经毒素切割脊髓腹角(ventral horn)神经元(例如与运动神经元相关)中的SNARE蛋白(例如SNAP25)。嵌合梭菌神经毒素可抑制所述神经元中(例如介质,优选疼痛介质)的分泌。所述SNARE蛋白的切割可以通过嵌合梭菌神经毒素从施用部位的神经元(例如逆向)运输而发生。

[0068] 在另一个优选的实施方案中,嵌合梭菌神经毒素在三叉神经节的神经元中,例如在其轴突中切割SNARE蛋白(例如SNAP25)。嵌合梭菌神经毒素可抑制所述神经元中(例如介质,优选疼痛介质)的分泌。所述SNARE蛋白的切割可以通过嵌合梭菌神经毒素从施用部位的神经元(例如逆向)运输而发生。可替代地,神经元可包含Aδ神经纤维或C神经纤维,嵌合梭菌神经毒素可与其结合,然后切割其SNARE蛋白(例如通过神经元细胞质的运输/扩散后)。最优选地,所述SNARE切割和分泌抑制可治疗偏头痛或偏头痛疼痛。

[0069] 已经描述了梭菌神经毒素的神经元(例如逆向)运输(参见Bomba-Warczak等人(2016),Cell Rep.,16(7),1974-1987),并且,不希望受理论束缚,据信梭菌神经毒素的神

经元运输通过以下而发生:梭菌神经毒素与非经典受体的结合(例如,在本案中通过结合除SYTI或SYTII之外的受体),掺入非酸化细胞器中,神经元(例如逆向)运输(例如,远离身体的外周朝向中枢神经系统),以及从神经元释放到细胞外空间中。在这种情况下,已经描述了梭菌神经毒素可以保持完整(即包含通过二硫键连接在一起的L-链和H-链的双链保持完整),允许通过标准中毒途径(canonical intoxication route)结合至第二神经元(例如在本发明的嵌合梭菌神经毒素的情况下通过SYTI或SYTII)。

[0070] 施用于受试者的嵌合梭菌神经毒素的一部分可结合至包含Aδ神经纤维或C神经纤维的神经元并抑制介质(例如疼痛介质)从所述神经元的释放,并且嵌合梭菌的一部分可在施用部位远端的部位发挥效应。在施用部位远端的部位发挥其效应的部分可以抑制中枢神经系统的神经元的分泌,优选抑制介质(例如神经递质)、更优选疼痛介质从中枢神经系统神经元的分泌。嵌合梭菌神经毒素可以通过神经元(例如逆向)运输到达中枢神经系统的神经元,并切割所述神经元的SNARE蛋白(例如SNAP25)。

[0071] 在一些实施方案中,嵌合梭菌神经毒素的神经元(例如逆向)运输可以包括嵌合梭菌神经毒素从一个神经元到另一个神经元的跨突触移动(例如转胞吞作用)。

[0072] 抑制神经元的分泌可以是部分或完全抑制,优选完全抑制。例如,嵌合梭菌神经毒素可以抑制至少40%、50%、60%、70%、80%、90%、95%或99%的神经元的分泌。优选地,嵌合梭菌神经毒素抑制100%的神经元的分泌。本文中的分泌优选是SNARE相关的(例如SNAP25相关的)分泌。

[0073] 在一个优选的实施方案中,本发明的嵌合梭菌神经毒素可通过抑制介质(例如疼痛介质)从包含Aδ神经纤维或C神经纤维的神经元的释放和通过抑制(例如介质,优选疼痛介质)从中枢神经系统的神经元的分泌来治疗本文所述的偏头痛或病症(优选疼痛),其中所述嵌合梭菌神经毒素分别结合至包含Aδ神经纤维或C神经纤维的神经元。

[0074] 梭菌属(Clostridia)的细菌产生高效且特异性的蛋白质毒素,其可使神经元和它们被递送至的其他细胞中毒。此类梭菌毒素的实例包括由破伤风梭菌(C.tetani,TeNT)和肉毒杆菌(C.botulinum,BoNT)血清型A-G和X产生的神经毒素(参见WO 2018/009903 A2),以及由巴氏梭菌(C.baratii)和丁酸梭菌(C.butyricum)产生的神经毒素。破伤风毒素和肉毒杆菌毒素都通过抑制受影响的神经元的功能,特别是神经递质的释放而起作用。虽然肉毒杆菌毒素通常作用于神经肌肉接头并抑制外周神经系统中的胆碱能传递,但破伤风毒素作用于中枢神经系统。

[0075] 在自然界中,梭菌神经毒素被合成为单链多肽,其在翻译后被蛋白水解切割事件修饰以形成通过二硫键连接在一起的两条多肽链。切割发生在特定切割位点,通常称为活化位点(例如活化环),其位于提供链间二硫键的半胱氨酸残基之间。正是这种双链形式是毒素的活性形式。这两条链称为重链(H-链),其分子量约为100kDa;和轻链(L-链),其分子量约为50kDa。H-链包含N-末端转位组分(H_N结构域)和C-末端靶向组分(H_C结构域)。切割位点位于L-链和转位结构域组分之间。在H_C结构域与其靶神经元结合并且结合的毒素通过内体内化到细胞中之后,H_N结构域使L-链转位穿过内体膜并进入胞质溶胶,以及L-链提供蛋白酶功能(也称为非细胞毒性蛋白酶)。

[0076] 非细胞毒性蛋白酶通过蛋白水解切割称为SNARE蛋白(例如SNAP25、VAMP或Syntaxin,优选SNAP25)的细胞内转运蛋白而起作用。首字母缩略词SNARE来源于术语可溶

性NSF附着受体(Soluble NSF Attachment Receptor),其中NSF是指N-乙基马来酰亚胺敏感因子(N-ethylmaleimide-Sensitive Factor)。SNARE蛋白是细胞内囊泡融合必不可少的,因此也是通过囊泡从细胞运输分泌分子必不可少的。蛋白酶功能是锌依赖性内肽酶活性,并表现出对SNARE蛋白的高底物特异性。因此,一旦递送至所需的靶细胞,非细胞毒性蛋白酶能够抑制来自靶细胞的细胞分泌。梭菌神经毒素的L-链蛋白酶是切割SNARE蛋白的非细胞毒性蛋白酶。

[0077] 鉴于SNARE蛋白的普遍存在的性质,梭菌神经毒素如肉毒杆菌毒素已经成功地应用于广泛的治疗中。

[0078] 有关肉毒杆菌和破伤风梭菌毒素产生的遗传基础的更多详细信息,请参见Henderson等人(1997)的The Clostridia:Molecular Biology and Pathogenesis,Academic press。

[0079] 下面更详细地描述梭菌神经毒素结构域。

[0080] L-链参考序列的实例包括:

[0081] A型肉毒杆菌神经毒素:氨基酸残基1-448

[0082] B型肉毒杆菌神经毒素:氨基酸残基1-440

[0083] 上述确定的参考序列应该被认为是指导,因为根据亚血清型可能发生轻微的变化。举例来说,US 2007/0166332(通过引用将其全文并入本文)引用了略微不同的梭菌序列:

[0084] A型肉毒杆菌神经毒素:氨基酸残基M1-K448

[0085] B型肉毒杆菌神经毒素:氨基酸残基M1-K441

[0086] 转位结构域是梭菌神经毒素的H-链的片段,大约相当于H-链的氨基末端一半,或者是对应于完整H-链中的该片段的结构域。

[0087] 参考转位结构域的实例包括:

[0088] A型肉毒杆菌神经毒素-氨基酸残基(449-871)

[0089] B型肉毒杆菌神经毒素-氨基酸残基(441-858)

[0090] 上述确定的参考序列应该被认为是指导,因为根据亚血清型可能发生轻微的变化。举例来说,US2007/0166332(通过引用并入本文)引用了略微不同的梭菌序列:

[0091] A型肉毒杆菌神经毒素-氨基酸残基(A449-K871)

[0092] B型肉毒杆菌神经毒素-氨基酸残基(A442-S858)

[0093] 在本发明的上下文中,包含转位结构域的多种BoNT/A H_N区可用于本发明的方面。来自BoNT/A重链的H_N区长度约为410-430个氨基酸,并包含转位结构域。研究表明,来自梭菌神经毒素重链的H_N区的全长对于转位结构域的转位活性不是必需的。因此,该实施方案的方面可以包括BoNT/A H_N区,其包含长度为例如至少350个氨基酸、至少375个氨基酸、至少400个氨基酸或至少425个氨基酸的转位结构域。该实施方案的其他方面可以包括BoNT/A H_N区,其包含长度为例如至多350个氨基酸、至多375个氨基酸、至多400个氨基酸或至多425个氨基酸的转位结构域。

[0094] 术语H_N涵盖天然存在的BoNT/A H_N部分,以及具有天然不存在的氨基酸序列和/或合成氨基酸残基的修饰的BoNT/A H_N部分。优选地,所述修饰的BoNT/A H_N部分仍然表现出上述转位功能。

[0095] 梭菌神经毒素受体结合结构域(H_C)参考序列的实例包括：

[0096] BoNT/A-N872-L1296

[0097] BoNT/B-E859-E1291

[0098] 梭菌神经毒素(例如BoNT)的约50kDa H_C结构域包含两个不同的结构特征,称为H_{CC}和H_{CN}结构域,通常每个为约25kDa。据信参与受体结合的氨基酸残基主要位于H_{CC}结构域中。天然梭菌神经毒素的H_C结构域可包含约400-440个氨基酸残基。这一事实由以下出版物证实,每份出版物均通过引用全文并入本文:Umland TC(1997)Nat.Struct.Biol.4:788-792;Herreros J(2000)Biochem.J.347:199-204;Halpern J(1993)J.Biol.Chem.268:15,pp.11188-11192;Rummel A(2007)PNAS104:359-364;Lacey DB(1998)Nat.Struct.Biol.5:898-902;Knapp(1998)Am.Cryst.Assoc.Abstract Papers 25:90;Swaminathan和Eswaramoorthy(2000)Nat.Struct.Biol.7:1751-1759;以及Rummel A(2004)Mol.Microbiol.51(3),631-643。

[0099] (参考)H_{CN}结构域的实例包括：

[0100] A型肉毒杆菌神经毒素-氨基酸残基(872-1110)

[0101] B型肉毒杆菌神经毒素-氨基酸残基(859-1097)

[0102] 上述序列位置可能根据血清型/亚型略有不同,(参考)H_{CN}结构域的其他实例包括：

[0103] A型肉毒杆菌神经毒素-氨基酸残基(874-1110)

[0104] B型肉毒杆菌神经毒素-氨基酸残基(861-1097)

[0105] (参考)H_{CC}结构域的实例包括：

[0106] A型肉毒杆菌神经毒素-氨基酸残基(Y1111-L1296)

[0107] B型肉毒杆菌神经毒素-氨基酸残基(Y1098-E1291)

[0108] WO 2017/191315 A1(其通过引用并入本文)教导了嵌合梭菌神经毒素及其制备和制造方法。因此,用于本发明的包含肉毒杆菌神经毒素A(BoNT/A)轻链和转位结构域(BoNT/A H_N)和BoNT/B受体结合结构域(H_C结构域)的嵌合梭菌神经毒素可以是WO 2017/191315A1中教导的一种。

[0109] 本文所用的术语“嵌合梭菌神经毒素”或“嵌合神经毒素”是指包含(优选由其组成)来自第一种梭菌神经毒素血清型的梭菌神经毒素轻链和转位结构域(H_N结构域)以及来自第二种不同梭菌神经毒素血清型的受体结合结构域(H_C结构域)的神经毒素。具体地,用于本发明的嵌合梭菌神经毒素包含肉毒杆菌神经毒素A(BoNT/A)轻链和转位结构域(H_N结构域)以及BoNT/B受体结合结构域(H_C结构域)。嵌合梭菌神经毒素的BoNT/A LH_N结构域与BoNT/B H_C结构域共价连接。本发明的嵌合梭菌神经毒素可称为嵌合肉毒杆菌神经毒素。所述嵌合梭菌神经毒素在本文中也称为“BoNT/AB”、“mrBoNT/AB”或“BoNT/AB嵌合体”。

[0110] L-链和H_N结构域(任选包括完整或部分活化环,例如当嵌合梭菌神经毒素为单链形式时的完整活化环和当为双链形式时的切割的/部分活化环)可统称为LH_N结构域。因此LH_N结构域也不包含H_C结构域。

[0111] 嵌合梭菌神经毒素可以基本上由肉毒杆菌神经毒素A(BoNT/A)轻链和转位结构域(H_N结构域)以及BoNT/B受体结合结构域(H_C结构域)组成。

[0112] 本文所用的术语“基本上由…组成”是指,例如当施用于受试者时,嵌合梭菌神经毒素也不包含赋予多肽额外的功能一个或多个氨基酸残基。换言之,“基本上由”肉毒杆菌

神经毒素A(BoNT/A)轻链和转位结构域(H_N结构域)以及BoNT/B受体结合结构域(H_C结构域)“组成”的多肽还可以包含一个或多个氨基酸残基(对于肉毒杆菌神经毒素A(BoNT/A)轻链和易位结构域(HN结构域)以及BoNT/B受体结合结构域(H_C结构域)的氨基酸残基),但例如当施用于受试者时,所述一个或多个另外的氨基酸残基不赋予多肽额外的功能。另外的功能可以包括酶活性、结合活性和/或任何生理活性。

[0113] 除了任何梭菌神经毒素序列之外,嵌合梭菌神经毒素可以包含非梭菌神经毒素序列,只要该非梭菌神经毒素序列不破坏嵌合梭菌神经毒素实现其治疗效果(优选治疗疼痛)的能力。优选地,非梭菌神经毒素序列不是具有催化活性(例如酶活性)的序列。在一个实施方案中,本发明的嵌合梭菌神经毒素不包含非梭菌催化活性结构域。在一个实施方案中,嵌合梭菌神经毒素不包含另外的催化活性结构域。在一个实施方案中,非梭菌序列不是与细胞受体结合的序列。换言之,在一个实施方案中,非梭菌序列不是细胞受体的配体。细胞受体可以是蛋白质细胞受体,如整合膜蛋白。细胞受体的实例可以在IUPHAR药理学数据库指南2019.4版中找到,网址为https://www.guidetopharmacology.org/download.jsp#db_reports。非梭菌神经毒素序列可以包括有助于纯化的标签,例如His-标签。在一个实施方案中,本发明的嵌合梭菌神经毒素不包含标记或用于添加标记的位点,例如分选酶受体或供体位点。

[0114] 优选地,嵌合梭菌神经毒素可以由肉毒杆菌神经毒素A(BoNT/A)轻链和转位结构域(H_N结构域)以及BoNT/B受体结合结构域(H_C结构域)组成。

[0115] 嵌合梭菌神经毒素包含轻链,该轻链能够表现出非细胞毒性蛋白酶活性并且能够在靶神经元的胞质溶胶中切割SNARE蛋白。如上所述,双链形式是梭菌神经毒素的活性形式。因此,本发明排除使用包含已经(例如通过一种或多种突变的方式)催化失活的轻链(“无催化活性的轻链”)的嵌合梭菌神经毒素。这种无催化活性的轻链(和包含它的梭菌神经毒素)是本领域已知的。无催化活性的L-链可以具有一个或多个使所述催化活性失活的突变。例如,无催化活性的L-链可包含活性位点残基的突变。突变可以是取代或缺失,特别是用化学上相似的氨基酸取代。谷氨酸可以被谷氨酰胺取代,组氨酸可以被酪氨酸取代,精氨酸可以被谷氨酰胺取代,和/或酪氨酸可以被苯丙氨酸取代。可替代地,任何残基可被丙氨酸取代。无催化活性的BoNT/A L-链可包含H223、E224、H227、E262、R363和/或Y366处的突变,例如,至少E224和H227处的突变。无催化活性的BoNT/A L-链可包含在E224处用谷氨酰胺取代(E224Q)和在H227处用酪氨酸取代(H227Y)。

[0116] 本文所用的关于梭菌神经毒素L-链的术语“无催化活性的”是指所述L-链基本上没有非细胞毒性蛋白酶活性,例如没有非细胞毒性蛋白酶活性。无催化活性的梭菌神经毒素L-链可以是不切割靶细胞中胞外融合装置的蛋白质的链。术语“基本上没有非细胞毒性蛋白酶活性”是指梭菌神经毒素L-链具有低于5%的催化活性梭菌神经毒素L-链(优选如SEQ ID NO:6所示的天然BoNT/A的L-链)的非细胞毒性蛋白酶活性,例如小于2%、1%或小于0.1%的催化活性梭菌神经毒素L-链的非细胞毒性蛋白酶活性。非细胞毒性蛋白酶活性可通过以下方式体外测定:将测试梭菌神经毒素L-链与SNARE蛋白一起孵育,并将由测试梭菌神经毒素L-链切割的SNARE蛋白的量与在相同条件下由催化活性的梭菌神经毒素L-链(优选如SEQ ID NO:6所示的天然BoNT/A的L-链)切割的SNARE蛋白的量进行比较。常规技术(例如SDS-PAGE和蛋白质印迹)可用于量化切割的SNARE蛋白的量。合适的体外测定描述于

WO 2019/145577 A1,其通过引用并入本文。

[0117] 基于细胞的体内测定也可用于确定包含L-链和功能性细胞结合和转位结构域的梭菌神经毒素是否具有非细胞毒性蛋白酶活性。诸如趾外展评分(Digit Abduction Score)(DAS)测定法、背根神经节(DRG)测定法、脊髓神经元(SCN)测定法和小鼠膈神经-偏侧膈(PNHD)测定法等测定法是本领域的常规测定法。用于确定非细胞毒性蛋白酶活性的合适测定法可以是Aoki KR,Toxicon 39:1815-1820;2001或Donald等人(2018),Pharmacol Res Perspect,e00446,1-14中描述的测定法,其通过引用并入本文。

[0118] 当施用于受试者时,嵌合梭菌神经毒素优选为其活性双链形式,其中轻链和重链通过二硫键连接在一起。当梭菌神经毒素(例如嵌合梭菌神经毒素)在本文中通过多肽序列(SEQ ID NO)方式定义时,序列(SEQ ID NO)的L-链部分可以构成双链梭菌神经毒素(例如,双链嵌合梭菌神经毒素)的第一链,并且H_N和H_C结构域一起可以构成双链梭菌神经毒素(例如,双链嵌合梭菌神经毒素)的第二链,其中第一链和第二链通过二硫键连接在一起。本领域技术人员将理解,蛋白酶可在梭菌神经毒素(例如嵌合梭菌神经毒素)的活化环内的一个或多个位置,优选在活化环内的两个位置切割。当切割发生在活化环中的一个以上的位置(优选在两个位置)时,双链梭菌神经毒素序列(例如双链嵌合梭菌神经毒素)中可能不存在该序列的C-末端L-链部分的小片段。鉴于此,双链梭菌神经毒素(例如双链嵌合梭菌神经毒素)的序列可能与相应的单链梭菌神经毒素(例如单链嵌合梭菌神经毒素)的序列略有不同。所述小片段可以是1-15个氨基酸。特别地,在一个实施方案中,当使用Lys-C将单链嵌合梭菌神经毒素转化为双链梭菌神经毒素时,所述不存在的序列的C-末端L-链部分的小片段可以是SEQ ID NO:15或16。

[0119] LH_N结构域的C-末端氨基酸残基可以对应于分隔BoNT/A的LH_N和H_C结构域的3_{10}螺旋的第一个氨基酸残基,并且H_C结构域的N-末端氨基酸残基可以对应于分隔BoNT/B的LH_N和H_C结构域的3_{10}螺旋的第二个氨基酸残基。

[0120] BoNT/A多肽序列的实例提供为SEQ ID NO:6。

[0121] BoNT/B多肽序列的实例提供为SEQ ID NO:7(UniProt登记号B1INP5)。

[0122] 本文提及的“分隔BoNT/A的LH_N和H_C结构域的3_{10}螺旋的第一个氨基酸残基”是指分隔LH_N和H_C结构域的3_{10}螺旋的N-末端残基。

[0123] 本文提及的“分隔BoNT/B的LH_N和H_C结构域的3_{10}螺旋的第二个氨基酸残基”是指在分隔LH_N和H_C结构域的3_{10}螺旋的N-末端残基之后的氨基酸残基。

[0124] “3_{10}螺旋”是在蛋白质和多肽中发现的一种类型的二级结构,还有α-螺旋、β-折叠和回折(reverse turn)。3_{10}螺旋中的氨基酸以右旋螺旋结构排列,其中每个完整的转角(turn)由三个残基和十个原子完成,所述三个残基和十个原子将它们之间的分子内氢键分开。每个氨基酸对应于螺旋中的120°转角(即螺旋每个转角有三个残基),以及沿螺旋轴平移2.0 Å (=0.2 nm),并且在通过氢键形成的环中具有10个原子。最重要的是,氨基酸的N-H基团先与前三个残基的氨基酸的C=O基团形成氢键;这种重复的i+3→i氢键限定了3_{10}螺旋。3_{10}螺旋是本领域技术人员熟悉的结构生物学中的标准概念。

[0125] 这种3_{10}螺旋对应于形成实际螺旋的四个残基和两个帽(或过渡)残基,这四个残基的每一端都有一个。本文所用的术语“分隔LH_N和H_C结构域的3_{10}螺旋”由6个残基组成。

[0126] 通过进行结构分析和序列比对,鉴定了分隔LH_N和H_C结构域的3_{10}螺旋。这种3_{10}螺旋

在其N-末端(即在LH_N结构域的C-末端部分)被α-螺旋包围,并在其C-末端(即在H_C结构域的N-末端部分)被β-链包围。3_{10}螺旋的第一个(N-末端)残基(帽或过渡残基)也对应于该α-螺旋的C-末端残基。

[0127] 分隔LH_N和H_C结构域的3_{10}螺旋可以例如由公开可用的肉毒杆菌神经毒素的晶体结构确定,例如3BTA(http://www.rcsb.org/pdb/explore/explore.do?structureId=3BTA)和1EPW(http://www.rcsb.org/pdb/explore/explore.do?structureId=1EPW)分别对应肉毒杆菌神经毒素A1和B1。

[0128] 公开可用的计算机建模和比对工具也可用于确定其他神经毒素中分隔LH_N和H_C结构域的3_{10}螺旋的位置,例如同源建模服务器LOOPP(学习、观察和输出蛋白质模式(Learning,Observing and Outputting Protein Patterns),http://loopp.org)、PHYRE(蛋白质同源/模拟识别引擎(Protein Homology/analogY Recognition Engine),http://www.sbg.bio.ic.ac.uk/phyre2/)和Rosetta(https://www.rosettacommons.org/)、蛋白质叠加服务器SuperPose(http://wishart.biology.ualberta.ca/superpose/)、比对程序Clustal Omega(http://www.clustal.org/omega/)以及分子和细胞生物学家的互联网资源(http://molbiol-tools.ca/)上列出的许多其他工具/服务。特别地,"H_N/H_{CN}"连接处周围的区域在结构上可以是高度保守的,这使得它成为叠加不同血清型的理想区域。

[0129] 例如,下列方法可用于测定其他神经毒素中这种3_{10}螺旋的序列:

[0130] 1.结构同源性模型工具LOOP(http://loopp.org)可用于获得基于BoNT/A1晶体结构的其他BoNT血清型的预测结构(3BTA.pdb);

[0131] 2.由此获得的结构(pdb)文件可被编辑以仅包括H_{CN}结构域的N-末端和其之前的约80个残基(其为H_N结构域的一部分),从而保留在结构上高度保守的"H_N/H_{CN}"区域;

[0132] 3.蛋白质叠加服务器SuperPose(http://wishart.biology.ualberta.ca/superpose/)可以用于将每种血清型叠加到3BTA.pdb结构上;

[0133] 4.可以检查重叠的pdb文件以将3_{10}螺旋定位在BoNT/A1的H_C结构域的起始处,然后可以鉴定其他血清型中的相应残基。

[0134] 5.其他的BoNT血清型序列可以与Clustal Omega进行比对,以检查相应的残基是否正确。

[0135] 通过该方法测定的LH_N、H_C和3_{10}螺旋结构域的实例如下所示:

[0136]

神经毒素	登记号（加上小数后的序列版本）	LH_N	H_C	3_{10} 螺旋
BoNT/A1 (SEQ ID NO: 6)	A5HZZ9.1	1-872	873-1296	^{872}NIINTS^{877}
BoNT/A2	X73423.3	1-872	873-1296	^{872}NIVNTS^{877}
BoNT/A3	DQ185900.1（也称为Q3LRX9.1）	1-872	873-1292	^{872}NIVNTS^{877}
BoNT/A4	EU341307.1（也称为Q3LRX8.1）	1-872	873-1296	^{872}NITNAS^{877}
BoNT/A5	EU679004.1（也称为C1IPK2.1）	1-872	873-1296	^{872}NIINTS^{877}
BoNT/A6	FJ981696.1	1-872	873-1296	^{872}NIINTS^{877}
BoNT/A7	JQ954969.1（也称为K4LN57.1）	1-872	873-1296	^{872}NIINTS^{877}
BoNT/A8	KM233166.1	1-872	873-1297	^{872}NITNTS^{877}
BoNT/B1 (SEQ ID NO: 7)	B1INP5.1	1-859	860-1291	^{859}EILNNI^{864}
BoNT/B2	AB084152.1（也称为Q8GR96.1）	1-859	860-1291	^{859}EILNNI^{864}
BoNT/B3	EF028400.1（也称为A2I2S2.1）	1-859	860-1291	^{859}EILNNI^{864}
BoNT/B4	EF051570.1（也称为A2I2W0.1）	1-859	860-1291	^{859}EILNNI^{864}
BoNT/B5	EF033130.1（也称为A2I2U6.1）	1-859	860-1291	^{859}DILNNI^{864}
BoNT/B6	AB302852.1（也称为A8R089.1）	1-859	860-1291	^{859}EILNNI^{864}

[0137]

神经毒素	登记号（加上小数后的序列版本）	LH_N	H_C	3_{10} 螺旋
BoNT/B7	JQ354985.1（也称为H9CNK9.1）	1-859	860-1291	^{859}EILNNI^{864}
BoNT/B8	JQ964806.1（也称为I6Z8G9.1）	1-859	860-1292	^{859}EILNNI^{864}

[0138] 通过结构分析和序列比对，发现分隔LH_N和H_C结构域的3_{10}螺旋后面的β链是所有肉毒杆菌神经毒素和破伤风神经毒素中的保守结构，并且当从分隔LH_N和H_C结构域的3_{10}螺旋的第一个残基开始时，所述β链从第8个残基开始(例如，BoNT/A1的残基879)。

[0139] BoNT/AB嵌合体可包含与来自BoNT/B的H_C结构域共价连接的来自BoNT/A的LH_N结构域，其中LH_N结构域的C-末端氨基酸残基对应于位于BoNT/A的H_C结构域的开头(N-末端)的β链N-末端的第八个氨基酸残基，并且其中H_C结构域的N-末端氨基酸残基对应于位于BoNT/B的H_C结构域的开头(N-末端)的β链N-末端的第七个氨基酸。

[0140] BoNT/AB嵌合体可包含与来自BoNT/B的H_C结构域共价连接的来自BoNT/A的LH_N结构

域,其中LH_N结构域的C-末端氨基酸残基对应于位于BoNT/A的LH_N结构域的末尾(C-末端)的α-螺旋的C-末端氨基酸残基,并且其中H_C结构域的N-末端氨基酸残基对应于紧邻位于BoNT/B的LH_N结构域的末尾(C-末端)的α-螺旋的C-末端氨基酸残基的C-末端氨基酸残基。

[0141] BoNT/AB嵌合体设计过程的基本原理是尽量确保二级结构不受影响,从而最大限度地减少对三级结构和每个结构域功能的任何改变。不希望受理论束缚,假设通过不破坏BoNT/AB嵌合体中3_{10}螺旋的四个中心氨基酸残基,确保嵌合神经毒素的最佳构象,从而允许嵌合神经毒素充分发挥他们的全部功能。事实上,令人惊讶地,仅保留BoNT/A的3_{10}螺旋的第一个氨基酸残基和BoNT/B的3_{10}螺旋之前的第二个氨基酸残基不仅允许产生可溶性和功能性BoNT/AB嵌合体,而且进一步导致相对于其他BoNT/AB嵌合体改善的性质,特别是增加的效力、增加的安全比和/或更长的作用持续时间(以及当与天然BoNT/A[例如SEQ ID NO:6]相比时增加的安全比和/或作用持续时间)。

[0142] 可以通过测量相关动物模型(例如小鼠,其中在施用七天内检测体重的损失)中的体重损失百分比而实验性地评估神经毒素的不良影响(由神经毒素从施用位点扩散引起)。相反地,可以通过趾外展评分(DAS)测定法(肌肉麻痹的测量)来实验性地评价神经毒素的期望的中靶效应(on-target effects)。DAS测定法可以通过将在明胶磷酸盐缓冲液中配制的20μL神经毒素注射到小鼠腓肠肌/比目鱼肌复合体中进行,然后使用Aoki的方法评估趾外展评分(Aoki KR,Toxicon 39:1815-1820;2001)。在DAS测定中,将小鼠尾部短暂悬挂,以引起特征性的惊跳反应(startle response),其中小鼠伸展其后肢并外展其后趾。注射神经毒素后,按五分制量表对不同程度的趾外展进行评分(0=正常至4=趾外展和腿伸展的最大程度减少)。

[0143] 然后神经毒素的安全比可以表示为小鼠体重下降10%所需的神经毒素的量(在小鼠施用后头七天内的峰值效应下测量)与DAS评分为2所需的神经毒素的量之间的比率。因此,需要高安全比评分,并且指示神经毒素能够有效地麻痹目标肌肉而几乎没有不期望的脱靶效应。

[0144] 高安全性比在治疗中特别有利,因为它代表治疗指数的增加。换言之,这意味着与备选的梭菌神经毒素治疗剂相比,可以使用减少的剂量和/或可以使用增加的剂量而没有任何额外的(例如有害的)作用。有害作用可包括全身毒性和/或不期望的向邻近肌肉的扩散。使用更高剂量的神经毒素而没有额外作用的可能性是特别有利的,因为更高剂量通常导致神经毒素作用的持续时间更长。

[0145] 嵌合梭菌神经毒素的效力可以表示为当施用于小鼠腓肠肌/比目鱼肌复合体时导致给定DAS评分的神经毒素的最小剂量,例如DAS评分为2(ED_{50}剂量)或DAS评分为4。嵌合梭菌神经毒素的效力也可以表示为在测量神经毒素对SNARE切割的细胞测定中的EC_{50}剂量,例如在测量嵌合梭菌神经毒素对SNAP25切割的细胞测定中的EC_{50}剂量。

[0146] 嵌合梭菌神经毒素的作用持续时间可以表示为在向小鼠腓肠肌/比目鱼肌复合体施用给定剂量的神经毒素(例如导致DAS评分为4的神经毒素的最小剂量)后恢复DAS评分为0所需的时间。

[0147] 所述嵌合梭菌神经毒素可具有大于7的安全比,其中所述安全比计算为:以pg/小鼠测量的-10%体重变化所需的毒素剂量除以以pg/小鼠测量的DAS EC_{50},其中EC_{50}=产生DAS评分为2所需的剂量。例如,嵌合梭菌神经毒素可具有至少8、9、10、15、20、25、30、35、40、

45或50的安全比。

[0148] 优选地,嵌合梭菌神经毒素具有至少10(例如安全比为10)、更优选至少12或13(例如14-15)的安全比。嵌合梭菌神经毒素可具有大于7至最多50的安全比,例如8-45、10-20或12-15。

[0149] 当与BoNT/A(例如,SEQ ID NO:6,例如双链形式的SEQ ID NO:6)相比时,本发明的嵌合梭菌神经毒素优选具有更长的作用持续时间(例如一种或多种症状改善至少5%、10%、25%或50%)。所述作用持续时间可以至少大1.25×、1.5×、1.75×、2.0×或2.25×。所述嵌合梭菌神经毒素的作用持续时间可为4.5至9个月或6至9个月。例如,作用持续时间可以是(从发作起)至少4.5个月、5.0个月、5.5个月、6个月、6.5个月、7.0个月、7.5个月、8.0个月、8.5个月或9.0个月。在具体实施方案中,作用持续时间可大于9.0个月。

[0150] 因此,在一个实施方案中,嵌合梭菌神经毒素可以治疗受试者的病症(例如疼痛或感觉障碍,优选疼痛)的持续时间(例如从施用开始)比用BoNT/A(例如,SEQ ID NO:6,例如双链形式的SEQ ID NO:6)治疗的受试者的持续时间更长。所述持续时间可以是从施用起的持续时间,其与本发明的嵌合梭菌神经毒素的作用持续时间一致。因此,嵌合梭菌神经毒素可以在施用后的持续时间中治疗受试者的病症,该持续时间比施用BoNT/A(例如,SEQ ID NO:6,例如双链形式的SEQ ID NO:6)后的治疗持续时间长至少1.25×、1.5×、1.75×、2.0×或2.25×。嵌合梭菌神经毒素可以治疗受试者的病症自施用起的持续时间为4.5至9个月或6至9个月,例如从施用起至少4.5个月、5.0个月、5.5个月、6个月、6.5个月、7.0个月、7.5个月、8.0个月、8.5个月或9.0个月。在具体实施方案中,嵌合梭菌神经毒素可以在自施用起大于9.0个月的持续时间内治疗受试者的病症。

[0151] 因此,在一个方面,本发明提供了一种用于治疗受试者的病症(例如疼痛或感觉障碍,优选疼痛)的方法,所述治疗的持续时间(例如从施用开始)比用BoNT/A(例如,SEQ ID NO:6,例如双链形式的SEQ ID NO:6)治疗的受试者的治疗持续时间更长,该方法包括向受试者施用嵌合梭菌神经毒素,其中嵌合梭菌神经毒素抑制来自包含Aδ神经纤维或C神经纤维的神经元的介质(例如疼痛介质)的释放,其中所述嵌合梭菌神经毒素分别结合至包含Aδ神经纤维或C神经纤维的神经元,并且其中所述嵌合梭菌神经毒素包含肉毒杆菌神经毒素A(BoNT/A)轻链和转位结构域(H_N结构域),以及BoNT/B受体结合结构域(H_C结构域)。

[0152] 在一个相关方面,本发明提供了一种嵌合梭菌神经毒素,其用于治疗受试者的病症(例如疼痛或感觉障碍,优选疼痛)的方法中,所述治疗的持续时间(例如从施用开始)比用BoNT/A(例如,SEQ ID NO:6,例如双链形式的SEQ ID NO:6)治疗的受试者的治疗持续时间更长,该方法包括向受试者施用嵌合梭菌神经毒素,其中嵌合梭菌神经毒素抑制来自包含Aδ神经纤维或C神经纤维的神经元的介质(例如疼痛介质)的释放,其中所述嵌合梭菌神经毒素分别结合至包含Aδ神经纤维或C神经纤维的神经元,并且其中所述嵌合梭菌神经毒素包含肉毒杆菌神经毒素A(BoNT/A)轻链和转位结构域(H_N结构域),以及BoNT/B受体结合结构域(H_C结构域)。

[0153] 在另一个相关方面,本发明提供了嵌合梭菌神经毒素在制备用于治疗受试者的病症(例如疼痛或感觉障碍,优选疼痛)的药物中的用途,所述治疗的持续时间(例如从施用开始)比用BoNT/A(例如,SEQ ID NO:6,例如双链形式的SEQ ID NO:6)治疗的受试者的治疗持续时间更长,其中嵌合梭菌神经毒素抑制来自包含Aδ神经纤维或C神经纤维的神经元的介

质(例如疼痛介质)的释放,其中所述嵌合梭菌神经毒素分别结合至包含Aδ神经纤维或C神经纤维的神经元,并且其中所述嵌合梭菌神经毒素包含肉毒杆菌神经毒素A(BoNT/A)轻链和转位结构域(H_N结构域),以及BoNT/B受体结合结构域(H_C结构域)。

[0154] 因此,在一个方面,本发明提供了一种用于治疗受试者的病症(例如疼痛或感觉障碍,优选疼痛)的方法,所述治疗的持续时间(例如从施用开始)比用BoNT/A(例如,SEQ ID NO:6,例如双链形式的SEQ ID NO:6)治疗的受试者的治疗持续时间更长,该方法包括向受试者施用嵌合梭菌神经毒素,其中嵌合梭菌神经毒素包含肉毒杆菌神经毒素A(BoNT/A)轻链和转位结构域(H_N结构域),以及BoNT/B受体结合结构域(H_C结构域)。

[0155] 在一个相关方面,本发明提供了一种嵌合梭菌神经毒素,其用于治疗受试者的病症(例如疼痛或感觉障碍,优选疼痛)的方法中,所述治疗的持续时间(例如从施用开始)比用BoNT/A(例如,SEQ ID NO:6,例如双链形式的SEQ ID NO:6)治疗的受试者的治疗持续时间更长,该方法包括向受试者施用嵌合梭菌神经毒素,其中嵌合梭菌神经毒素包含肉毒杆菌神经毒素A(BoNT/A)轻链和转位结构域(H_N结构域),以及BoNT/B受体结合结构域(H_C结构域)。

[0156] 在另一个相关方面,本发明提供了嵌合梭菌神经毒素在制备用于治疗受试者的病症(例如疼痛或感觉障碍,优选疼痛)的药物中的用途,所述治疗的持续时间(例如从施用开始)比用BoNT/A(例如,SEQ ID NO:6,例如双链形式的SEQ ID NO:6)治疗的受试者的治疗持续时间更长,其中嵌合梭菌神经毒素包含肉毒杆菌神经毒素A(BoNT/A)轻链和转位结构域(H_N结构域),以及BoNT/B受体结合结构域(H_C结构域)。

[0157] 所述病症优选为偏头痛或偏头痛疼痛。

[0158] 术语“治疗(treat)受试者的病症持续时间(例如从施用开始)比用BoNT/A治疗的受试者的治疗持续时间更长”或“治疗(treating)受试者的病症持续时间(例如从施用开始)比用BoNT/A治疗的受试者的治疗持续时间更长”可以指与施用BoNT/A相比,在施用本发明的嵌合梭菌神经毒素后,受试者的病症的一种或多种症状在更长时间内减轻。所述作用持续时间可以至少大1.25×、1.5×、1.75×、2.0×或2.25×。嵌合梭菌神经毒素的作用持续时间可以在6至9个月之间。例如,作用持续时间可以是至少:(从发作起)4.5个月、5.0个月、5.5个月、6个月、6.5个月、7.0个月、7.5个月、8.0个月、8.5个月或9.0个月。在具体实施方案中,作用持续时间可大于9.0个月。所述减轻可以通过与已经用BoNT/A治疗的表现出等同症状的等同对照受试者进行比较来确定。在对照受试者的一种或多种症状的严重性与BoNT/A治疗前基本相同(例如相同)的时间段,与用嵌合梭菌神经毒素治疗前的一种或多种症状的严重性相比,用根据本发明的嵌合梭菌神经毒素治疗的受试者可表现出等同的一种或多种症状改善至少5%、10%、25%或50%。

[0159] 在一个实施方案中,嵌合梭菌神经毒素可以治疗受试者的病症(例如疼痛或感觉障碍,优选疼痛)的疗效比用BoNT/A(例如,SEQ ID NO:6,例如双链形式的SEQ ID NO:6)治疗的受试者的疗效更好。

[0160] 因此,在一个方面,本发明提供了一种用于治疗受试者的病症(例如疼痛或感觉障碍,优选疼痛)的方法,所述治疗的疗效比用BoNT/A(例如,SEQ ID NO:6,例如双链形式的SEQ ID NO:6)治疗的受试者的治疗疗效更好,该方法包括向受试者施用嵌合梭菌神经毒素,其中嵌合梭菌神经毒素抑制来自包含Aδ神经纤维或C神经纤维的神经元的介质(例如疼

痛介质)的释放,其中所述嵌合梭菌神经毒素分别结合至包含Aδ神经纤维或C神经纤维的神经元,并且其中所述嵌合梭菌神经毒素包含肉毒杆菌神经毒素A(BoNT/A)轻链和转位结构域(H_N结构域),以及BoNT/B受体结合结构域(H_C结构域)。

[0161] 在一个相关方面,本发明提供了一种嵌合梭菌神经毒素,其用于治疗受试者的病症(例如疼痛或感觉障碍,优选疼痛)的方法中,所述治疗的疗效比用BoNT/A(例如,SEQ ID NO:6,例如双链形式的SEQ ID NO:6)治疗的受试者的治疗疗效更好,该方法包括向受试者施用嵌合梭菌神经毒素,其中嵌合梭菌神经毒素抑制来自包含Aδ神经纤维或C神经纤维的神经元的介质(例如疼痛介质)的释放,其中所述嵌合梭菌神经毒素分别结合至包含Aδ神经纤维或C神经纤维的神经元,并且其中所述嵌合梭菌神经毒素包含肉毒杆菌神经毒素A(BoNT/A)轻链和转位结构域(H_N结构域),以及BoNT/B受体结合结构域(H_C结构域)。

[0162] 在另一个相关方面,本发明提供了嵌合梭菌神经毒素在制备用于治疗受试者的病症(例如疼痛或感觉障碍,优选疼痛)的药物中的用途,所述治疗的疗效比用BoNT/A(例如,SEQ ID NO:6,例如双链形式的SEQ ID NO:6)治疗的受试者的治疗疗效更好,其中嵌合梭菌神经毒素抑制来自包含Aδ神经纤维或C神经纤维的神经元的介质(例如疼痛介质)的释放,其中所述嵌合梭菌神经毒素分别结合至包含Aδ神经纤维或C神经纤维的神经元,并且其中所述嵌合梭菌神经毒素包含肉毒杆菌神经毒素A(BoNT/A)轻链和转位结构域(H_N结构域),以及BoNT/B受体结合结构域(H_C结构域)。

[0163] 因此,在一个方面,本发明提供了一种用于治疗受试者的病症(例如疼痛或感觉障碍,优选疼痛)的方法,所述治疗的疗效比用BoNT/A(例如,SEQ ID NO:6,例如双链形式的SEQ ID NO:6)治疗的受试者的治疗疗效更好,该方法包括向受试者施用嵌合梭菌神经毒素,其中嵌合梭菌神经毒素包含肉毒杆菌神经毒素A(BoNT/A)轻链和转位结构域(H_N结构域),以及BoNT/B受体结合结构域(H_C结构域)。

[0164] 在一个相关方面,本发明提供了一种嵌合梭菌神经毒素,其用于治疗受试者的病症(例如疼痛或感觉障碍,优选疼痛)的方法中,所述治疗的疗效比用BoNT/A(例如,SEQ ID NO:6,例如双链形式的SEQ ID NO:6)治疗的受试者的治疗疗效更好,该方法包括向受试者施用嵌合梭菌神经毒素,其中嵌合梭菌神经毒素包含肉毒杆菌神经毒素A(BoNT/A)轻链和转位结构域(H_N结构域),以及BoNT/B受体结合结构域(H_C结构域)。

[0165] 在另一个相关方面,本发明提供了嵌合梭菌神经毒素在制备用于治疗受试者的病症(例如疼痛或感觉障碍,优选疼痛)的药物中的用途,所述治疗的疗效比用BoNT/A(例如,SEQ ID NO:6,例如双链形式的SEQ ID NO:6)治疗的受试者的治疗疗效更好,其中嵌合梭菌神经毒素包含肉毒杆菌神经毒素A(BoNT/A)轻链和转位结构域(H_N结构域),以及BoNT/B受体结合结构域(H_C结构域)。

[0166] 所述病症优选为偏头痛或偏头痛疼痛。

[0167] 术语“治疗(treat)受试者的病症的疗效比用BoNT/A治疗的受试者的治疗疗效更好”或“治疗(treating)受试者的病症的疗效比用BoNT/A治疗的受试者的治疗疗效更好”可以指与施用BoNT/A相比,在施用本发明的嵌合梭菌神经毒素后,受试者的病症的一种或多种症状在更大程度上减轻。所述减轻可以通过与已经用BoNT/A治疗的表现出等同症状的等同对照受试者进行比较来确定。在施用后的给定时间段,与施用BoNT/A(例如,SEQ ID NO:6,例如双链形式的SEQ ID NO:6)后的相同时间段的对照受试者的等同的一种或多种症状

的严重性相比,用根据本发明的嵌合梭菌神经毒素治疗的受试者可以表现出一种或多种症状的严重性减轻至少5%、10%、25%或50%。在另一个实施方案中,更好的疗效可以指用嵌合梭菌神经毒素治疗的受试者的一种或多种症状的严重性的最大减轻程度大于用BoNT/A(例如,SEQ ID NO:6,例如双链形式的SEQ ID NO:6)治疗的对照受试者的等同的一种或多种症状的严重性的最大减轻程度。

[0168] 在一个实施方案中,与用BoNT/A(例如,SEQ ID NO:6,例如双链形式的SEQ ID NO:6)治疗的受试者相比,嵌合梭菌神经毒素可以更大程度地减轻受试者的疼痛(例如偏头痛)。

[0169] 因此,在一个方面,本发明提供了一种用于减轻受试者的疼痛的方法中,与用BoNT/A(例如,SEQ ID NO:6,例如双链形式的SEQ ID NO:6)治疗的受试者相比,其更大程度地减轻受试者的疼痛,该方法包括向受试者施用嵌合梭菌神经毒素,其中嵌合梭菌神经毒素抑制来自包含Aδ神经纤维或C神经纤维的神经元的疼痛介质的释放,其中所述嵌合梭菌神经毒素分别结合至包含Aδ神经纤维或C神经纤维的神经元,并且其中所述嵌合梭菌神经毒素包含肉毒杆菌神经毒素A(BoNT/A)轻链和转位结构域(H_N结构域),以及BoNT/B受体结合结构域(H_C结构域)。

[0170] 在一个相关方面,本发明提供了一种嵌合梭菌神经毒素,其用于与用BoNT/A(例如,SEQ ID NO:6,例如双链形式的SEQ ID NO:6)治疗的受试者相比更大程度地减轻受试者的疼痛的方法中,该方法包括向受试者施用嵌合梭菌神经毒素,其中嵌合梭菌神经毒素抑制来自包含Aδ神经纤维或C神经纤维的神经元的疼痛介质的释放,其中所述嵌合梭菌神经毒素分别结合至包含Aδ神经纤维或C神经纤维的神经元,并且其中所述嵌合梭菌神经毒素包含肉毒杆菌神经毒素A(BoNT/A)轻链和转位结构域(H_N结构域),以及BoNT/B受体结合结构域(H_C结构域)。

[0171] 在另一个相关方面,本发明提供了嵌合梭菌神经毒素在制备用于减轻受试者的疼痛的药物中的用途,与用BoNT/A(例如,SEQ ID NO:6,例如双链形式的SEQ ID NO:6)治疗的受试者相比,其更大程度地减轻受试者的疼痛,其中嵌合梭菌神经毒素抑制来自包含Aδ神经纤维或C神经纤维的神经元的疼痛介质的释放,其中所述嵌合梭菌神经毒素分别结合至包含Aδ神经纤维或C神经纤维的神经元,并且其中所述嵌合梭菌神经毒素包含肉毒杆菌神经毒素A(BoNT/A)轻链和转位结构域(H_N结构域),以及BoNT/B受体结合结构域(H_C结构域)。

[0172] 因此,在一个方面,本发明提供了一种用于减轻受试者的疼痛的方法中,与BoNT/A(例如,SEQ ID NO:6,例如双链形式的SEQ ID NO:6)治疗的受试者相比,其更大程度地减轻受试者的疼痛,该方法包括向受试者施用嵌合梭菌神经毒素,其中嵌合梭菌神经毒素包含肉毒杆菌神经毒素A(BoNT/A)轻链和转位结构域(H_N结构域),以及BoNT/B受体结合结构域(H_C结构域)。

[0173] 在一个相关方面,本发明提供了一种嵌合梭菌神经毒素,其用于减轻受试者的疼痛的方法中,与用BoNT/A(例如,SEQ ID NO:6,例如双链形式的SEQ ID NO:6)治疗的受试者相比,其更大程度地减轻受试者的疼痛,该方法包括向受试者施用嵌合梭菌神经毒素,其中嵌合梭菌神经毒素包含肉毒杆菌神经毒素A(BoNT/A)轻链和转位结构域(H_N结构域),以及BoNT/B受体结合结构域(H_C结构域)。

[0174] 在另一个相关方面,本发明提供了嵌合梭菌神经毒素在制备用于减轻受试者的疼

痛的药物中的用途,与用BoNT/A(例如,SEQ ID NO:6,例如双链形式的SEQ ID NO:6)治疗的受试者相比,其更大程度地减轻受试者的疼痛,其中嵌合梭菌神经毒素包含肉毒杆菌神经毒素A(BoNT/A)轻链和转位结构域(H_N结构域),以及BoNT/B受体结合结构域(H_C结构域)。

[0175] 所述疼痛优选为偏头痛疼痛。

[0176] 术语"与用BoNT/A治疗的受试者相比更大程度地减轻(reduce)受试者的疼痛"或"与用BoNT/A治疗的受试者相比更大程度地减轻(reducing)受试者的疼痛"可以指与施用BoNT/A相比,在施用本发明的嵌合梭菌神经毒素后,受试者的疼痛更大程度地减轻。所述减轻可以通过与已经用BoNT/A治疗的表现出等同疼痛的等同对照受试者进行比较来确定。在施用后的给定时间段,与施用BoNT/A(例如,SEQ ID NO:6,例如双链形式的SEQ ID NO:6)后的相同时间段的对照受试者的等同的疼痛的严重性相比,用根据本发明的嵌合梭菌神经毒素治疗的受试者可以表现出疼痛减轻至少5%、10%、25%或50%。在另一个实施方案中,用嵌合梭菌神经毒素治疗的受试者的疼痛的最大减轻程度大于用BoNT/A(例如,SEQ ID NO:6,例如双链形式的SEQ ID NO:6)治疗的对照受试者的等同疼痛的最大减轻程度。

[0177] 在一个实施方案中,与用BoNT/A(例如,SEQ ID NO:6,例如双链形式的SEQ ID NO:6)治疗的受试者的相同生物流体和/或脑中相同疼痛介质减少的量相比,嵌合梭菌神经毒素可以更大程度地减少受试者的生物流体和/或脑中的疼痛介质(例如偏头痛疼痛介质)的量。

[0178] 因此,在一个方面,本发明提供了一种方法,其用于减少受试者的生物流体和/或脑中疼痛介质的量的方法中,与用BoNT/A(例如,SEQ ID NO:6,例如双链形式的SEQ ID NO:6)治疗的受试者的相同生物流体和/或脑中相同疼痛介质减少的量相比,其更大程度地减少受试者的生物流体和/或脑中的疼痛介质的量,该方法包括向受试者施用嵌合梭菌神经毒素,其中嵌合梭菌神经毒素抑制来自包含Aδ神经纤维或C神经纤维的神经元的疼痛介质的释放,其中所述嵌合梭菌神经毒素分别结合至包含Aδ神经纤维或C神经纤维的神经元,并且其中所述嵌合梭菌神经毒素包含肉毒杆菌神经毒素A(BoNT/A)轻链和转位结构域(H_N结构域),以及BoNT/B受体结合结构域(H_C结构域)。

[0179] 在一个相关方面,本发明提供了一种嵌合梭菌神经毒素,其用于减少受试者的生物流体和/或脑中疼痛介质的量的方法中,与用BoNT/A(例如,SEQ ID NO:6,例如双链形式的SEQ ID NO:6)治疗的受试者的相同生物流体和/或脑中相同疼痛介质减少的量相比,其更大程度地减少受试者的生物流体和/或脑中的疼痛介质的量,该方法包括向受试者施用嵌合梭菌神经毒素,其中嵌合梭菌神经毒素抑制来自包含Aδ神经纤维或C神经纤维的神经元的疼痛介质的释放,其中所述嵌合梭菌神经毒素分别结合至包含Aδ神经纤维或C神经纤维的神经元,并且其中所述嵌合梭菌神经毒素包含肉毒杆菌神经毒素A(BoNT/A)轻链和转位结构域(H_N结构域),以及BoNT/B受体结合结构域(H_C结构域)。

[0180] 在另一个相关方面,本发明提供了嵌合梭菌神经毒素在制备用于减少受试者的生物流体和/或脑中疼痛介质的量的药物中的用途,与用BoNT/A(例如,SEQ ID NO:6,例如双链形式的SEQ ID NO:6)治疗的受试者的相同生物流体和/或脑中相同疼痛介质减少的量相比,其更大程度地减少受试者的生物流体和/或脑中的疼痛介质的量,其中嵌合梭菌神经毒素抑制来自包含Aδ神经纤维或C神经纤维的神经元的疼痛介质的释放,其中所述嵌合梭菌神经毒素分别结合至包含Aδ神经纤维或C神经纤维的神经元,并且其中所述嵌合梭菌神经

毒素包含肉毒杆菌神经毒素A(BoNT/A)轻链和转位结构域(H_N结构域),以及BoNT/B受体结合结构域(H_C结构域)。

[0181] 因此,在一个方面,本发明提供了一种方法,其用于减少受试者的生物流体和/或脑中疼痛介质的量的方法,与用BoNT/A(例如,SEQ ID NO:6,例如双链形式的SEQ ID NO:6)治疗的受试者的相同生物流体和/或脑中相同疼痛介质减少的量相比,其更大程度地减少受试者的生物流体和/或脑中的疼痛介质的量,该方法包括向受试者施用嵌合梭菌神经毒素,其中嵌合梭菌神经毒素包含肉毒杆菌神经毒素A(BoNT/A)轻链和转位结构域(H_N结构域),以及BoNT/B受体结合结构域(H_C结构域)。

[0182] 在一个相关方面,本发明提供了一种嵌合梭菌神经毒素,其用于减少受试者的生物流体和/或脑中疼痛介质的量的方法中,与用BoNT/A(例如,SEQ ID NO:6,例如双链形式的SEQ ID NO:6)治疗的受试者的相同生物流体和/或脑中相同疼痛介质减少的量相比,其更大程度地减少受试者的生物流体和/或脑中的疼痛介质的量,该方法包括向受试者施用嵌合梭菌神经毒素,其中嵌合梭菌神经毒素包含肉毒杆菌神经毒素A(BoNT/A)轻链和转位结构域(H_N结构域),以及BoNT/B受体结合结构域(H_C结构域)。

[0183] 在另一个相关方面,本发明提供了嵌合梭菌神经毒素在制备用于减少受试者的生物流体和/或脑中疼痛介质的量的药物中的用途,与用BoNT/A(例如,SEQ ID NO:6,例如双链形式的SEQ ID NO:6)治疗的受试者的相同生物流体和/或脑中相同疼痛介质减少的量相比,其更大程度地减少受试者的生物流体和/或脑中的疼痛介质的量,其中嵌合梭菌神经毒素包含肉毒杆菌神经毒素A(BoNT/A)轻链和转位结构域(H_N结构域),以及BoNT/B受体结合结构域(H_C结构域)。

[0184] 术语“与用BoNT/A治疗的受试者的相同生物流体和/或脑中的相同疼痛介质减少的量相比更大程度地减少(reduce)受试者的生物流体和/或脑中的疼痛介质的量”或“与用BoNT/A治疗的受试者的相同生物流体和/或脑中的相同疼痛介质减少的量相比更大程度地减少(reducing)受试者的生物流体和/或脑中的疼痛介质的量”可以指与施用BoNT/A相比,在施用本发明的嵌合梭菌神经毒素后,受试者的疼痛介质的量更大程度地减少。所述减少可以通过与已经用BoNT/A治疗的等同对照受试者的相同生物流体和/或脑中的相同疼痛介质的量进行比较来确定。在施用后的给定时间段,与施用BoNT/A(例如,SEQ ID NO:6,例如双链形式的SEQ ID NO:6)后的相同时间段的对照受试者的相同生物流体和/或脑中的相同疼痛介质的量相比,用根据本发明的嵌合梭菌神经毒素治疗的受试者可以表现出其相同生物流体和/或脑中的疼痛介质的量减少至少5%、10%、25%或50%。在另一个实施方案中,用嵌合梭菌神经毒素治疗的受试者的生物流体和/或脑中的疼痛介质的量的最大减少量(maximal reduction)大于用BoNT/A(例如,SEQ ID NO:6,例如双链形式的SEQ ID NO:6)治疗的对照受试者的相同生物流体和/或脑中的相同疼痛介质的最大减少量。所述疼痛介质可以是偏头痛疼痛介质。所述疼痛介质优选为CGRP。优选地,生物流体是血液(包括其一部分)。

[0185] CGRP可用作评估镇痛剂(例如止痛药)疗效的相关标志物。CGRP因此可用作生物标志物,用于确定梭菌神经毒素治疗疼痛(例如偏头痛疼痛)的适用性。因此,在一个方面,本发明提供了确定梭菌神经毒素是否适合治疗疼痛的方法,所述方法包括:

[0186] (a)比较第一样品中包含的CGRP水平与第二样品中包含的CGRP水平,其中第一样

品已在施用梭菌神经毒素之前从受试者获得,并且其中第二样品已在施用梭菌神经毒素之后从相同受试者获得;和

[0187] (b)当第二样品中的CGRP水平低于第一样品中的CGRP水平时,确定梭菌神经毒素适合治疗疼痛;或者

[0188] (c)当第二样品中的CGRP水平不低于(例如,高于或等于)第一样品中的CGRP水平时,确定梭菌神经毒素不适合治疗疼痛。本文中使用的术语"低于"优选意指统计学上显著低于,而"不低于"优选意指无统计学上显著差异(例如相同)或统计学上显著高于。

[0189] 梭菌神经毒素可以是本领域已知的任何合适的梭菌神经毒素,例如本文所述的嵌合梭菌神经毒素。所述梭菌神经毒素可以是BoNT/A、BoNT/B、BoNT/C、BoNT/D、BoNT/E、BoNT/F、BoNT/G、BoNT/X或破伤风神经毒素(TeNT)。

[0190] BoNT/A可包含与SEQ ID NO:6具有至少70%序列同一性的多肽序列。例如,BoNT/A可包含与SEQ ID NO:6具有至少80%、90%、95%或99.9%序列同一性的多肽序列。优选地,BoNT/A可以包含SEQ ID NO:6(更优选由其组成)。

[0191] BoNT/B可包含与SEQ ID NO:7具有至少70%序列同一性的多肽序列。例如,BoNT/B可包含与SEQ ID NO:7具有至少80%、90%、95%或99.9%序列同一性的多肽序列。优选地,BoNT/B可以包含SEQ ID NO:7(更优选由其组成)。

[0192] BoNT/C可包含与SEQ ID NO:8具有至少70%序列同一性的多肽序列。例如,BoNT/C可包含与SEQ ID NO:8具有至少80%、90%、95%或99.9%序列同一性的多肽序列。优选地,BoNT/C可以包含SEQ ID NO:8(更优选由其组成)。

[0193] BoNT/D可包含与SEQ ID NO:9具有至少70%序列同一性的多肽序列。例如,BoNT/D可包含与SEQ ID NO:9具有至少80%、90%、95%或99.9%序列同一性的多肽序列。优选地,BoNT/D可以包含SEQ ID NO:9(更优选由其组成)。

[0194] BoNT/E可包含与SEQ ID NO:10具有至少70%序列同一性的多肽序列。例如,BoNT/E可包含与SEQ ID NO:10具有至少80%、90%、95%或99.9%序列同一性的多肽序列。优选地,BoNT/E可以包含SEQ ID NO:10(更优选由其组成)。

[0195] BoNT/F可包含与SEQ ID NO:11具有至少70%序列同一性的多肽序列。例如,BoNT/F可包含与SEQ ID NO:11具有至少80%、90%、95%或99.9%序列同一性的多肽序列。优选地,BoNT/F可以包含SEQ ID NO:11(更优选由其组成)。

[0196] BoNT/G可包含与SEQ ID NO:12具有至少70%序列同一性的多肽序列。例如,BoNT/G可包含与SEQ ID NO:12具有至少80%、90%、95%或99.9%序列同一性的多肽序列。优选地,BoNT/G可以包含SEQ ID NO:12(更优选由其组成)。

[0197] BoNT/X可包含与SEQ ID NO:13具有至少70%序列同一性的多肽序列。例如,BoNT/X可包含与SEQ ID NO:13具有至少80%、90%、95%或99.9%序列同一性的多肽序列。优选地,BoNT/X可以包含SEQ ID NO:13(更优选由其组成)。

[0198] TeNT可包含与SEQ ID NO:14具有至少70%序列同一性的多肽序列。例如,TeNT可包含与SEQ ID NO:14具有至少80%、90%、95%或99.9%序列同一性的多肽序列。优选地,TeNT可以包含SEQ ID NO:14(更优选由其组成)。

[0199] 在一个实施方案中,在确定梭菌神经毒素是否适合治疗疼痛的方法中使用梭菌神经毒素之前,将梭菌神经毒素转化为其双链形式,例如如本文所述的。

[0200] 第一样品和第二样品可以是血液样品,任选地经受一个或多个处理步骤。第一样品和第二样品优选是等同的(例如,相同类型并且任选地已经经受相同的处理步骤)。CGRP的水平可以使用任何合适的技术来确定,包括定量蛋白质印迹法和/或质谱法。

[0201] BoNT/A轻链、BoNT/A转位结构域和/或BoNT/B H_C结构域可以是修饰的BoNT/A轻链、BoNT/A转位结构域和/或BoNT/B H_C结构域或其衍生物,包括但不限于下文描述的那些。修饰的BoNT/A轻链、BoNT/A转位结构域和/或BoNT/B H_C结构域或衍生物可以含有与BoNT/A轻链、BoNT/A转位结构域和/或BoNT/B H_C结构域的天然(未修饰)形式相比已经修饰的一个或多个氨基酸,或者可以含有一个或多个在BoNT/A轻链、BoNT/A转位结构域和/或BoNT/B H_C结构域的天然(未修饰)形式中不存在的插入氨基酸。举例来说,相对于天然的(未修饰的)BoNT/A轻链、BoNT/A转位结构域和/或BoNT/B H_C结构域序列,修饰的BoNT/A轻链、BoNT/A转位结构域和/或BoNT/B H_C结构域可以在一个或多个结构域中具有修饰的氨基酸序列。这样的修饰可以修饰其功能方面,例如生物活性或持久性。因此,在一个实施方案中,BoNT/A轻链、BoNT/A转位结构域和/或BoNT/B H_C结构域是修饰的BoNT/A轻链、BoNT/A转位结构域和/或BoNT/B H_C结构域,或修饰的BoNT/A轻链、BoNT/A转位结构域和/或BoNT/B H_C结构域的衍生物。

[0202] 修饰的BoNT/B H_C结构域可具有修饰与靶神经细胞结合的一个或多个修饰,例如当与天然(未修饰的)BoNT/B H_C结构域相比时提供更高或更低亲和力的结合。BoNT/B H_C结构域中的此类修饰可以包括修饰H_C结构域的神经节苷脂结合位点或蛋白质(例如突触结合蛋白)结合位点中的残基,所述修饰改变与靶神经细胞的神经节苷脂受体和/或蛋白质受体的结合。此类修饰的神经毒素的实例描述于WO 2006/027207和WO 2006/114308中,两者均通过引用整体并入本文。

[0203] 修饰的轻链可以在其氨基酸序列中具有一处或多处修饰,例如底物结合或催化结构域中的修饰,其可以改变或修饰被修饰的轻链的SNARE蛋白特异性,条件是所述修饰不使所述轻链催化失活。此类修饰的神经毒素的实例描述于WO 2010/120766和US2011/0318385中,两者均通过引用整体并入本文。

[0204] 来自BoNT/A的LH_N结构域可对应于SEQ ID NO:6的氨基酸残基1至872,或与其具有至少70%序列同一性的多肽序列。来自BoNT/A的LH_N结构域可对应于SEQ ID NO:6的氨基酸残基1至872,或与其具有至少80%、90%或95%序列同一性的多肽序列。优选地,来自BoNT/A的LH_N结构域对应于SEQ ID NO:6的氨基酸残基1至872。

[0205] 来自BoNT/B的H_C结构域可对应于SEQ ID NO:7的氨基酸残基860至1291,或与其具有至少70%序列同一性的多肽序列。来自BoNT/B的H_C结构域可对应于SEQ ID NO:7的氨基酸残基860至1291,或与其具有至少80%、90%或95%序列同一性的多肽序列。优选地,来自BoNT/B的H_C结构域对应于SEQ ID NO:7的氨基酸残基860至1291。

[0206] 优选地,BoNT/AB嵌合体包含BoNT/A1 LH_N结构域和BoNT/B1 H_C结构域。更优选地,LH_N结构域对应于BoNT/A1(SEQ ID NO:6)的氨基酸残基1至872并且H_C结构域对应于BoNT/B1(SEQ ID NO:7)的氨基酸残基860至1291。

[0207] 最优选地,BoNT/B H_C结构域还包含H_{CC}亚结构域中的至少一个氨基酸残基取代、插入、插入缺失(indel)或缺失,其具有与天然BoNT/B序列相比增加BoNT/B神经毒素对人Syt II的结合亲和力的作用。BoNT/B H_{CC}亚结构域中合适的氨基酸残基取代、插入、插入缺失

(indel)或缺失已经公开于WO 2013/180799和WO 2016/154534(均通过引用并入本文)。

[0208] BoNT/B H_{CC}亚结构域中合适的氨基酸残基取代、插入、插入缺失(indel)或缺失可以包括选自以下的取代突变:V1118M、Y1183M、E1191M、E1191I、E1191Q、E1191T、S1199Y、S1199F、S1199L、S1201V、E1191C、E1191V、E1191L、E1191Y、S1199W、S1199E、S1199H、W1178Y、W1178Q、W1178A、W1178S、Y1183C、Y1183P及其组合。

[0209] BoNT/B H_{CC}亚结构域中合适的氨基酸残基取代、插入、插入缺失(indel)或缺失还可包括选自由以下的两个取代突变的组合:E1191M和S1199L,E1191M和S1199Y,E1191M和S1199F,E1191Q和S1199L,E1191Q和S1199Y,E1191Q和S1199F,E1191M和S1199W,E1191M和W1178Q,E1191C和S1199W,E1191C和S1199Y,E1191C和W1178Q,E1191Q和S1199W,E1191V和S1199W,E1191V和S1199Y,或E1191V和W1178Q。

[0210] 在BoNT/B H_{CC}亚结构域中合适的氨基酸残基取代、插入、插入缺失(indel)或缺失也可包括三种取代突变的组合,其为E1191M、S1199W和W1178Q。

[0211] 优选地,BoNT/B H_{CC}亚结构域中的氨基酸残基取代、插入、插入缺失(indel)或缺失包括两种取代突变的组合,其为E1191M和S1199Y。此类修饰存在于嵌合梭菌神经毒素SEQ ID NO:1和SEQ ID NO:4中。E1191M可对应于SEQ ID NO:1的位置1204,并且S1199Y可对应于位置1212。因此,SEQ ID NO:1可包含1204M和1212Y。

[0212] 所述修饰可以是与如SEQ ID NO:7所示的未修饰的BoNT/B相比时的修饰,其中氨基酸残基编号通过与SEQ ID NO:7的比对来确定。由于SEQ ID NO:7(以及对应于本文所述的嵌合梭菌神经毒素多肽的SEQ ID NO)的位置1处甲硫氨酸残基的存在是任选的,在确定氨基酸残基编号时,本领域技术人员将甲硫氨酸残基的存在/不存在纳入考虑。例如,当SEQ ID NO:7包括甲硫氨酸时,位置编号将如上定义(例如E1191将是SEQ ID NO:7的E1191)。可替代地,当SEQ ID NO:7中不存在甲硫氨酸时,氨基酸残基编号应修改为-1(例如E1191将是SEQ ID NO:7的E1190)。因此,嵌合梭菌神经毒素的多肽序列的起始甲硫氨酸氨基酸残基可以是任选的或不存在。当本文所述的其他多肽序列的1位甲硫氨酸存在/不存在时,类似的考虑也适用,本领域技术人员使用本领域常规技术容易确定正确的氨基酸残基编号。可以使用本文所述的用于确定序列同源性和/或%序列同一性的任何方法进行比对。

[0213] 本文所用的术语“缺失”是指去除多肽的一个或多个氨基酸残基,而不在缺失位点置换一个或多个氨基酸残基。因此,当从具有x个氨基酸残基(例如)的多肽序列中缺失一个氨基酸残基时,所得多肽具有x-1个氨基酸残基。

[0214] 本文所用的术语“插入缺失(indel)”是指缺失多肽的一个或多个氨基酸残基,并在缺失位点插入与缺失的氨基酸残基数目相比不同数目的氨基酸残基(更多或更少的氨基酸残基)。因此,(例如)对于其中从具有x个氨基酸残基的多肽序列中缺失两个氨基酸残基的插入缺失(indel),所得多肽具有x-1个氨基酸残基或x+≥1个氨基酸残基。插入和缺失可以以任何顺序、依次或同时进行。

[0215] 本文所用的术语“取代”是指在相同位点用相同数量的氨基酸残基替换一个或多个氨基酸残基。因此,(例如)对于具有x个氨基酸残基的多肽序列的取代,所得多肽也具有x个氨基酸残基。优选地,取代是在单个氨基酸位置的取代。

[0216] 本文所用的术语“插入”是指在插入位点添加多肽的一个或多个氨基酸残基而不缺失多肽的一个或多个氨基酸残基。因此,(例如)当一个氨基酸残基被插入到具有x个氨基

酸残基的多肽序列中时,所得多肽具有x+1个氨基酸残基。

[0217] 通过氨基酸残基的取代、插入、缺失或通过插入缺失(indel)修饰蛋白质的方法是本领域已知的。举例来说,可以通过修饰编码多肽的核酸序列(例如DNA序列)来引入氨基酸修饰。这可以使用标准分子克隆技术来实现,例如通过使用聚合酶的定点诱变,其中用编码所需氨基酸的短链DNA(寡核苷酸)来替换原始编码序列,或通过使用各种酶(例如连接酶和限制性内切核酸酶)插入/缺失部分基因。可替代地,可以化学合成修饰的基因序列。通常,修饰可以通过修饰编码天然梭菌神经毒素(或其部分)的核酸来进行,使得由所述核酸编码的经修饰的嵌合梭菌神经毒素(或其部分)包含所述修饰。可替代地,可以合成编码包含修饰的经修饰的梭菌神经毒素(或其部分)的核酸。

[0218] 用于本发明的嵌合梭菌神经毒素可包含与选自SEQ ID NO:1-5的多肽序列具有至少70%序列同一性的多肽序列。例如,所述嵌合梭菌神经毒素可包含与选自SEQ ID NO:1-5的多肽序列具有至少80%、90%、95%或99.9%序列同一性的多肽序列。优选地,用于本发明的嵌合梭菌神经毒素可包含选自SEQ ID NO:1-5的多肽序列(更优选由其组成)。在所述嵌合梭菌神经毒素中,SEQ ID NO:1是优选的。

[0219] 因此,所述嵌合梭菌神经毒素优选包含与SEQ ID NO:1具有至少70%序列同一性的多肽序列。更优选地,所述嵌合梭菌神经毒素可包含与SEQ ID NO:1具有至少80%、90%、95%或99.9%序列同一性的多肽序列。最优选地,用于本发明的嵌合梭菌神经毒素可包含SEQ ID NO:1(更优选由其组成)。

[0220] 本发明的双链嵌合梭状芽孢杆菌神经毒素可包含与SEQ ID NO:1-5中任一项具有至少70%、80%、90%、95%、99.9%或100%序列同一性的多肽序列的L-链部分,其构成双链嵌合梭菌神经毒素的第一链,并且可以包含与SEQ ID NO:1-5中的任一项具有至少70%、80%、90%、95%、99.9%或100%序列同一性的多肽序列的H_N和H_C结构域,其一起构成双链嵌合梭菌神经毒素的第二链,其中第一链和第二链通过二硫键连接在一起。

[0221] 其中切割发生在嵌合梭菌神经毒素的活化环内的一个以上位置(优选两个位置)处,所述嵌合梭菌神经毒素包含与SEQ ID NO:1-5中任一项具有至少70%、80%、90%、95%、99.9%或100%序列同一性的多肽序列,与SEQ ID NO:1-5中任一项具有至少70%、80%、90%、95%、99.9%或100%序列同一性的序列的C-末端L-链部分的小片段可以不存在于所述双链嵌合梭菌神经毒素中。鉴于此,双链嵌合梭菌神经毒素的序列(例如包含与SEQ ID NO:1-5中任一项具有至少70%、80%、90%、95%、99.9%或100%序列同一性的多肽序列)可以与相应的单链嵌合梭菌神经毒素略有不同,所述单链嵌合梭菌神经毒素包含与SEQ ID NO:1-5中任一项具有至少70%、80%、90%、95%、99.9%或100%序列同一性的多肽序列。所述小片段可以是1-15个氨基酸。特别地,在一个实施方案中,当使用Lys-C将单链嵌合梭菌神经毒素转化为双链梭菌神经毒素时,所述不存在的序列的C-末端L-链部分的小片段可以是SEQ ID NO:15或16。

[0222] 优选地,本发明的双链嵌合梭状芽孢杆菌神经毒素可包含与SEQ ID NO:1具有至少70%、80%、90%、95%、99.9%或100%序列同一性的多肽序列的L-链部分,其构成双链嵌合梭菌神经毒素的第一链,并且可以包含与SEQ ID NO:1具有至少70%、80%、90%、95%、99.9%或100%序列同一性的多肽序列的H_N和H_C结构域,其一起构成双链嵌合梭菌神经毒素的第二链,其中第一链和第二链通过二硫键连接在一起。

[0223] 其中切割发生在嵌合梭菌神经毒素的活化环内的一个以上位置(优选两个位置)处,所述嵌合梭菌神经毒素包含与SEQ ID NO:1具有至少70%、80%、90%、95%、99.9%或100%序列同一性的多肽序列,与SEQ ID NO:1具有至少70%、80%、90%、95%、99.9%或100%序列同一性的序列的C-末端L-链部分的小片段可以不存在于所述双链嵌合梭菌神经毒素中。鉴于此,双链嵌合梭菌神经毒素的序列(例如包含与SEQ ID NO:1具有至少70%、80%、90%、95%、99.9%或100%序列同一性的多肽序列)可以与相应的单链嵌合梭菌神经毒素略有不同,所述单链嵌合梭菌神经毒素包含与SEQ ID NO:1具有至少70%、80%、90%、95%、99.9%或100%序列同一性的多肽序列。所述小片段可以是1-15个氨基酸。特别地,在一个实施方案中,当使用Lys-C将单链嵌合梭菌神经毒素转化为双链梭菌神经毒素时,所述不存在的序列的C-末端L-链部分的小片段可以是SEQ ID NO:15或16。

[0224] 在一个特别优选的实施方案中,双链嵌合梭菌神经毒素包含轻链和重链(或由轻链和重链组成),所述轻链包含与SEQ ID NO:17或18(优选SEQ ID NO:17)具有至少70%、80%、90%、95%或99.9%序列同一性的多肽序列,所述重链包含与SEQ ID NO:19具有至少70%、80%、90%、95%或99.9%序列同一性的多肽序列,其中所述轻链和重链通过二硫键连接在一起。更优选地,双链嵌合梭菌神经毒素包含轻链和重链(或由轻链和重链组成),所述轻链包含SEQ ID NO:17或18(优选SEQ ID NO:17),所述重链包含SEQ ID NO:19,其中所述轻链和重链通过二硫键连接在一起。甚至更优选地,双链嵌合梭菌神经毒素包含轻链和重链(或由轻链和重链组成),所述轻链具有SEQ ID NO:17,所述重链具有SEQ ID NO:19,其中所述轻链和重链通过二硫键连接在一起。二硫键优选由SEQ ID NO:17或18的半胱氨酸残基429与SEQ ID NO:19的半胱氨酸残基6形成和/或位于其之间。

[0225] 在优选的实施方案中,本发明的嵌合梭菌神经毒素不包含除所述轻链和重链之外的治疗剂或诊断剂(例如核酸、蛋白质、肽或小分子治疗剂或诊断剂)。例如,在一个实施方案中,所述嵌合梭菌神经毒素可以不包含共价或非共价结合的治疗剂或诊断剂。因此,本发明的嵌合梭菌神经毒素优选不作为其他治疗剂或诊断剂的递送媒介物。

[0226] 在实施方案中,本文所述的嵌合梭菌神经毒素具有用于纯化的标签(例如His标签)和/或接头,所述标签和/或接头是任选的。

[0227] 本发明的嵌合梭菌神经毒素可以不含存在于天然存在的梭菌神经毒素复合物中的复合蛋白。

[0228] 本发明的嵌合梭菌神经毒素可以使用重组核酸技术产生。因此,在一个实施方案中,嵌合梭菌神经毒素(如本文所述)是重组嵌合梭菌神经毒素。

[0229] 在一个实施方案中,提供了包含编码嵌合梭菌神经毒素的核酸序列的核酸(例如DNA)。在一个实施方案中,核酸序列被制备为包含启动子和终止子的DNA载体的一部分。核酸序列可选自本文所述的任何核酸序列。

[0230] 在一个优选的实施方案中,载体具有选自以下的启动子:

[0231]

启动子	诱导剂	典型诱导条件
Tac(杂合)	IPTG	0.2 mM (0.05-2.0mM)
AraBAD	L-阿拉伯糖	0.2% (0.002-0.4%)
T7-lac 操纵子	IPTG	0.2 mM (0.05-2.0mM)

[0232] 在另一个优选的实施方案中,载体具有选自以下的启动子:

启动子	诱导剂	典型诱导条件
Tac(杂合)	IPTG	0.2 mM (0.05-2.0mM)

[0233]

AraBAD	L-阿拉伯糖	0.2% (0.002-0.4%)
T7-lac 操纵子	IPTG	0.2 mM (0.05-2.0mM)
T5-lac 操纵子	IPTG	0.2 mM (0.05-2.0mM)

[0234] 核酸分子可以使用本领域已知的任何合适的方法来制备。因此,可以使用化学合成技术制备核酸分子。可替代地,可以使用分子生物学技术制备本发明的核酸分子。

[0235] 本发明的DNA构建体优选在计算机上设计,然后通过常规DNA合成技术合成。

[0236] 根据要采用的最终宿主细胞(例如大肠杆菌)表达系统,任选地修改上述核酸序列信息以用于密码子偏好。

[0237] 术语“核苷酸序列”和“核酸”在本文中同义使用。优选地,核苷酸序列是DNA序列。

[0238] 本发明的嵌合梭菌神经毒素可以作为单链或作为双链存在。然而,优选嵌合梭菌神经毒素作为双链存在,其中L-链通过二硫键与H-链(或其组分,例如H_N结构域)连接。

[0239] 具有轻链和重链的单链嵌合梭菌神经毒素的产生可以使用包括以下步骤的方法来实现:在表达宿主中表达编码嵌合梭菌神经毒素的核酸,裂解宿主细胞以提供含有单链嵌合梭菌神经毒素的宿主细胞匀浆,并分离单链嵌合梭菌神经毒素。本文所述的单链嵌合梭菌神经毒素可以使用包括使单链嵌合梭菌神经毒素与蛋白酶(例如Lys-C)接触的方法进行蛋白水解加工,所述蛋白酶水解嵌合梭菌神经毒素的活化环中的肽键,从而将单链嵌合梭菌神经毒素转化为相应的双链嵌合梭菌神经毒素(例如其中轻链和重链通过二硫键连接在一起)。双链嵌合梭菌神经毒素优选可通过这种方法获得。

[0240] 因此,本发明中使用的嵌合梭菌神经毒素优选是由单链BoNT/A产生的双链嵌合梭菌神经毒素,其中单链BoNT/A包含本文所述的多肽序列或由本文所述的多肽序列组成。例如,优选地,本发明中使用的嵌合梭菌神经毒素是由包含与SEQ ID NO:1具有至少70%(例如至少80%、90%、95%或99.9%)序列同一性的多肽序列的多肽产生的双链嵌合梭菌神经毒素。最优选地,用于本发明的嵌合梭菌神经毒素是由包含SEQ ID NO:1(甚至更优选由其组成)的多肽产生的双链嵌合梭菌神经毒素。因此,在一些实施方案中,嵌合梭菌神经毒素是双链嵌合梭菌神经毒素,其中轻链(L-链)通过可由以下方法获得的二硫键连接至重链(H-链),该方法包括将包含SEQ ID NO:1的单链嵌合梭菌神经毒素与水解其活化环中的肽键的蛋白酶接触,从而将单链嵌合梭菌神经毒素转化为相应的双链嵌合梭菌神经毒素。在一些实施方案中,嵌合梭菌神经毒素是双链嵌合梭菌神经毒素,其中L-链通过可由以下方法获得的二硫键连接至H-链,该方法包括将由SEQ ID NO:1组成的单链嵌合梭菌神经毒素与水解其活化环中的肽键的蛋白酶接触,从而将单链嵌合梭菌神经毒素转化为相应的双链嵌合梭菌神经毒素。

[0241] 本文所用的术语“可获得的(obtainable)”还涵盖术语“获得的(obtained)”。在一个实施方案中,术语“可获得的(obtainable)”是指获得的。

[0242] 用于切割活化环的蛋白酶优选是Lys-C。用于切割活化环以产生双链梭菌神经毒素的合适的蛋白酶和方法教导于WO 2014/080206、WO2014/079495和EP2677029A2,其通过

引用并入本文。Lys-C可以将活化环C-末端切割为其中存在的一个或多个赖氨酸残基。当Lys-C不止一次地切割该活化环时,本领域技术人员将理解,当与本文所示的SEQ ID NO相比时,双链修饰的BoNT/A的活化环的小肽可以不存在(优选SEQ ID NO:15或16可以不存在)。

[0243] 本文所用的术语“一个或多个”可以指至少2、3、4、5、6、7、8、9、10、15或20个。在一个实施方案中,其中“一个或多个”在列表之前,“一个或多个”可以指列表的所有成员。类似地,本文使用的术语“至少一个”可以指至少2、3、4、5、6、7、8、9、10、15或20个。在一个实施方案中,其中“至少一个”在列表之前,“至少一个”可以指列表的所有成员。

[0244] 本文所用的“受试者”可以是哺乳动物,例如人或其他哺乳动物。优选地,“受试者”是指人类受试者。

[0245] 根据本发明进行治疗的受试者可以是不适合用非嵌合梭菌神经毒素治疗的受试者。所述受试者可以是对用非嵌合梭菌神经毒素治疗有抗性的受试者。抗性可能由于受试者对梭菌神经毒素的免疫应答的发展而产生,包括抗梭菌神经毒素抗体的产生。在一个实施方案中,根据本发明进行治疗的受试者可以是不适合用BoNT/A治疗的受试者。所述受试者可能对用BoNT/A治疗有抗性。

[0246] 本文所用的术语“病症(disorder)”还涵盖“疾病(disease)”。在一个实施方案中,所述病症是疾病。

[0247] 本文所用的术语“治疗(treat)”或“治疗(treating)”涵盖预防性治疗(例如预防疼痛发作)以及矫正性治疗(例如治疗已经患有疼痛的受试者)。优选地,如本文所用的“治疗(treat)”或“治疗(treating)”意指矫正性治疗。

[0248] 在一个实施方案中,将嵌合梭菌神经毒素施用于在治疗时未经历疼痛或病症症状的受试者。此类施用可适于实现本文所述的疼痛或病症的预防性治疗。在一个实施方案中,偏头痛(例如偏头痛疼痛)的治疗可以是偏头痛的预防性治疗。在一个实施方案中,对在治疗时未经历偏头痛或偏头痛症状的受试者施用嵌合梭菌神经毒素。

[0249] 本文所用的术语“治疗(treat)”或“治疗(treating)”是指病症(优选疼痛)和/或其症状。因此,本发明的嵌合梭菌神经毒素可以治疗有效量或预防有效量施用于受试者。优选地,本发明的嵌合梭菌神经毒素以治疗有效量施用于受试者。

[0250] “治疗有效量”是嵌合梭菌神经毒素的任何量,当单独或与另一种药剂组合(优选单独)施用于受试者以治疗所述病症(优选疼痛)(或其症状)时,该量足以实现所述病症(优选疼痛)或其症状的这种治疗。

[0251] “预防有效量”是嵌合梭菌神经毒素的任何量,当单独或与另一种药剂组合(优选单独)施用于受试者时,其抑制或延迟病症(优选疼痛)(或其症状)的发作或复发。在一些实施方案中,预防有效量完全预防病症(优选疼痛)的发作或复发。“抑制”发作是指减少发作(优选疼痛)(或其症状)的可能性、预防病症(优选疼痛)的峰值效应的大小和/或完全预防发作。

[0252] 嵌合梭菌神经毒素可以治疗疼痛而不治疗引起所述疼痛的潜在病症。

[0253] 除了治疗疼痛外,嵌合梭菌神经毒素还可治疗病症的一种或多种其他症状。在一个实施方案中,嵌合梭菌神经毒素可治疗一种或多种与神经元分泌相关的其他症状,例如本文所述的介质(例如疼痛介质)的释放。例如,CGRP可能涉及许多与偏头痛有关的症状,例

如畏光。因此,根据本发明的对偏头痛或偏头痛性疼痛的治疗还可以治疗偏头痛的一种或多种其他症状,例如畏光。

[0254] 本发明的嵌合梭菌神经毒素可以以任何合适的方式配制以施用于受试者,例如作为药物组合物的一部分。这种药物组合物可包含本发明的嵌合梭菌神经毒素和药学上可接受的载体、赋形剂、佐剂、推进剂和/或盐。

[0255] 本发明的嵌合梭菌神经毒素可以配制用于口服、肠胃外、连续输注、吸入或局部应用。适合于注射的组合物可以是溶液、悬浮液或乳液或干粉的形式,其在使用前溶解或悬浮在合适的媒介物中。

[0256] 在一个方面,本发明提供了用于治疗疼痛的嵌合梭菌神经毒素的单位剂型,所述单位剂型包含:

[0257] a.0.2单位至最多707单位的嵌合梭菌神经毒素,其中1单位是对应于计算的小鼠半数致死剂量(LD_{50})的嵌合梭菌神经毒素的量;或

[0258] b.5pg至17,000pg的嵌合梭菌神经毒素;和

[0259] c.任选地药学上可接受的载体、赋形剂、佐剂和/或盐。

[0260] 优选单位剂型的嵌合梭菌神经毒素包含与SEQ ID NO:1具有至少70%序列同一性的多肽序列。例如,与SEQ ID NO:1具有至少80%、90%、95%或99.9%序列同一性的多肽序列。最优选地,所述嵌合梭菌神经毒素可包含SEQ ID NO:1(更优选由其组成)。

[0261] 用于治疗疼痛的单位剂型可包含0.2单位至最多707单位的嵌合梭菌神经毒素。所述范围的上限可以是700、650、600、550、500、450、400、350、300、250、200、150或100单位的嵌合梭菌神经毒素,优选上限是666单位。所述范围的下限可以是40、45、50、60、65、70、75、80、85、90、100、150、200、250、300、350、400、450、500、550、600、650或700单位的嵌合梭菌神经毒素,优选下限为42单位或31单位。所述范围的下限可以大于125个单位。优选地,单位剂型包含31单位至707单位的嵌合梭菌神经毒素。更优选地,单位剂型包含42单位至666单位的嵌合梭菌神经毒素,例如200单位至400单位的嵌合梭菌神经毒素或41单位至229单位如83单位至188单位、83单位至125单位(例如104单位)或145单位至188单位的嵌合梭菌神经毒素。优选地,单位剂型包含166单位的嵌合梭菌神经毒素。单位剂型可包含47单位至707单位的嵌合梭菌神经毒素,例如187单位至282单位的嵌合梭菌神经毒素,或47至258单位,例如94单位至211单位、94单位至141单位(例如117单位)或164至211单位的嵌合梭菌神经毒素。单位剂型可包含188单位的嵌合梭菌神经毒素。

[0262] 用于治疗疼痛的单位剂型可包含5pg至17,000pg的嵌合梭菌神经毒素。所述范围的上限可以是16,500、15,500、14,500、13,500、12,500、11,500、10,500、9,500、8,500、7,500、6,500、5,500、4,500、3,500、2,500、1,500或500pg的嵌合梭菌神经毒素,优选上限是16,000pg。所述范围的下限可以是750、850、950、1000、1500、2000、2,500、3,000、3,500、4,000、4,500或5,000pg嵌合梭菌神经毒素,优选下限是1000pg或750pg。所述范围的下限可以大于3,000pg。优选地,单位剂型包含750pg至17,000pg的嵌合梭菌神经毒素。更优选地,单位剂型包含1000pg至16,000pg的嵌合梭菌神经毒素,例如4,000pg至6,000pg的嵌合梭菌神经毒素或1,000至5,500pg如2,000pg至4,500pg、2,000pg至3,000pg(例如2,500pg)或3,500至4,500pg的嵌合梭菌神经毒素。优选地,单位剂型包含4,000pg的嵌合梭菌神经毒素。

[0263] 在一些实施方案中,用于治疗疼痛的单位剂型可以用于治疗头痛疼痛(例如偏头

痛疼痛)或偏头痛,并且可以包含:

[0264] a.42单位至最多258单位(例如42至229单位)的嵌合梭菌神经毒素,其中1单位是对应于计算的小鼠半数致死剂量(LD_{50})的嵌合梭菌神经毒素的量;或者

[0265] b.1,000pg至5,500pg的嵌合梭菌神经毒素;和

[0266] c.任选地药学上可接受的载体、赋形剂、佐剂和/或盐。

[0267] 根据本发明使用的嵌合梭菌神经毒素的效力可以根据标准技术通过小鼠LD_{50}测定来确定。在所述测定中,1单位定义为对应于计算的小鼠半数致死剂量(LD_{50})的嵌合梭菌神经毒素的量。优选地,计算的小鼠半数致死腹膜内剂量。

[0268] 所述测定中对应于1单位的嵌合梭菌神经毒素的量可以是20-24.04pg,例如21.3pg或24.04pg。优选地,所述测定中对应于1单位的嵌合梭菌神经毒素的量可以是24.04pg。

[0269] 当在本文中提及单位时,单位优选为LD_{50}单位。

[0270] 在另一个方面,本发明提供了用于治疗头痛疼痛(例如偏头痛疼痛)或偏头痛的单位剂型,该单位剂型包含:

[0271] a.42单位至最多258单位(例如42至229单位)的嵌合梭菌神经毒素,其中1单位是对应于计算的小鼠半数致死剂量(LD_{50})的嵌合梭菌神经毒素的量;或者

[0272] b.1,000pg至5,500pg的嵌合梭菌神经毒素;和

[0273] c.任选地药学上可接受的载体、赋形剂、佐剂和/或盐。

[0274] 优选单位剂型的嵌合梭菌神经毒素包含与SEQ ID NO:1具有至少70%序列同一性的多肽序列。例如,与SEQ ID NO:1具有至少80%、90%、95%或99.9%序列同一性的多肽序列。最优选地,嵌合梭菌神经毒素可包含SEQ ID NO:1(更优选由其组成)。

[0275] 用于治疗头痛疼痛(例如偏头痛疼痛)或偏头痛的单位剂型可以是42单位至229单位。单位剂型的上限可以是225、220、215、210、205、200、190、180、170、160、150、125、100或83单位的嵌合梭菌神经毒素,优选上限是212单位,更优选208单位。单位剂型的下限可以是46、50、55、60、65、70、75、80或90、100、110、120、130、140、150、160或166单位的嵌合梭菌神经毒素,优选下限是58单位,更优选62单位。所述范围的下限可以大于125个单位。单位剂型可包含58单位至212单位(例如62单位至208单位)、83单位至212单位、125至212单位或125至166单位的嵌合梭菌神经毒素。单位剂型可包含大于125单位至最多229单位的嵌合梭菌神经毒素。优选地,单位剂型包含83单位至188单位、83单位至125单位(例如104单位)或145单位至188单位的嵌合梭菌神经毒素。优选地,单位剂型包含166单位的嵌合梭菌神经毒素。单位剂型可包含47单位至258单位的嵌合梭菌神经毒素,例如94单位至211单位、94单位至141单位(例如117单位)或164至211单位的嵌合梭菌神经毒素。单位剂型可包含188单位的嵌合梭菌神经毒素。

[0276] 用于治疗头痛疼痛(例如偏头痛疼痛)或偏头痛的单位剂型可以是1,000pg至5,500pg。单位剂型的上限可以是5,250、5,200、5,100、5,000、4,500、4,000、3,500、3,000、2,500或2,000pg的嵌合梭菌神经毒素,优选上限是5,100pg,更优选5,000pg。单位剂型的下限可以是1,100、1,200、1,250、1,300、1,350、1,400或1,450、1,500、2,000、2,500、3,000、3,500或4,000pg的嵌合梭菌神经毒素,优选下限是1,400pg,更优选1,500pg。所述范围的下限可以大于3,000pg。单位剂型可包含1,400pg至5,100pg、2,000pg至5,100pg、3,000pg至5,

100pg或3,000pg至4,000pg的嵌合梭菌神经毒素。单位剂型可包含大于3,000pg至最多5,500pg的嵌合梭菌神经毒素。优选地,单位剂型包含2,000pg至4,500pg、2,000pg至3,000pg(例如2,500pg)或3,500pg至4,500pg的嵌合梭菌神经毒素。优选地,单位剂型包含4,000pg的嵌合梭菌神经毒素。

[0277] 在待局部递送嵌合梭菌神经毒素的情况下,嵌合梭菌神经毒素可以配制为乳膏(例如用于局部施用)或用于皮下注射。

[0278] 局部递送装置可包括气雾剂或其他喷雾剂(例如喷雾器)。在这方面,嵌合梭菌神经毒素的气雾剂制剂能够递送至肺和/或其他鼻和/或支气管或气道。

[0279] 嵌合梭菌神经毒素可以施用于受试者的面部、颈部和/或颅骨。例如,嵌合梭菌神经毒素可以施用至其肌肉和/或真皮组分。嵌合梭菌神经毒素可以施用于面部、颈部和颅骨中的两个或多个,优选施用于面部、颈部和颅骨。特别地,嵌合梭菌神经毒素可以施用于受试者的面部、颈部和/或颅骨区域。

[0280] 本发明的嵌合梭菌神经毒素可以通过鞘内或硬膜外注射在参与受影响器官的神经支配的脊柱节段的水平上施用于受试者。

[0281] 施用途径可以是通过腹腔镜和/或局部注射。在一个实施方案中,本发明的嵌合梭菌神经毒素在待治疗部位处或附近施用,优选在待治疗部位处施用。例如,嵌合梭菌神经毒素可以鞘内或脊柱内施用。在一个实施方案中,本发明的嵌合梭菌神经毒素的施用途径可以是脊柱内和/或鞘内。

[0282] 在一个实施方案中,本发明的嵌合梭菌神经毒素可以外周施用。在一个实施方案中,嵌合梭菌神经毒素可以皮下施用。

[0283] 本发明的嵌合梭菌神经毒素可以通过注射施用。嵌合梭菌神经毒素可以在每次治疗期间至少5、10、15、20、25或30个注射部位施用。嵌合梭菌神经毒素可以通过在每次治疗期间最多50、45、40、35、30、25或20个注射部位注射来施用。嵌合梭菌神经毒素可以通过在每次治疗期间最多20或15个注射部位注射来施用。优选地,嵌合梭菌神经毒素可以通过在每次治疗期间最多10(例如最多9、8、7、6、5、4、3或2)个注射部位注射来施用,。在一个实施方案中,嵌合梭菌神经毒素可以在每次治疗期间1-40、5-40、8-38、30-40(例如35)或15-25(例如20)个注射部位施用。在一个实施方案中,嵌合梭菌神经毒素可以在每次治疗的1-10、3-10、5-10或7-10个注射部位施用。在一个实施方案中,嵌合梭菌神经毒素可以在每次治疗期间25-35(例如31)个注射部位施用。优选地,嵌合梭菌神经毒素可以在每次治疗期间25-30(例如28)个注射部位施用。

[0284] 本发明的嵌合梭菌神经毒素可以皮内施用,例如皮内注射。嵌合梭菌神经毒素可以通过在每次治疗期间至少5、10、15、20、25或30个注射部位皮内注射施用。嵌合梭菌神经毒素可以通过在每次治疗期间最多50、45、40、35、30、25或20个注射部位皮内注射施用。嵌合梭菌神经毒素可以通过在每次治疗期间最多20或15个注射部位皮内注射施用。优选地,嵌合梭菌神经毒素可以通过在每次治疗期间最多10(例如最多9、8、7、6、5、4、3或2)个注射部位皮内注射施用。在一个实施方案中,嵌合梭菌神经毒素可以在每次治疗期间1-40、5-40、8-38、30-40(例如35)或15-25(例如20)个注射部位施用。在一个实施方案中,嵌合梭菌神经毒素可以在每次治疗的1-10、3-10、5-10或7-10个注射部位施用。在一个实施方案中,嵌合梭菌神经毒素可以在每次治疗期间25-35(例如31)个注射部位施用。优选地,嵌合梭菌

神经毒素可以在每次治疗期间25-30(例如28)个注射部位施用。皮内注射可以在肌肉区域进行,例如本文所述的肌肉。在一个实施方案中,可以对覆盖肌肉的皮肤进行皮内注射。

[0285] 最优选地,嵌合梭菌神经毒素可以肌内施用,例如通过肌内注射。施用嵌合梭菌神经毒素的具体肌肉将取决于待治疗病症(优选疼痛)的性质和位置。

[0286] 嵌合梭菌神经毒素可以被施用于受试者的一块或多块肌肉,所述肌肉选自:额肌(frontalis)、皱眉肌(corrugator)(例如皱眉肌(corrugator supercilii))、降眉间肌(例如降眉间肌-鼻肌(procerus nasalis))、枕肌、颞肌、斜方肌、咬肌、鼻肌、眼轮匝肌、颈椎旁肌、颞筋膜、耳上肌、耳前肌、耳后肌、胸锁乳突肌、颈阔肌、前鼻孔扩张肌、后鼻孔扩张肌、降鼻中隔肌、颏肌、口轮匝肌、颧肌、笑肌、颊肌、枕额肌、提上唇肌、降下唇肌、降口角肌、甲状舌骨肌、肩胛舌骨肌、胸骨舌骨肌、颈夹肌、头夹肌、颈半棘肌、头半棘肌、肩胛提肌、二腹肌或斜角肌。

[0287] 例如,所述嵌合梭菌神经毒素可以被施用于受试者的一块或多块肌肉,所述肌肉选自:额肌(frontalis)、皱眉肌(corrugator)、降眉间肌(例如降眉间肌-鼻肌(procerus nasalis))、枕肌、颞肌、斜方肌、咬肌、鼻肌、眼轮匝肌、颈椎旁肌、颞筋膜、耳上肌、耳前肌、耳后肌、胸锁乳突肌、颈阔肌、前鼻孔扩张肌、后鼻孔扩张肌、降鼻中隔肌、颏肌、口轮匝肌、颧肌、笑肌、颊肌、枕额肌、提上唇肌、降下唇肌、降口角肌、甲状舌骨肌、肩胛舌骨肌、胸骨舌骨肌、颈夹肌、肩胛提肌、二腹肌和斜角肌。

[0288] 当所述病症是头痛疼痛(例如偏头痛疼痛)或偏头痛时,本发明可以包括将嵌合梭菌神经毒素施用于:额肌(frontalis)、皱眉肌(corrugator)(例如皱眉肌(corrugator supercilii))和降眉间肌(例如降眉间肌-鼻肌(procerus nasalis))、枕肌、颞肌、斜方肌、咬肌、鼻肌、眼轮匝肌、颈椎旁肌、颞筋膜、耳上肌、耳前肌、耳后肌、胸锁乳突肌、颈阔肌、前鼻孔扩张肌、后鼻孔扩张肌、降鼻中隔肌、颏肌、口轮匝肌、颧肌、笑肌、颊肌、枕额肌、提上唇肌、降下唇肌、降口角肌、甲状舌骨肌、肩胛舌骨肌、胸骨舌骨肌、颈夹肌、肩胛提肌、二腹肌和斜角肌。当所述病症是头痛疼痛(例如偏头痛疼痛)或偏头痛时,所述嵌合梭菌神经毒素可以被施用于受试者的一块或多块肌肉,所述肌肉选自:额肌(frontalis)、皱眉肌(corrugator)(例如皱眉肌(corrugator supercilii))和降眉间肌(例如降眉间肌-鼻肌(procerus nasalis))、枕肌、颞肌、斜方肌、咬肌、鼻肌、眼轮匝肌、颈椎旁肌、颞筋膜、耳上肌、耳前肌、耳后肌、胸锁乳突肌、颈阔肌、前鼻孔扩张肌、后鼻孔扩张肌、降鼻中隔肌、颏肌、口轮匝肌、颧肌、笑肌、颊肌、枕额肌、提上唇肌、降下唇肌、降口角肌、甲状舌骨肌、肩胛舌骨肌、胸骨舌骨肌、颈夹肌、肩胛提肌、二腹肌和斜角肌。优选地,将嵌合梭菌神经毒素施用于以下的一块或多块:降眉间肌、皱眉肌、咬肌、颞肌、枕肌和斜方肌。当存在两种形式的相同肌肉(例如,两块枕肌肌肉)时,可根据受试者的需要将嵌合梭菌神经毒素施用至所述肌肉中的一块或两块。优选地,将嵌合梭菌神经毒素施用至两块所述肌肉。

[0289] 嵌合梭菌神经毒素可以肌内施用,例如通过肌内注射。施用嵌合梭菌神经毒素的具体肌肉将取决于待治疗病症(优选疼痛)的性质和位置。当所述病症是头痛疼痛(例如偏头痛疼痛)或偏头痛时,本发明可以包括将嵌合梭菌神经毒素施用于:额肌(frontalis)、皱眉肌(corrugator)(例如皱眉肌(corrugator supercilii))、降眉间肌(例如降眉间肌-鼻肌(procerus nasalis))、枕肌、颞肌、斜方肌、咬肌、鼻肌、眼轮匝肌、颈椎旁肌、颞筋膜、耳上肌、耳前肌、耳后肌、胸锁乳突肌、颈阔肌、前鼻孔扩张肌、后鼻孔扩张肌、降鼻中隔肌、颏

肌、口轮匝肌、颧肌、笑肌、颊肌、枕额肌、提上唇肌、降下唇肌、降口角肌、甲状舌骨肌、肩胛舌骨肌、胸骨舌骨肌、颈夹肌、头夹肌、颈半棘肌、头半棘肌、肩胛提肌、二腹肌或斜角肌。当所述病症是头痛疼痛(例如偏头痛疼痛)或偏头痛时,所述嵌合梭菌神经毒素可以被施用于受试者的一块或多块肌肉,所述肌肉选自:额肌(frontalis)、皱眉肌(corrugator)(例如皱眉肌(corrugator supercilii))、降眉间肌(例如降眉间肌-鼻肌(procerus nasalis))、枕肌、颞肌、斜方肌、咬肌、鼻肌、眼轮匝肌、颈椎旁肌、颞筋膜、耳上肌、耳前肌、耳后肌、胸锁乳突肌、颈阔肌、前鼻孔扩张肌、后鼻孔扩张肌、降鼻中隔肌、颏肌、口轮匝肌、颧肌、笑肌、颊肌、枕额肌、提上唇肌、降下唇肌、降口角肌、甲状舌骨肌、肩胛舌骨肌、胸骨舌骨肌、颈夹肌、头夹肌、颈半棘肌、头半棘肌、肩胛提肌、二腹肌和斜角肌。优选地,将嵌合梭菌神经毒素施用于以下的一块或多块:降眉间肌、皱眉肌、咬肌、颞肌、枕肌和斜方肌。当存在两种形式的相同肌肉(例如,两块枕肌肌肉)时,可根据受试者的需要将嵌合梭菌神经毒素施用至所述肌肉中的一块或两块。优选地,将嵌合梭菌神经毒素施用至两块所述肌肉。

[0290] 本发明可包括将嵌合梭菌神经毒素施用至以下的至少一块:额肌肌肉、皱眉肌(例如皱眉肌(corrugator supercilii))肌肉、降眉间肌(例如降眉间肌-鼻肌(procerus nasalis))、枕肌(例如上或下枕肌)肌肉、颞肌肌肉、斜方肌(例如上、中或下斜方肌)肌肉、咬肌肌肉、鼻肌肌肉、眼轮匝肌肌肉、颈椎旁肌肌肉、颞筋膜肌肉、耳上肌肌肉、耳前肌肌肉、耳后肌肌肉、胸锁乳突肌肌肉、颈阔肌肌肉、前鼻孔扩张肌肌肉、后鼻孔扩张肌肌肉、降鼻中隔肌肌肉、颏肌肌肉、口轮匝肌肌肉、颧肌肌肉、笑肌肌肉、颊肌肌肉、枕额肌肌肉、提上唇肌肌肉、降下唇肌肌肉、降口角肌肌肉、甲状舌骨肌肌肉、肩胛舌骨肌肌肉、胸骨舌骨肌肌肉、颈夹肌肌肉、头夹肌肌肉、颈半棘肌肌肉、头半棘肌肌肉、肩胛提肌肌肉、二腹肌肌肉或斜角肌肌肉。优选地,本发明可包括将嵌合梭菌神经毒素施用至以下至少一块:额肌肌肉、皱眉肌(例如皱眉肌(corrugator supercilii))肌肉、鼻肌肌肉、眼轮匝肌肌肉、颞肌肌肉、枕肌肌肉或斜方肌肌肉。更优选地,本发明可包括将嵌合梭菌神经毒素施用至:额肌肌肉、皱眉肌(例如皱眉肌(corrugator supercilii))肌肉、鼻肌肌肉、眼轮匝肌肌肉、颞肌肌肉、枕肌肌肉和斜方肌肌肉。当存在两种形式的相同肌肉(例如,两块枕肌肌肉)时,可根据受试者的需要将嵌合梭菌神经毒素施用至所述肌肉中的一块或两块。优选地,将嵌合梭菌神经毒素施用至两块所述肌肉。所述施用在治疗头痛疼痛(例如偏头痛疼痛)或偏头痛中可能特别相关。

[0291] 当疼痛是关节炎疼痛时,可以将嵌合梭菌神经毒素施用于受试者的手、腕、膝和/或脚的一块或多块肌肉,例如根据关节炎和/或关节炎疼痛的位置。当施用至受试者的手、腕、膝或脚时,施用可以是单侧的(例如,当关节炎或关节炎疼痛仅存在于一只手、腕、膝和/或脚时)或双侧的(例如,当关节炎或关节炎疼痛存在于双手、双腕、双膝和/或双脚时)。嵌合梭菌神经毒素可以施用于受试者手的一块或多块肌肉,所述肌肉选自:拇短屈肌、骨间掌侧肌、拇短展肌、拇短屈肌、拇展肌、拇对掌肌、背侧骨间肌、小指展肌、小指屈肌和小指对掌肌(优选选自以下的一块或多块:拇短屈肌、骨间掌侧肌、拇短展肌、拇短屈肌和拇展肌)。嵌合梭菌神经毒素可以施用于受试者手腕的一块或多块肌肉,所述肌肉选自:拇短伸肌,拇长展肌,小指伸肌,尺侧腕伸肌,尺侧腕屈肌,指伸肌,桡侧腕伸肌和肱桡肌。嵌合梭菌神经毒素可以施用于受试者膝部的一块或多块肌肉,所述肌肉选自:sartorious、股内侧肌、股外侧肌、腓肠肌、跖肌、半膜肌、会阴长肌、腓肠肌、胫骨前肌、股直肌、腓骨长肌、髂腰肌、耻骨

肌、内收肌、大收肌、股薄肌、股二头肌、比目鱼肌、比目鱼肌、指长伸肌、拇长伸肌、腓骨短肌和趾长屈肌(优选选自以下的一块或多块:sartorious、股内侧肌、股外侧肌、腓肠肌、跖肌、半膜肌、会阴长肌、腓肠肌、胫骨前肌、股直肌和腓骨长肌)。嵌合梭菌神经毒素可以施用于受试者的一块或多块足部肌肉,所述肌肉选自:趾短伸肌和拇短伸肌。

[0292] 嵌合梭菌神经毒素可以通过在每次治疗期间至少5、10、15、20、25或30个注射部位肌内注射施用。嵌合梭菌神经毒素可以通过在每次治疗期间最多50、45、40、35、30、25或20个注射部位肌内注射施用。嵌合梭菌神经毒素可以通过在每次治疗期间最多20或15个注射部位肌内注射施用。优选地,嵌合梭菌神经毒素可以通过在每次治疗期间最多10(例如最多9、8、7、6、5、4、3或2)个注射部位肌内注射施用。在一个实施方案中,嵌合梭菌神经毒素可以在每次治疗期间1-40、5-40、8-38、30-40(例如35)或15-25(例如20)个注射部位施用。在一个实施方案中,嵌合梭菌神经毒素可以在每次治疗的1-10、3-10、5-10或7-10个注射部位施用。在一个实施方案中,嵌合梭菌神经毒素可以在每次治疗期间25-35(例如31)个注射部位施用。优选地,嵌合梭菌神经毒素可以在每次治疗期间25-30(例如28)个注射部位施用。

[0293] 嵌合梭菌神经毒素可以通过(例如每个注射部位)每次注射单位剂量的方式施用。

[0294] 嵌合梭菌神经毒素可以经神经内、经神经周或通过神经节周施用来施用。

[0295] 嵌合梭菌神经毒素可施用于三叉神经、三叉神经节、蝶腭神经节、半月神经节、中间神经、舌咽神经、迷走神经、耳神经节和/或经由枕神经施用于上颈根。优选地,将嵌合梭菌神经毒素施用于三叉神经、三叉神经节和/或蝶腭神经节。

[0296] 嵌合梭菌神经毒素可以关节内施用。嵌合梭菌神经毒素可以在关节附近肌内和/或皮内施用。

[0297] 嵌合梭菌神经毒素可通过血管周施用来施用。

[0298] 本发明的嵌合梭菌神经毒素的施用剂量范围是产生期望的治疗和/或预防效果的剂量范围。

[0299] 流体剂型通常利用嵌合梭菌神经毒素和无热原的无菌媒介物制备。根据所用的媒介物和浓度,嵌合梭菌神经毒素可以溶解或悬浮在媒介物中。在制备溶液时,可将嵌合梭菌神经毒素溶解在媒介物中,必要时通过加入氯化钠使溶液等渗,并使用无菌技术通过无菌过滤器过滤进行灭菌,然后填充到合适的无菌小瓶或安瓿中并密封。可替代地,如果溶液稳定性足够,可通过高压灭菌对密封容器中的溶液进行灭菌。有利地,可以将添加剂例如缓冲剂、增溶剂、稳定剂、防腐剂或杀菌剂、悬浮剂或乳化剂和/或局部麻醉剂溶解在媒介物中。

[0300] 在使用前溶解或悬浮在合适的媒介物中的干粉可以通过在无菌区域使用无菌技术将预灭菌的成分填充到无菌容器中来制备。可替代地,可以在无菌区域使用无菌技术将成分溶解到合适的容器中。然后将产物冷冻干燥并将容器无菌密封。

[0301] 适于本文所述施用途径的不经肠道的悬浮液以基本上相同的方式制备,除了将无菌组分悬浮在无菌媒介物中而不是溶解,并且灭菌不能通过过滤完成。这些组分可以以无菌状态分离,或者可替代地可以在分离后例如通过γ辐射进行灭菌。

[0302] 有利地,组合物中包含悬浮剂例如聚乙烯吡咯烷酮(polyvinylpyrrolidone)以促进组分的均匀分布。

[0303] 根据本发明的施用可以利用多种递送技术,包括微粒包封或高压气溶胶冲击。

[0304] 与常规梭菌神经毒素(例如天然BoNT/A)不同,本发明的嵌合梭菌神经毒素具有改

善的安全性和/或改善的活性,例如,如与常规神经毒素相比时改善的安全比所证明的(其他细节参见WO 2017/191315 A1)。鉴于此,嵌合梭菌神经毒素可以低剂量施用,同时仍然表现出治疗功效,并且可以高剂量施用,而不引起不希望的毒性相关的副作用。因此,本发明提供了用于治疗病症(优选疼痛)的宽范围的合适剂量范围。

[0305] 为了方便医生,嵌合梭菌神经毒素可以以单位剂量的方式施用。所述单位剂量可以在单个部位施用,或者可替代地,小于单位剂量可以在施用部位施用(例如,其中存在两个或更多个施用部位并且剂量在所述部位之间(相等或不相等地)分配)。在一个实施方案中,当实施本发明时,可以对每个治疗的肌肉和/或神经元施用单个单位剂量。

[0306] 在一个实施方案中,当实施本发明时,可以对肌肉和/或神经元施用至少一个单位剂量。例如,当实施本发明时,可以对肌肉和/或神经元施用1-20、1-10、1-7或1-5个单位剂量。

[0307] 在一个实施方案中,每次注射(例如,每个注射部位)可施用至少0.25、0.5、1或2个单位剂量。例如,每次注射(例如,每个注射部位)可施用0.25、0.5、1或2个单位剂量。优选地,每次注射(例如,每个注射部位)施用1个单位剂量。

[0308] 当施用单位剂量(或其分数或倍数)时,这可以指施用基本上所有的单位剂量(或其分数或倍数)。例如,单位剂量(或其分数或倍数)的残余量(例如至多1%、0.1%或0.01%)可以保留在从中取出嵌合梭菌神经毒素的小瓶中(例如其中嵌合梭菌神经毒素已经重构)。然而,优选(例如在一个或多个注射部位)施用所有单位剂量(或其分数或倍数)。

[0309] 合适的单位剂量可以是5pg到17,000pg的嵌合梭菌神经毒素。单位剂量范围的上限可以是16,500、15,500、14,500、13,500、12,500、11,500、10,500、9,500、8,500、7,500、6,500、5,500、4,500、3,500、2,500、1,500或500pg的嵌合梭菌神经毒素,优选上限是16,000pg。单位剂量范围的下限可以是10、20、30、50、100、200、250、350、450、550、650、750、850、950、1,000、1,500、2,000、2,500、3,000、3,500、4,000、4,500或5,000pg的嵌合梭菌神经毒素,优选下限是1,000pg或750pg。所述范围的下限可以大于3,000pg。优选地,单位剂量是750pg至17,000pg的嵌合梭菌神经毒素。嵌合梭菌神经毒素的单位剂量可以是3,640pg至17,000pg。更优选地,嵌合梭菌神经毒素的单位剂量是1,000pg至16,000pg的嵌合梭菌神经毒素,例如4,000pg至6,000pg的嵌合梭菌神经毒素或1,000至5,500pg如2,000pg至4,500pg、2,000pg至3,000pg(例如2,500pg)或3,500至4,500pg的嵌合梭菌神经毒素。优选地,单位剂量包含4,000pg的嵌合梭菌神经毒素。

[0310] 合适的单位剂量可以是0.2单位至最多707单位的嵌合梭菌神经毒素。所述范围的上限可以是700、650、600、550、500、450、400、350、300、250、200、150或100单位的嵌合梭菌神经毒素,优选上限是666单位。所述范围的下限可以是40、45、50、60、65、70、75、80、85、90、100、150、200、250、300、350、400、450、500、550、600、650或700单位的嵌合梭菌神经毒素,优选下限为42单位或31单位。所述范围的下限可以大于125个单位。优选地,单位剂量是31单位至707单位的嵌合梭菌神经毒素。嵌合梭菌神经毒素的单位剂量可以是166单位至707单位。更优选地,单位剂量是42单位至666单位的嵌合梭菌神经毒素,例如200单位至400单位的嵌合梭菌神经毒素或41单位至229单位如83单位至188单位、83单位至125单位(例如104单位)或145单位至188单位的嵌合梭菌神经毒素。优选地,单位剂量是166单位的嵌合梭菌神经毒素。单位剂量可以是47单位至707单位的嵌合梭菌神经毒素,例如187单位至282单位

的嵌合梭菌神经毒素,或47至258单位,例如94单位至211单位、94单位至141单位(例如117单位)或164至211单位的嵌合梭菌神经毒素。单位剂量可以是188单位的嵌合梭菌神经毒素。

[0311] 合适的单位剂量可以是1,000pg至5,500pg。单位剂量范围的上限可以是5,250、5,200、5,100、5,000、4,500、4,000、3,500、3,000、2,500或2,000pg的嵌合梭菌神经毒素,优选上限是5,100pg,更优选5,000pg。单位剂量范围的下限可以是1,100、1,200、1,250、1,300、1,350、1,400或1,450、1,500、2,000、2,500、3,000、3,500或4,000pg的嵌合梭菌神经毒素,优选下限是1,400pg,更优选1,500pg。所述范围的下限可以大于3,000pg。单位剂量可以是1,400pg至5,100pg(例如1,500pg至5,000pg)、2,000pg至5,100pg、3,000至5,100pg或3,000至4,000pg的嵌合梭菌神经毒素。单位剂量可包含大于3,000pg至最多5,500pg的嵌合梭菌神经毒素。嵌合梭菌神经毒素的单位剂量可以是2,000pg至4,500pg、2,000pg至3,000pg(例如2,500pg)或3,500至4,500pg的嵌合梭菌神经毒素。优选地,单位剂量包含4,000pg的嵌合梭菌神经毒素。

[0312] 合适的单位剂量可以是42单位至258单位(例如至多229单位)。单位剂量范围的上限可以是225、220、215、210、205、200、190、180、170、160、150、125、100或83单位的嵌合梭菌神经毒素,优选上限是212单位,更优选208单位。单位剂量范围的下限可以是46、50、55、60、65、70、75、80或90、100、110、120、130、140、150、160或166单位的嵌合梭菌神经毒素,优选下限是58单位,更优选62单位。所述范围的下限可以大于125个单位。单位剂量可以是58单位至212单位(例如62单位至208单位)、83单位至212单位、125至212单位或125至166单位的嵌合梭菌神经毒素。单位剂量可包含大于125单位至最多229单位的嵌合梭菌神经毒素。嵌合梭菌神经毒素的单位剂量可以是83单位至188单位、83单位至125单位(例如104单位)或146单位至188单位的嵌合梭菌神经毒素。优选地,单位剂量包含166单位的嵌合梭菌神经毒素。单位剂量可包含47单位至258单位的嵌合梭菌神经毒素,例如94单位至211单位、94单位至141单位(例如117单位)或164至211单位的嵌合梭菌神经毒素。单位剂量可以是188单位的嵌合梭菌神经毒素。

[0313] 每次治疗期间施用的总剂量可以是至多255,000pg的嵌合梭菌神经毒素。这可以对应于15×单位剂量。施用的总剂量可以是至多255,000pg的嵌合梭菌神经毒素并且对应于单位剂量的28×、31×或39×。换言之,在给定治疗期间施用的嵌合梭菌神经毒素的总量可以是至多255,000pg。总剂量可以是至多240,000、220,000、200,000、180,000、160,000、140,000、110,000、100,000、90,000、80,000、70,000、60,000、50,000、40,0 00、30,000、20,000、10,000或5,000pg。优选地,总剂量可以是至多240,000pg嵌合梭菌神经毒素。总剂量可以是至少900、1,000、2,000、3,000、4,000、5,000、7,500、10,000、12,500、15,000、20,000、30,000、40,000、50,000、60,000、70,000、80,000、90,000、100,000、120,000、150,000、175,000、200,000或220,000pg。优选地,总剂量可以是至少1,500pg,更优选至少2,000pg的嵌合梭菌神经毒素,更优选大于3,000pg,例如至少12,000pg。总剂量可以是3,640pg到255,000pg的嵌合梭菌神经毒素。总剂量可以是2,000-240,000pg,优选128,000-240,000pg。更优选地,施用的总剂量为15,000-240,000pg。总剂量可以是75,000pg或115,000pg。总剂量可以是70,000pg或112,000pg。

[0314] 每次治疗期间施用的总剂量可以是至多10,607单位的嵌合梭菌神经毒素。这可以

对应于15×单位剂量。施用的总剂量可以是至多10,607单位的嵌合梭菌神经毒素并且对应于单位剂量的28×、31×或39×。换言之,在给定治疗期间施用的嵌合梭菌神经毒素的总量可以是至多10,607单位。总剂量可以是至多10,500、10,000、9,500、9,000、8,500、8,000、7,500、7,000、6,500、6,000、5,500、5,000、4,500、4,000、3,500、3,000、2,500、2,000、1,500、1,000、500或207单位。优选地,总剂量可以是至多11,268或9,983单位的嵌合梭菌神经毒素。总剂量可以是至少37、50、100、150、200、250、500、1,000、1,500、2,000、2,500、3,000、3,500、4,000、4,500、5,000、5,500、6,000、6,500、7,000,7,500、8,000、8,500、9,000、9,500、9,151、10,000或10,328单位。优选地,总剂量可以是至少62单位或70单位,更优选至少83单位或94单位的嵌合梭菌神经毒素,更优选大于125单位或141单位,例如至少499单位或563单位。总剂量可以是165单位至10,607单位或171单位至10,607单位的嵌合梭菌神经毒素。总剂量可以是83-9,983单位或94-10,607单位,优选5,324-9,983单位或6,009-10,607单位。更优选地,施用的总剂量为624-9,983单位或704-10,607单位。总剂量可以是3,120或4,784单位。总剂量可以是2,911或4,659单位。总剂量可以是3,521单位或5,399单位。总剂量可以是3,286单位或5,258单位。

[0315] 合适的单位剂量可以是2,500pg,并且总剂量可以是至多70,000pg。例如,合适的单位剂量可以是2,500pg,并且总剂量可以是70,000pg。合适的单位剂量可以是4,000pg,并且总剂量可以是至多112,000pg。例如,合适的单位剂量可以是4,000pg,并且总剂量可以是112,000pg。合适的单位剂量可以是5,000pg,并且总剂量可以是至多155,000pg。例如,合适的单位剂量可以是5,000pg,并且总剂量可以是155,000pg。

[0316] 合适的单位剂量可以是104单位,并且总剂量可以是至多2,912单位。例如,合适的单位剂量可以是104单位,并且总剂量可以是2,912单位。合适的单位剂量可以是166单位,并且总剂量可以是至多4,659单位。例如,合适的单位剂量可以是166单位,并且总剂量可以是4,659单位。合适的单位剂量可以是208单位,并且总剂量可以是至多6,448单位。例如,合适的单位剂量可以是208单位,并且总剂量可以是6,448单位。

[0317] 合适的单位剂量可以是117单位,并且总剂量可以是至多3,286单位。例如,合适的单位剂量可以是117单位,并且总剂量可以是3,286单位。合适的单位剂量可以是188单位,并且总剂量可以是至多5,258单位。例如,合适的单位剂量可以是188单位,并且总剂量可以是5,258单位。合适的单位剂量可以是235单位,并且总剂量可以是至多7,277单位。例如,合适的单位剂量可以是235单位,并且总剂量可以是7,277单位。

[0318] 在给定治疗中施用的单位剂量的总数可以是至多15×单位剂量。例如,施用的单位剂量的总数可以是至多14×、13×、12×、11×、10×、9×、8×或7×。施用的单位剂量的总数可以是单位剂量的至少2×、3×、4×、5×、6×、7×,优选至少2×。施用的单位剂量的总数可以是2×至15×、7×至15×或10×至14×。优选地,施用的单位剂量的数目是15×。

[0319] 在给定治疗中施用的单位剂量的总数可以是至多39×单位剂量(只要在治疗期间给予的总剂量不超过255,000pg或10,607单位的上限)。例如,施用的单位剂量的总数可以是至多35×、31×、30×、29×、28×、27×、26×、25×或20×单位剂量,优选施用的单位剂量的总数是至多28×单位剂量。施用的单位剂量的总数可以是单位剂量的至少2×、3×、4×、5×、6×、7×,优选至少2×。施用的单位剂量的总数可以是2×至39×、15×至31×或28×至31×。施用的单位剂量的总数可以是28×、31×或39×。

[0320] 因此,每次治疗期间施用的总剂量可以是至多192,500pg的嵌合梭菌神经毒素。例如,施用的总剂量可以是至多180,000pg,或至多177,000pg(例如至多175,000pg)。

[0321] 因此,每次治疗期间施用的总剂量可以是至多8,007单位的嵌合梭菌神经毒素。例如,施用的总剂量可以是至多7,488单位,或至多7,363单位(例如至多7,280单位)。每次治疗期间施用的总剂量可以是至多9,037单位的嵌合梭菌神经毒素。例如,施用的总剂量可以是至多8,451单位,或至多8,310单位(例如至多8,216单位)。

[0322] 每次治疗期间施用的总剂量可以是至多110,000pg的嵌合梭菌神经毒素。例如,施用的总剂量可以是至多105,000pg,至多102,000pg(例如至多100,000pg)。

[0323] 每次治疗期间施用的总剂量可以是至多4,576单位的嵌合梭菌神经毒素。例如,施用的总剂量可以是至多4,368单位,优选至多4,243单位(更优选至多4,160单位)。每次治疗期间施用的总剂量可以是至多5,165单位的嵌合梭菌神经毒素。例如,施用的总剂量可以是至多4,929单位,优选至多4,789单位(更优选至多4,695单位)。

[0324] 术语“至多”在用于提及数值(例如至多255,000pg)时意指至多并包括所述数值。因此,作为实例,提及施用“至多255,000pg”的嵌合梭菌神经毒素涵盖施用255,000pg嵌合梭菌神经毒素以及施用少于255,000pg的嵌合梭菌神经毒素。

[0325] 当病症是头痛疼痛(例如偏头痛疼痛)或偏头痛时,可以将至少单位剂量的嵌合梭菌神经毒素施用于额肌、皱眉肌、鼻肌、眼轮匝肌、颞肌、枕肌和斜方肌中的一块或多块。优选地,可以将至少单位剂量的嵌合梭菌神经毒素施用于额肌、皱眉肌、鼻肌、眼轮匝肌、颞肌、枕肌和斜方肌。在一些实施方案中,将多个单位剂量施用至以下的一块或多块:额肌、皱眉肌、鼻肌、眼轮匝肌、颞肌、枕肌和斜方肌。在一个实施方案中,将单个单位剂量施用于皱眉肌、鼻肌和眼轮匝肌,并且将多个单位剂量施用于额肌、颞肌、枕肌和斜方肌。多个单位剂量可以是2-10个单位剂量,例如2-8个单位剂量,优选2-5个单位剂量,例如2-4个单位剂量。

[0326] 更优选地,所述方法包括将嵌合梭菌神经毒素施用于以下的至少一块:额肌肌肉、皱眉肌(例如皱眉肌(corrugator supercilii))肌肉、鼻肌肌肉、眼轮匝肌肌肉、颞肌肌肉、枕肌肌肉或斜方肌肌肉。所述方法可包括将嵌合梭菌神经毒素施用至:额肌肌肉、皱眉肌(例如皱眉肌(corrugator supercilii))肌肉、鼻肌肌肉、眼轮匝肌肌肉、颞肌肌肉、枕肌肌肉和斜方肌肌肉。

[0327] 头痛疼痛(例如偏头痛疼痛)或偏头痛治疗可包括施用:

[0328] (i)2个单位剂量至额肌肌肉(优选每块额肌肌肉2个单位剂量);

[0329] (ii)1个单位剂量至皱眉肌肌肉(优选每块皱眉肌肌肉1个单位剂量);

[0330] (iii)1个单位剂量至鼻肌肌肉(优选每块鼻肌肌肉1个单位剂量);

[0331] (iv)1个单位剂量至眼轮匝肌肌肉(优选每块眼轮匝肌肌肉1个单位剂量);

[0332] (v)4个单位剂量至颞肌肌肉(优选每块颞肌肌肉4个单位剂量);

[0333] (vi)3个单位剂量至枕肌肌肉(优选每块枕肌肌肉3个单位剂量);和/或

[0334] (vii)2个单位剂量至斜方肌肌肉(优选每块斜方肌肌肉2个单位剂量)

[0335] 头痛疼痛(例如偏头痛疼痛)或偏头痛治疗可包括施用:

[0336] (i)2个单位剂量至额肌肌肉(优选每块额肌肌肉2个单位剂量);

[0337] (ii)1个单位剂量至皱眉肌肌肉(优选每块皱眉肌肌肉1个单位剂量);

[0338] (iii)1个单位剂量至鼻肌肌肉(优选每块鼻肌肌肉1个单位剂量);

[0339] (iv)1个单位剂量至眼轮匝肌肌肉(优选每块眼轮匝肌肌肉1个单位剂量);

[0340] (v)4个单位剂量至颞肌肌肉(优选每块颞肌肌肉4个单位剂量);

[0341] (vi)3个单位剂量至枕肌肌肉(优选每块枕肌肌肉3个单位剂量);和

[0342] (vii)2个单位剂量至斜方肌肌肉(优选每块斜方肌肌肉2个单位剂量)。

[0343] 头痛疼痛(例如偏头痛疼痛)或偏头痛的治疗可包括双侧施用单位剂量的嵌合梭菌神经毒素。头痛疼痛(例如偏头痛疼痛)或偏头痛治疗可包括施用:

[0344] (i)4个单位剂量至额肌肌肉(优选2个单位剂量至面部第一侧额肌肌肉,以及2个单位剂量至面部第二侧额肌肌肉);

[0345] (ii)2个单位剂量至皱眉肌肌肉(优选1个单位剂量至面部第一侧的皱眉肌肌肉,以及1个单位剂量至面部第二侧的皱眉肌肌肉);

[0346] (iii)2个单位剂量至鼻肌肌肉(优选1个单位剂量至面部第一侧的鼻肌肌肉,以及1个单位剂量至面部第二侧的鼻肌肌肉);

[0347] (iv)2个单位剂量至眼轮匝肌肌肉(优选1个单位剂量至面部第一侧的眼轮匝肌肌肉,以及1个单位剂量至面部第二侧的眼轮匝肌肌肉);

[0348] (v)8个单位剂量至颞肌肌肉(优选4个单位剂量至头部第一侧的颞肌肌肉,以及4个单位剂量至头部第二侧的颞肌肌肉);

[0349] (vi)6个单位剂量至枕肌肌肉(优选3个单位剂量至头部第一侧的枕肌肌肉,以及3个单位剂量至头部第二侧的枕肌肌肉);和/或

[0350] (vii)4个单位剂量至斜方肌肌肉(优选2个单位剂量至颈部第一侧的斜方肌肌肉,以及2个单位剂量至颈部第二侧的斜方肌肌肉)。

[0351] 优选地,头痛疼痛(例如偏头痛疼痛)或偏头痛治疗包括施用:

[0352] (i)4个单位剂量至额肌肌肉(优选2个单位剂量至面部第一侧额肌肌肉,以及2个单位剂量至面部第二侧额肌肌肉);

[0353] (ii)2个单位剂量至皱眉肌肌肉(优选1个单位剂量至面部第一侧的皱眉肌肌肉,以及1个单位剂量至面部第二侧的皱眉肌肌肉);

[0354] (iii)2个单位剂量至鼻肌肌肉(优选1个单位剂量至面部第一侧的鼻肌肌肉,以及1个单位剂量至面部第二侧的鼻肌肌肉);

[0355] (iv)2个单位剂量至眼轮匝肌肌肉(优选1个单位剂量至面部第一侧的眼轮匝肌肌肉,以及1个单位剂量至面部第二侧的眼轮匝肌肌肉);

[0356] (v)8个单位剂量至颞肌肌肉(优选4个单位剂量至头部第一侧的颞肌肌肉,以及4个单位剂量至头部第二侧的颞肌肌肉);

[0357] (vi)6个单位剂量至枕肌肌肉(优选3个单位剂量至头部第一侧的枕肌肌肉,以及3个单位剂量至头部第二侧的枕肌肌肉);和

[0358] (vii)4个单位剂量至斜方肌肌肉(优选2个单位剂量至颈部第一侧的斜方肌肌肉,以及2个单位剂量至颈部第二侧的斜方肌肌肉)。

[0359] 当如前述实施方案中所述治疗头痛(例如偏头痛疼痛)或偏头痛时,优选每次注射(例如注射部位)施用一个单位剂量。因此,嵌合梭菌神经毒素的施用可包括:

[0360] (i)注射2次至额肌肌肉(优选每块额肌肌内注射2次);

[0361] (ii)注射1次至皱眉肌肌肉(优选每块皱眉肌肌内注射1次);

[0362] (iii)注射1次至鼻肌肌肉(优选每块鼻肌肌内注射1次);

[0363] (iv)注射1次至眼轮匝肌肌肉(优选每块眼轮匝肌肌内注射1次);

[0364] (v)注射4次至颞肌肌肉(优选每块颞肌肌内注射4次);

[0365] (vi)注射3次至枕肌肌肉(优选每块枕肌肌内注射3次);和/或

[0366] (vii)注射2次至斜方肌肌肉(优选每块斜方肌肌内注射2次)。

[0367] 嵌合梭菌神经毒素的施用可包括:

[0368] (i)注射2次至额肌肌肉(优选每块额肌肌内注射2次);

[0369] (ii)注射1次至皱眉肌肌肉(优选每块皱眉肌肌内注射1次);

[0370] (iii)注射1次至鼻肌肌肉(优选每块鼻肌肌内注射1次);

[0371] (iv)注射1次至眼轮匝肌肌肉(优选每块眼轮匝肌肌内注射1次);

[0372] (v)注射4次至颞肌肌肉(优选每块颞肌肌内注射4次);

[0373] (vi)注射3次至枕肌肌肉(优选每块枕肌肌内注射3次);和

[0374] (vii)注射2次至斜方肌肌肉(优选每块斜方肌肌内注射2次)。

[0375] 嵌合梭菌神经毒素的施用可包括:

[0376] (i)注射4次至额肌肌肉(优选注射2次至面部第一侧的额肌肌肉,以及注射2次至面部第二侧的额肌肌肉);

[0377] (ii)注射2次至皱眉肌肌肉(优选注射1次至面部第一侧的皱眉肌肌肉,以及注射1次至面部第二侧的皱眉肌肌肉);

[0378] (iii)注射2次至鼻肌肌肉(优选注射1次至面部第一侧的鼻肌肌肉,以及注射1次至面部第二侧的鼻肌肌肉);

[0379] (iv)注射2次至眼轮匝肌肌肉(优选注射1次至面部第一侧的眼轮匝肌肌肉,以及注射1次至面部第二侧的眼轮匝肌肌肉);

[0380] (v)注射8次至颞肌肌肉(优选注射4次至头部第一侧的颞肌肌肉,以及注射4次至头部第二侧的颞肌肌肉);

[0381] (vi)注射6次至枕肌肌肉(优选注射3次至头部第一侧的枕肌肌肉,以及注射3次至头部第二侧的枕肌肌肉);和/或

[0382] (vii)注射4次至斜方肌肌肉(优选注射2次至颈部第一侧的斜方肌肌肉,以及注射2次至颈部第二侧的斜方肌肌肉)。

[0383] 优选地,嵌合梭菌神经毒素的施用包括:

[0384] (i)注射4次至额肌肌肉(优选注射2次至面部第一侧的额肌肌肉,以及注射2次至面部第二侧的额肌肌肉);

[0385] (ii)注射2次至皱眉肌肌肉(优选注射1次至面部第一侧的皱眉肌肌肉,以及注射1次至面部第二侧的皱眉肌肌肉);

[0386] (iii)注射2次至鼻肌肌肉(优选注射1次至面部第一侧的鼻肌肌肉,以及注射1次至面部第二侧的鼻肌肌肉);

[0387] (iv)注射2次至眼轮匝肌肌肉(优选注射1次至面部第一侧的眼轮匝肌肌肉,以及注射1次至面部第二侧的眼轮匝肌肌肉);

[0388] (v)注射8次至颞肌肌肉(优选注射4次至头部第一侧的颞肌肌肉,以及注射4次至头部第二侧的颞肌肌肉);

[0389] (vi)注射6次至枕肌肌肉(优选注射3次至头部第一侧的枕肌肌肉,以及注射3次至头部第二侧的枕肌肌肉);和

[0390] (vii)注射4次至斜方肌肌肉(优选注射2次至颈部第一侧的斜方肌肌肉,以及注射2次至颈部第二侧的斜方肌肌肉)。

[0391] 当病症是头痛疼痛(例如偏头痛疼痛)或偏头痛时,可以将至少单位剂量的嵌合梭菌神经毒素肌内或皮内施用至额肌、降眉间肌、皱眉肌、颞肌、枕肌、斜方肌和颈椎旁肌群肌肉中的一块或多块。优选地,可以将至少单位剂量的嵌合梭菌神经毒素肌内或皮内施用至额肌、降眉间肌、皱眉肌、颞肌、枕肌、斜方肌和颈椎旁肌群肌肉(例如,向每块颈椎旁肌群肌肉施用至少单位剂量)。

[0392] 当病症是头痛疼痛(例如偏头痛疼痛)或偏头痛时,可以将至少单位剂量的嵌合梭菌神经毒素施用至额肌、降眉间肌、皱眉肌、颞肌、枕肌、斜方肌和颈椎旁肌群肌肉中的一块或多块。优选地,可以将至少单位剂量的嵌合梭菌神经毒素施用至额肌、降眉间肌、皱眉肌、颞肌、枕肌、斜方肌和颈椎旁肌群肌肉(例如,向每块颈椎旁肌群肌肉施用至少单位剂量)。在一个实施方案中:

[0393] (i)将单个单位剂量施用至以下的一块或多块:降眉间肌肌肉;和皱眉肌肌肉(优选将单个单位剂量施用于面部第一侧(如左侧)的皱眉肌肌肉,以及将第二单位剂量施用于面部第二侧(如右侧)的皱眉肌肌肉);和/或(优选和)

[0394] (iii)将多个单位剂量施用至以下一块或多块:额肌肌肉;颞肌肌肉;枕肌肌肉;斜方肌肌肉;和颈椎旁肌群(例如,其中向颈椎旁肌群的每块肌肉施用单个或双单位剂量)。多个单位剂量可以是2-8个单位剂量,例如2-5个单位剂量。

[0395] 头痛疼痛(例如偏头痛疼痛)或偏头痛的治疗可包括双侧肌内或皮内施用单位剂量的嵌合梭菌神经毒素。头痛疼痛(例如偏头痛疼痛)或偏头痛治疗可包括施用:

[0396] (i)2个单位剂量至面部第一侧额肌肌肉,和/或2个单位剂量至面部第二侧额肌肌肉;

[0397] (ii)1个单位剂量至降眉间肌肌肉;

[0398] (iii)1个单位剂量至面部第一侧皱眉肌肌肉,和/或1个单位剂量至面部第二侧皱眉肌肌肉;

[0399] (iv)4个单位剂量至头部第一侧颞肌肌肉,和/或4个单位剂量至头部第二侧颞肌肌肉;

[0400] (v)3个单位剂量至颈部/头部(优选头部)第一侧枕肌肌肉,和/或3个单位剂量至颈部/头部(优选头部)第二侧枕肌肌肉;

[0401] (vi)3个单位剂量至颈部第一侧颞肌肌肉,和/或3个单位剂量至面部第二侧颞肌肌肉;和/或

[0402] (vii)4个单位剂量至颈部第一侧的颈椎旁肌群,和/或4个单位剂量至颈部第二侧的颈椎旁肌群(例如其中每块颈椎旁肌群肌肉施用1个单位剂量);或2个单位剂量至颈部第一侧的的颈椎旁肌群,和/或2个单位剂量至颈部第二侧的颈椎旁肌群(例如其中每块颈椎旁肌群肌肉施用2个单位剂量)。

[0403] 头痛疼痛(例如偏头痛疼痛)或偏头痛治疗可包括施用:

[0404] (i)2个单位剂量至面部第一侧额肌肌肉,以及2个单位剂量至面部第二侧额肌肌

肉;

[0405] (ii)1个单位剂量至降眉间肌肌肉;

[0406] (iii)1个单位剂量至面部第一侧皱眉肌肌肉,以及1个单位剂量至面部第二侧皱眉肌肌肉;(iv)4个单位剂量至头部第一侧颞肌肌肉,以及4个单位剂量至头部第二侧颞肌肌肉;

[0407] (v)3个单位剂量至颈部/头部(优选头部)第一侧枕肌肌肉,以及3个单位剂量至颈部/头部(优选头部)第二侧枕肌肌肉;

[0408] (vi)3个单位剂量至颈部第一侧颞肌肌肉,以及3个单位剂量至面部第二侧颞肌肌肉;和/或

[0409] (vii)4个单位剂量至颈部第一侧的颈椎旁肌群,以及4个单位剂量至颈部第二侧的颈椎旁肌群(例如其中每块颈椎旁肌群肌肉施用1个单位剂量);或2个单位剂量至颈部第一侧的的颈椎旁肌群,以及2个单位剂量至颈部第二侧的颈椎旁肌群(例如其中每块颈椎旁肌群肌肉施用2个单位剂量)。

[0410] 头痛疼痛(例如偏头痛疼痛)或偏头痛治疗可包括施用:

[0411] (i)2个单位剂量至面部第一侧额肌肌肉,以及2个单位剂量至面部第二侧额肌肌肉;

[0412] (ii)1个单位剂量至降眉间肌肌肉;

[0413] (iii)1个单位剂量至面部第一侧皱眉肌肌肉,以及1个单位剂量至面部第二侧皱眉肌肌肉;

[0414] (iv)4个单位剂量至头部第一侧颞肌肌肉,以及4个单位剂量至头部第二侧颞肌肌肉;

[0415] (v)3个单位剂量至颈部/头部(优选头部)第一侧枕肌肌肉,以及3个单位剂量至颈部/头部(优选头部)第二侧枕肌肌肉;

[0416] (vi)3个单位剂量至颈部第一侧颞肌肌肉,以及3个单位剂量至面部第二侧颞肌肌肉;和

[0417] (vii)4个单位剂量至颈部第一侧的颈椎旁肌群,以及4个单位剂量至颈部第二侧的颈椎旁肌群(例如其中每块颈椎旁肌群肌肉施用1个单位剂量);或2个单位剂量至颈部第一侧的的颈椎旁肌群,以及2个单位剂量至颈部第二侧的颈椎旁肌群(例如其中每块颈椎旁肌群肌肉施用2个单位剂量)。

[0418] 头痛疼痛(例如偏头痛疼痛)或偏头痛治疗可包括施用:

[0419] (i)1个单位剂量至额肌肌肉(优选每块额肌肌肉1个单位剂量);

[0420] (ii)1个单位剂量至降眉间肌肌肉(优选每块降眉间肌肌肉1个单位剂量);

[0421] (iii)1个单位剂量至皱眉肌肌肉(优选每块皱眉肌肌肉1个单位剂量);

[0422] (iv)4个单位剂量至颞肌肌肉(优选每块颞肌肌肉4个单位剂量);

[0423] (v)3个单位剂量至枕肌肌肉(优选每块枕肌肌肉3个单位剂量);

[0424] (vi)3个单位剂量至斜方肌肌肉(优选每块斜方肌肌肉3个单位剂量);和/或

[0425] (vii)4个单位剂量至颈椎旁肌群(优选每个颈椎旁肌群4个单位剂量),或2个单位剂量至颈椎旁肌群(优选每个颈椎旁肌群2个单位剂量)。

[0426] 头痛疼痛(例如偏头痛疼痛)或偏头痛治疗可包括施用:

[0427] (i)1个单位剂量至额肌肌肉(优选每块额肌肌肉1个单位剂量);

[0428] (ii)1个单位剂量至降眉间肌肌肉(优选每块降眉间肌肌肉1个单位剂量);

[0429] (iii)1个单位剂量至皱眉肌肌肉(优选每块皱眉肌肌肉1个单位剂量);

[0430] (iv)4个单位剂量至颞肌肌肉(优选每块颞肌肌肉4个单位剂量);

[0431] (v)3个单位剂量至枕肌肌肉(优选每块枕肌肌肉3个单位剂量);

[0432] (vi)3个单位剂量至斜方肌肌肉(优选每块斜方肌肌肉3个单位剂量);和

[0433] (vii)4个单位剂量至颈椎旁肌群(优选每个颈椎旁肌群4个单位剂量),或2个单位剂量至颈椎旁肌群(优选每个颈椎旁肌群2个单位剂量)。

[0434] 头痛疼痛(例如偏头痛疼痛)或偏头痛治疗可包括施用:

[0435] (i)4个单位剂量至额肌肌肉(优选2个单位剂量至面部第一侧额肌肌肉,以及2个单位剂量至面部第二侧额肌肌肉);

[0436] (ii)1个单位剂量至降眉间肌肌肉;

[0437] (iii)2个单位剂量至皱眉肌肌肉(优选1个单位剂量至面部第一侧的皱眉肌肌肉,以及1个单位剂量至面部第二侧的皱眉肌肌肉);

[0438] (iv)8个单位剂量至颞肌肌肉(优选4个单位剂量至头部第一侧的颞肌肌肉,以及4个单位剂量至头部第二侧的颞肌肌肉);

[0439] (v)6个单位剂量至枕肌肌肉(优选3个单位剂量至头部第一侧的枕肌肌肉,以及3个单位剂量至头部第二侧的枕肌肌肉);

[0440] (vi)6个单位剂量至斜方肌肌肉(优选3个单位剂量至颈部第一侧的斜方肌肌肉,以及3个单位剂量至颈部第二侧的斜方肌肌肉);和/或

[0441] (vii)8个单位剂量至颈椎旁肌群(优选4个单位剂量至颈部第一侧的颈椎旁肌群,以及4个单位剂量至颈部第二侧的颈椎旁肌群(例如其中每块颈椎旁肌群肌肉施用1个单位剂量));或4个单位剂量至颈椎旁肌群(优选2个单位剂量至颈部第一侧的的颈椎旁肌群,以及2个单位剂量至颈部第二侧的颈椎旁肌群(例如其中每块颈椎旁肌群肌肉施用2个单位剂量))。

[0442] 头痛疼痛(例如偏头痛疼痛)或偏头痛治疗可包括施用:

[0443] (i)4个单位剂量至额肌肌肉(优选2个单位剂量至面部第一侧额肌肌肉,以及2个单位剂量至面部第二侧额肌肌肉);

[0444] (ii)1个单位剂量至降眉间肌肌肉;

[0445] (iii)2个单位剂量至皱眉肌肌肉(优选1个单位剂量至面部第一侧的皱眉肌肌肉,以及1个单位剂量至面部第二侧的皱眉肌肌肉);

[0446] (iv)8个单位剂量至颞肌肌肉(优选4个单位剂量至头部第一侧的颞肌肌肉,以及4个单位剂量至头部第二侧的颞肌肌肉);

[0447] (v)6个单位剂量至枕肌肌肉(优选3个单位剂量至头部第一侧的枕肌肌肉,以及3个单位剂量至头部第二侧的枕肌肌肉);

[0448] (vi)6个单位剂量至斜方肌肌肉(优选3个单位剂量至颈部第一侧的斜方肌肌肉,以及3个单位剂量至颈部第二侧的斜方肌肌肉);和

[0449] (vii)8个单位剂量至颈椎旁肌群(优选4个单位剂量至颈部第一侧的颈椎旁肌群,以及4个单位剂量至颈部第二侧的颈椎旁肌群(例如其中每块颈椎旁肌群肌肉施用1个单位

剂量));或4个单位剂量至颈椎旁肌群(优选2个单位剂量至颈部第一侧的的颈椎旁肌群,以及2个单位剂量至颈部第二侧的颈椎旁肌群(例如其中每块颈椎旁肌群肌肉施用2个单位剂量))。

[0450] 当如前述实施方案中所述治疗头痛疼痛(例如偏头痛疼痛)或偏头痛时,优选每个注射部位施用一个单位剂量。因此,所述治疗可包括施用嵌合梭菌神经毒素至:

[0451] (i)面部第一侧额肌肌肉的2个注射部位和/或面部第二侧额肌肌肉的2个注射部位;

[0452] (ii)降眉间肌肌肉的1个注射部位;

[0453] (iii)面部第一侧皱眉肌肌肉的1个注射部位和/或面部第二侧皱眉肌肌肉的1个注射部位;

[0454] (iv)头部第一侧颞肌肌肉的4个注射部位和/或头部第二侧颞肌肌肉的4个注射部位;

[0455] (v)颈部/头部(优选头部)第一侧枕肌肌肉的3个注射部位和/或颈部/头部(优选头部)第二侧枕肌肌肉的3个注射部位;

[0456] (vi)颈部第一侧斜方肌肌肉的3个注射部位和/或颈部第二侧斜方肌肌肉的3个注射部位;和/或

[0457] (vii)颈部第一侧的颈椎旁肌群的4个注射部位和/或颈部第二侧的颈椎旁肌群的4个注射部位(例如,其中每块颈椎旁肌群肌肉有1个注射部位),或颈部第一侧的颈椎旁肌群的2个注射部位和/或颈部第二侧的颈椎旁肌群的2个注射部位(例如,其中每个颈椎旁肌群肌肉有2个注射部位)。

[0458] 所述治疗可包括施用嵌合梭菌神经毒素至:

[0459] (i)面部第一侧额肌肌肉的2个注射部位和面部第二侧额肌肌肉的2个注射部位;

[0460] (ii)降眉间肌肌肉的1个注射部位;

[0461] (iii)面部第一侧皱眉肌肌肉的1个注射部位和面部第二侧皱眉肌肌肉的1个注射部位;

[0462] (iv)头部第一侧颞肌肌肉的4个注射部位和头部第二侧颞肌肌肉的4个注射部位;

[0463] (v)颈部/头部(优选头部)第一侧枕肌肌肉的3个注射部位和颈部/头部(优选头部)第二侧枕肌肌肉的3个注射部位;

[0464] (vi)颈部第一侧斜方肌肌肉的3个注射部位和颈部第二侧斜方肌肌肉的3个注射部位;和/或

[0465] (vii)颈部第一侧的颈椎旁肌群的4个注射部位和颈部第二侧的颈椎旁肌群的4个注射部位(例如,其中每块颈椎旁肌群肌肉有1个注射部位),或颈部第一侧的颈椎旁肌群的2个注射部位和颈部第二侧的颈椎旁肌群的2个注射部位(例如,其中每个颈椎旁肌群肌肉有2个注射部位)。

[0466] 所述治疗可包括施用嵌合梭菌神经毒素至:

[0467] (i)面部第一侧额肌肌肉的2个注射部位和面部第二侧额肌肌肉的2个注射部位;

[0468] (ii)降眉间肌肌肉的1个注射部位;

[0469] (iii)面部第一侧皱眉肌肌肉的1个注射部位和面部第二侧皱眉肌肌肉的1个注射部位;

[0470] (iv)头部第一侧颞肌肌肉的4个注射部位和头部第二侧颞肌肌肉的4个注射部位;

[0471] (v)颈部/头部(优选头部)第一侧枕肌肌肉的3个注射部位和颈部/头部(优选头部)第二侧枕肌肌肉的3个注射部位;

[0472] (vi)颈部第一侧斜方肌肌肉的3个注射部位和颈部第二侧斜方肌肌肉的3个注射部位;和

[0473] (vii)颈部第一侧的颈椎旁肌群的4个注射部位和颈部第二侧的颈椎旁肌群的4个注射部位(例如,其中每块颈椎旁肌群肌肉有1个注射部位),或颈部第一侧的颈椎旁肌群的2个注射部位和颈部第二侧的颈椎旁肌群的2个注射部位(例如,其中每个颈椎旁肌群肌肉有2个注射部位)。

[0474] 嵌合梭菌神经毒素的施用可包括:

[0475] (i)注射2次至额肌肌肉(优选每块额肌肌内注射2次);

[0476] (ii)注射1次至降眉间肌肌肉(优选每块降眉间肌肌内注射1次);

[0477] (iii)注射1次至皱眉肌肌肉(优选每块皱眉肌肌内注射1次);

[0478] (iv)注射4次至颞肌肌肉(优选每块颞肌肌内注射4次);

[0479] (v)注射3次至枕肌肌肉(优选每块枕肌肌内注射3次);

[0480] (vi)注射3次至斜方肌肌肉(优选每块斜方肌肌内注射3次);和/或

[0481] (vii)注射4次至颈椎旁肌群(优选每个颈椎旁肌群注射2次)。

[0482] 嵌合梭菌神经毒素的施用可包括:

[0483] (i)注射2次至额肌肌肉(优选每块额肌肌内注射2次);

[0484] (ii)注射1次至降眉间肌肌肉(优选每块降眉间肌肌内注射1次);

[0485] (iii)注射1次至皱眉肌肌肉(优选每块皱眉肌肌内注射1次);

[0486] (iv)注射4次至颞肌肌肉(优选每块颞肌肌内注射4次);

[0487] (v)注射3次至枕肌肌肉(优选每块枕肌肌内注射3次);

[0488] (vi)注射3次至斜方肌肌肉(优选每块斜方肌肌内注射3次);和

[0489] (vii)注射4次至颈椎旁肌群(优选每个颈椎旁肌群注射2次)。

[0490] 嵌合梭菌神经毒素的施用可包括:

[0491] (i)注射4次至额肌肌肉(优选注射2次至面部第一侧的额肌肌肉,注射2次至面部第二侧的额肌肌肉);

[0492] (ii)注射1次至降眉间肌肌肉;

[0493] (iii)注射2次至皱眉肌肌肉(优选注射1次至面部第一侧的皱眉肌肌肉,以及注射1次至面部第二侧的皱眉肌肌肉);

[0494] (iv)注射8次至颞肌肌肉(优选注射4次至头部第一侧的颞肌肌肉,以及注射4次至头部第二侧的颞肌肌肉);

[0495] (v)注射6次至枕肌肌肉(优选注射3次至头部第一侧的枕肌肌肉,以及注射3次至头部第二侧的枕肌肌肉);

[0496] (vi)注射6次至斜方肌肌肉(优选注射3次至颈部第一侧的斜方肌肌肉,以及注射3次至颈部第二侧的斜方肌肌肉);和/或

[0497] (vii)注射8次至颈椎旁肌群(优选注射4次至颈部第一侧颈椎旁肌群,以及注射4次至颈部第二侧颈椎旁肌群(例如每块颈椎旁肌群肌内注射1次));或注射4次至颈椎旁肌

群(优选注射2次至颈部第一侧颈椎旁肌群,以及注射2次至颈部第二侧颈椎旁肌群(例如每块颈椎旁肌群肌内注射2次))。

[0498] 嵌合梭菌神经毒素的施用可包括:

[0499] (i)注射4次至额肌肌肉(优选注射2次至面部第一侧的额肌肌肉,注射2次至面部第二侧的额肌肌肉);

[0500] (ii)注射1次至降眉间肌肌肉;

[0501] (iii)注射2次至皱眉肌肌肉(优选注射1次至面部第一侧的皱眉肌肌肉,以及注射1次至面部第二侧的皱眉肌肌肉);

[0502] (iv)注射8次至颞肌肌肉(优选注射4次至头部第一侧的颞肌肌肉,以及注射4次至头部第二侧的颞肌肌肉);

[0503] (v)注射6次至枕肌肌肉(优选注射3次至头部第一侧的枕肌肌肉,以及注射3次至头部第二侧的枕肌肌肉);

[0504] (vi)注射6次至斜方肌肌肉(优选注射3次至颈部第一侧斜方肌肌肉,以及注射3次至颈部第二侧的斜方肌肌肉);和

[0505] (vii)注射8次至颈椎旁肌群(优选注射4次至颈部第一侧颈椎旁肌群,以及注射4次至颈部第二侧颈椎旁肌群(例如,每块颈椎旁肌群肌肉有1个注射部位));或注射4次至颈椎旁肌群(优选注射2次至颈部第一侧颈椎旁肌群,以及注射2次至颈部第二侧颈椎旁肌群(例如每块颈椎旁肌群肌内注射2次))。

[0506] 头痛疼痛(例如偏头痛疼痛)或偏头痛治疗可包括施用:

[0507] (i)2个单位剂量至额肌肌肉(优选每块额肌肌肉2个单位剂量);

[0508] (ii)1个单位剂量至降眉间肌肌肉;

[0509] (iii)1个单位剂量至皱眉肌肌肉(优选每块皱眉肌肌肉1个单位剂量);

[0510] (iv)5个单位剂量至颞肌肌肉(优选每块颞肌肌肉5个单位剂量);

[0511] (v)4个单位剂量至枕肌肌肉(优选每块枕肌肌肉4个单位剂量);

[0512] (vi)5个单位剂量至斜方肌肌肉(优选每块斜方肌肌肉5个单位剂量);和/或

[0513] (vii)4个单位剂量至颈椎旁肌群(优选每个颈椎旁肌群4个单位剂量),或2个单位剂量至颈椎旁肌群(优选每个颈椎旁肌群2个单位剂量)。

[0514] 头痛疼痛(例如偏头痛疼痛)或偏头痛治疗可包括施用:

[0515] (i)2个单位剂量至额肌肌肉(优选每块额肌肌肉2个单位剂量);

[0516] (ii)1个单位剂量至降眉间肌肌肉(优选每块降眉间肌肌肉1个单位剂量);

[0517] (iii)1个单位剂量至皱眉肌肌肉(优选每块皱眉肌肌肉1个单位剂量);

[0518] (iv)5个单位剂量至颞肌肌肉(优选每块颞肌肌肉5个单位剂量);

[0519] (v)4个单位剂量至枕肌肌肉(优选每块枕肌肌肉4个单位剂量);

[0520] (vi)5个单位剂量至斜方肌肌肉(优选每块斜方肌肌肉5个单位剂量);和

[0521] (vii)4个单位剂量至颈椎旁肌群(优选每个颈椎旁肌群4个单位剂量),或2个单位剂量至颈椎旁肌群(优选每个颈椎旁肌群2个单位剂量)。

[0522] 头痛疼痛(例如偏头痛疼痛)或偏头痛治疗可包括施用:

[0523] (i)4个单位剂量至额肌肌肉(优选2个单位剂量至面部第一侧额肌肌肉,以及2个单位剂量至面部第二侧额肌肌肉);

[0524] (ii)1个单位剂量至降眉间肌肌肉;

[0525] (iii)2个单位剂量至皱眉肌肌肉(优选1个单位剂量至面部第一侧的皱眉肌肌肉,以及1个单位剂量至面部第二侧的皱眉肌肌肉);

[0526] (iv)10个单位剂量至颞肌肌肉(优选5个单位剂量至头部第一侧的颞肌肌肉,以及5个单位剂量至头部第二侧的颞肌肌肉);

[0527] (v)8个单位剂量至枕肌肌肉(优选4个单位剂量至头部第一侧的枕肌肌肉,以及4个单位剂量至头部第二侧的枕肌肌肉);

[0528] (vi)10个单位剂量至斜方肌肌肉(优选4个单位剂量至颈部第一侧的斜方肌肌肉,以及4个单位剂量至颈部第二侧的斜方肌肌肉);和/或

[0529] (vii)4个单位剂量至颈椎旁肌群(例如,其中每块颈椎旁肌群肌肉施用1或2个单位剂量)。

[0530] 头痛疼痛(例如偏头痛疼痛)或偏头痛治疗可包括施用:

[0531] (i)4个单位剂量至额肌肌肉(优选2个单位剂量至面部第一侧额肌肌肉,以及2个单位剂量至面部第二侧额肌肌肉);

[0532] (ii)1个单位剂量至降眉间肌肌肉;

[0533] (iii)2个单位剂量至皱眉肌肌肉(优选1个单位剂量至面部第一侧的皱眉肌肌肉,以及1个单位剂量至面部第二侧的皱眉肌肌肉);

[0534] (iv)10个单位剂量至颞肌肌肉(优选5个单位剂量至头部第一侧的颞肌肌肉,以及5个单位剂量至头部第二侧的颞肌肌肉);

[0535] (v)8个单位剂量至枕肌肌肉(优选4个单位剂量至头部第一侧的枕肌肌肉,以及4个单位剂量至头部第二侧的枕肌肌肉);

[0536] (vi)10个单位剂量至斜方肌肌肉(优选5个单位剂量至颈部第一侧的斜方肌肌肉,以及5个单位剂量至颈部第二侧的斜方肌肌肉);和

[0537] (vii)4个单位剂量至颈椎旁肌群(例如,其中每块颈椎旁肌群肌肉施用1或2个单位剂量)。

[0538] 当治疗头痛疼痛(例如偏头痛疼痛)或偏头痛时,嵌合梭菌神经毒素的施用可以包括:

[0539] (i)注射2次至额肌肌肉(优选每块额肌肌内注射2次);

[0540] (ii)注射1次至降眉间肌肌肉;

[0541] (iii)注射1次至皱眉肌肌肉(优选每块皱眉肌肌内注射1次);

[0542] (iv)注射5次至颞肌肌肉(优选每块颞肌肌内注射5次);

[0543] (v)注射4次至枕肌肌肉(优选每块枕肌肌内注射4次);

[0544] (vi)注射5次至斜方肌肌肉(优选每块斜方肌肌内注射5次);和/或

[0545] (vii)注射4次至颈椎旁肌群(例如,其中每块颈椎旁肌群肌内注射1或2次)。

[0546] 施用可包括:

[0547] (i)注射2次至额肌肌肉(优选每块额肌肌内注射2次);

[0548] (ii)注射1次至降眉间肌肌肉;

[0549] (iii)注射1次至皱眉肌肌肉(优选每块皱眉肌肌内注射1次);

[0550] (iv)注射5次至颞肌肌肉(优选每块颞肌肌内注射5次);

[0551] (v)注射4次至枕肌肌肉(优选每块枕肌肌内注射4次);

[0552] (vi)注射5次至斜方肌肌肉(优选每块斜方肌肌内注射5次);和

[0553] (vii)注射4次至颈椎旁肌群(例如,其中每块颈椎旁肌群肌内注射1或2次)。

[0554] 施用可包括:

[0555] (i)注射4次至额肌肌肉(优选注射2次至面部第一侧的额肌肌肉,以及注射2次至面部第二侧的额肌肌肉);

[0556] (ii)注射1次至降眉间肌肌肉;

[0557] (iii)注射2次至皱眉肌肌肉(优选注射1次至面部第一侧的皱眉肌肌肉,以及注射1次至面部第二侧的皱眉肌肌肉);

[0558] (iv)注射10次至颞肌肌肉(优选注射5次至头部第一侧的颞肌肌肉,以及注射5次至头部第二侧的颞肌肌肉);

[0559] (v)注射8次至枕肌肌肉(优选注射4次至头部第一侧的枕肌肌肉,以及注射4次至头部第二侧的枕肌肌肉);

[0560] (vi)注射10次至斜方肌肌肉(优选注射4次至颈部第一侧的斜方肌肌肉,以及注射4次至颈部第二侧的斜方肌肌肉);和/或

[0561] (vii)注射4次至颈椎旁肌群(例如,其中每块颈椎旁肌群肌内注射1或2次)。

[0562] 施用可包括:

[0563] (i)注射4次至额肌肌肉(优选注射2次至面部第一侧的额肌肌肉,以及注射2次至面部第二侧的额肌肌肉);

[0564] (ii)注射1次至降眉间肌肌肉;

[0565] (iii)注射2次至皱眉肌肌肉(优选注射1次至面部第一侧的皱眉肌肌肉,以及注射1次至面部第二侧的皱眉肌肌肉);

[0566] (iv)注射10次至颞肌肌肉(优选注射5次至头部第一侧的颞肌肌肉,以及注射5次至头部第二侧的颞肌肌肉);

[0567] (v)注射8次至枕肌肌肉(优选注射4次至头部第一侧的枕肌肌肉,以及注射4次至头部第二侧的枕肌肌肉);

[0568] (vi)注射10次至斜方肌肌肉(优选注射4次至颈部第一侧斜方肌肌肉,以及注射4次至颈部第二侧的斜方肌肌肉);和

[0569] (vii)注射4次至颈椎旁肌群(例如,其中每块颈椎旁肌群肌内注射1或2次)。

[0570] 当病症是头痛疼痛(例如偏头痛疼痛)或偏头痛时,可以将至少单位剂量的嵌合梭菌神经毒素施用于额肌、皱眉肌、鼻肌、眼轮匝肌、咬肌、颞肌、枕肌和斜方肌中的一块或多块。在一个实施方案中,可以将至少单位剂量的嵌合梭菌神经毒素施用于额肌、皱眉肌、鼻肌、眼轮匝肌、咬肌、颞肌、枕肌和斜方肌。在一个实施方案中:

[0571] (i)将单个单位剂量施用至以下的一块或多块:降眉间肌肌肉;皱眉肌肌肉(优选将单个单位剂量施用于面部第一侧(如左侧)的皱眉肌肌肉,将第二单位剂量施用于面部第二侧(如右侧)的皱眉肌肌肉);眼轮匝肌肌肉;咬肌肌肉;和/或(优选和)上斜方肌肌肉;

[0572] (ii)将单位剂量的一半施用至鼻肌;和/或(优选和)

[0573] (iii)将多个单位剂量施用给以下一块或多块:颞肌肌肉;枕肌肌肉;和/或(优选和)下斜方肌肌肉。多个单位剂量可以是2-6个单位剂量,例如2-5个单位剂量。

[0574] 头痛疼痛(例如偏头痛疼痛)或偏头痛治疗可包括施用:

[0575] (i)1个单位剂量至额肌肌肉(优选每块额肌肌肉1个单位剂量);

[0576] (ii)1个单位剂量至皱眉肌肌肉(优选每块皱眉肌肌肉1个单位剂量);

[0577] (iii)0.5个单位剂量至鼻肌肌肉(优选每块鼻肌肌肉0.5个单位剂量);

[0578] (iv)1个单位剂量至眼轮匝肌肌肉(优选每块眼轮匝肌肌肉1个单位剂量);

[0579] (v)1个单位剂量至咬肌肌肉(优选每块咬肌肌肉1个单位剂量);

[0580] (vi)6个单位剂量至颞肌肌肉(优选每块颞肌肌肉6个单位剂量);

[0581] (vii)6个单位剂量至枕肌肌肉(优选每块枕肌肌肉6个单位剂量);

[0582] (viii)1个单位剂量至上斜方肌肌肉(优选每块上斜方肌肌肉1个单位剂量);和/或

[0583] (ix)2个单位剂量至下斜方肌肌肉(优选每块下斜方肌肌肉2个单位剂量);

[0584] 头痛疼痛(例如偏头痛疼痛)或偏头痛治疗可包括施用:

[0585] (i)1个单位剂量至额肌肌肉(优选每块额肌肌肉1个单位剂量);

[0586] (ii)1个单位剂量至皱眉肌肌肉(优选每块皱眉肌肌肉1个单位剂量);

[0587] (iii)0.5个单位剂量至鼻肌肌肉(优选每块鼻肌肌肉0.5个单位剂量);

[0588] (iv)1个单位剂量至眼轮匝肌肌肉(优选每块眼轮匝肌肌肉1个单位剂量);

[0589] (v)1个单位剂量至咬肌肌肉(优选每块咬肌肌肉1个单位剂量);

[0590] (vi)6个单位剂量至颞肌肌肉(优选每块颞肌肌肉6个单位剂量);

[0591] (vii)6个单位剂量至枕肌肌肉(优选每块枕肌肌肉6个单位剂量);

[0592] (viii)1个单位剂量至上斜方肌肌肉(优选每块上斜方肌肌肉1个单位剂量);和

[0593] (ix)2个单位剂量至下斜方肌肌肉(优选每块下斜方肌肌肉2个单位剂量);

[0594] 头痛疼痛(例如偏头痛疼痛)或偏头痛治疗可包括施用:

[0595] (i)2个单位剂量至额肌肌肉(优选1个单位剂量至面部第一侧额肌肌肉,以及1个单位剂量至面部第二侧额肌肌肉);

[0596] (ii)2个单位剂量至皱眉肌肌肉(优选1个单位剂量至面部第一侧的皱眉肌肌肉,以及1个单位剂量至面部第二侧的皱眉肌肌肉);

[0597] (iii)1个单位剂量至鼻肌肌肉(优选0.5个单位剂量至面部第一侧的鼻肌肌肉,以及0.5个单位剂量至面部第二侧的鼻肌肌肉);

[0598] (iv)2个单位剂量至眼轮匝肌肌肉(优选1个单位剂量至面部第一侧的眼轮匝肌肌肉,以及1个单位剂量至面部第二侧的眼轮匝肌肌肉);

[0599] (v)2个单位剂量至咬肌肌肉(优选1个单位剂量至面部第一侧咬肌肌肉,以及1个单位剂量至面部第二侧额肌肌肉);

[0600] (vi)12个单位剂量至颞肌肌肉(优选6个单位剂量至头部第一侧的颞肌肌肉,以及6个单位剂量至头部第二侧的颞肌肌肉);

[0601] (vii)12个单位剂量至枕肌肌肉(优选6个单位剂量至头部第一侧的枕肌肌肉,以及6个单位剂量至头部第二侧的枕肌肌肉);

[0602] (viii)2个单位剂量至上斜方肌肌肉(优选1个单位剂量至颈部第一侧的上斜方肌肌肉,以及1个单位剂量至颈部第二侧的上斜方肌肌肉);和/或

[0603] (ix)4个单位剂量至下斜方肌肌肉(优选2个单位剂量至颈部第一侧的下斜方肌肌

肉,和/或2个单位剂量至颈部第二侧的下斜方肌肌肉)。

[0604] 头痛疼痛(例如偏头痛疼痛)或偏头痛治疗可包括施用:

[0605] (i)2个单位剂量至额肌肌肉(优选1个单位剂量至面部第一侧额肌肌肉,以及1个单位剂量至面部第二侧额肌肌肉);

[0606] (ii)2个单位剂量至皱眉肌肌肉(优选1个单位剂量至面部第一侧的皱眉肌肌肉,以及1个单位剂量至面部第二侧的皱眉肌肌肉);

[0607] (iii)1个单位剂量至鼻肌肌肉(优选0.5个单位剂量至面部第一侧的鼻肌肌肉,以及0.5个单位剂量至面部第二侧的鼻肌肌肉);

[0608] (iv)2个单位剂量至眼轮匝肌肌肉(优选1个单位剂量至面部第一侧的眼轮匝肌肌肉,以及1个单位剂量至面部第二侧的眼轮匝肌肌肉);

[0609] (v)2个单位剂量至咬肌肌肉(优选1个单位剂量至面部第一侧咬肌肌肉,以及1个单位剂量至面部第二侧额肌肌肉);

[0610] (vi)12个单位剂量至颞肌肌肉(优选6个单位剂量至头部第一侧的颞肌肌肉,以及6个单位剂量至头部第二侧的颞肌肌肉);

[0611] (vii)12个单位剂量至枕肌肌肉(优选6个单位剂量至头部第一侧的枕肌肌肉,以及6个单位剂量至头部第二侧的枕肌肌肉);

[0612] (viii)2个单位剂量至上斜方肌肌肉(优选1个单位剂量至颈部第一侧的上斜方肌肌肉,以及1个单位剂量至颈部第二侧的上斜方肌肌肉);和

[0613] (ix)4个单位剂量至下斜方肌肌肉(优选2个单位剂量至颈部第一侧的下斜方肌肌肉,和/或2个单位剂量至颈部第二侧的下斜方肌肌肉)。

[0614] 嵌合梭菌神经毒素的施用可包括:

[0615] (i)注射1次至额肌肌肉(优选每块额肌肌内注射1次);

[0616] (ii)注射1次至皱眉肌肌肉(优选每块皱眉肌肌内注射1次);

[0617] (iii)注射1次至鼻肌肌肉(优选每块鼻肌肌内注射1次);

[0618] (iv)注射1次至眼轮匝肌肌肉(优选每块眼轮匝肌肌内注射1次);

[0619] (v)注射1次至咬肌肌肉(优选每块咬肌肌内注射1次);

[0620] (vi)注射3次至颞肌肌肉(优选每块颞肌肌内注射3次);

[0621] (vii)注射3次至枕肌肌肉(优选每块枕肌肌内注射3次);

[0622] (viii)注射1次至上斜方肌肌肉(优选每块上斜方肌肌内注射1次);和/或

[0623] (ix)注射1次至下斜方肌肌肉(优选每块下斜方肌肌内注射1次);

[0624] 嵌合梭菌神经毒素的施用可包括:

[0625] (i)注射1次至额肌肌肉(优选每块额肌肌内注射1次);

[0626] (ii)注射1次至皱眉肌肌肉(优选每块皱眉肌肌内注射1次);

[0627] (iii)注射1次至鼻肌肌肉(优选每块鼻肌肌内注射1次);

[0628] (iv)注射1次至眼轮匝肌肌肉(优选每块眼轮匝肌肌内注射1次);

[0629] (v)注射1次至咬肌肌肉(优选每块咬肌肌内注射1次);

[0630] (vi)注射3次至颞肌肌肉(优选每块颞肌肌内注射3次);

[0631] (vii)注射3次至枕肌肌肉(优选每块枕肌肌内注射3次);

[0632] (viii)注射1次至上斜方肌肌肉(优选每块上斜方肌肌内注射1次);和

[0633] (ix)注射1次至下斜方肌肌肉(优选每块下斜方肌肌内注射1次)；

[0634] 嵌合梭菌神经毒素的施用可包括：

[0635] (i)注射2次至额肌肌肉(优选注射1次至面部第一侧的额肌肌肉,以及注射1次至面部第二侧的额肌肌肉)；

[0636] (ii)注射2次至皱眉肌肌肉(优选注射1次至面部第一侧的皱眉肌肌肉,以及注射1次至面部第二侧的皱眉肌肌肉)；

[0637] (iii)注射2次至鼻肌肌肉(优选注射1次至面部第一侧的鼻肌肌肉,以及注射1次至面部第二侧的鼻肌肌肉))；

[0638] (iv)注射2次至眼轮匝肌肌肉(优选注射1次至面部第一侧的眼轮匝肌肌肉,以及注射1次至面部第二侧的眼轮匝肌肌肉)；

[0639] (v)注射2次至咬肌肌肉(优选注射1次至面部第一侧的咬肌肌肉,以及注射1次至面部第二侧的咬肌肌肉)；

[0640] (vi)注射6次至颞肌肌肉(优选注射3次至头部第一侧的颞肌肌肉,以及注射3次至头部第二侧的颞肌肌肉)；

[0641] (vii)注射6次至枕肌肌肉(优选注射3次至头部第一侧的枕肌肌肉,以及注射3次至头部第二侧的枕肌肌肉)；

[0642] (viii)注射2次至上斜方肌肌肉(优选注射1次至颈部第一侧的上斜方肌肌肉,以及注射1次至颈部第二侧的上斜方肌肌肉)；和/或

[0643] (ix)注射2次至下斜方肌肌肉(优选注射1次至颈部第一侧的下斜方肌肌肉,以及注射1次至颈部第二侧的下斜方肌肌肉)。

[0644] 嵌合梭菌神经毒素的施用可包括：

[0645] (i)注射2次至额肌肌肉(优选注射1次至面部第一侧的额肌肌肉,以及注射1次至面部第二侧的额肌肌肉)；

[0646] (ii)注射2次至皱眉肌肌肉(优选注射1次至面部第一侧的皱眉肌肌肉,以及注射1次至面部第二侧的皱眉肌肌肉)；

[0647] (iii)注射2次至鼻肌肌肉(优选注射1次至面部第一侧的鼻肌肌肉,以及注射1次至面部第二侧的鼻肌肌肉))；

[0648] (iv)注射2次至眼轮匝肌肌肉(优选注射1次至面部第一侧的眼轮匝肌肌肉,以及注射1次至面部第二侧的眼轮匝肌肌肉)；

[0649] (v)注射2次至咬肌肌肉(优选注射1次至面部第一侧的咬肌肌肉,以及注射1次至面部第二侧的咬肌肌肉)；

[0650] (vi)注射6次至颞肌肌肉(优选注射3次至头部第一侧的颞肌肌肉,以及注射3次至头部第二侧的颞肌肌肉)；

[0651] (vii)注射6次至枕肌肌肉(优选注射3次至头部第一侧的枕肌肌肉,以及注射3次至头部第二侧的枕肌肌肉)；

[0652] (viii)注射2次至上斜方肌肌肉(优选注射1次至颈部第一侧的上斜方肌肌肉,以及注射1次至颈部第二侧的上斜方肌肌肉)；和

[0653] (ix)注射2次至下斜方肌肌肉(优选注射1次至颈部第一侧的下斜方肌肌肉,以及注射1次至颈部第二侧的下斜方肌肌肉)。

[0654] 当病症是头痛疼痛(例如偏头痛疼痛)或偏头痛时,可以将至少单位剂量的嵌合梭菌神经毒素施用于额肌、皱眉肌、鼻肌、眼轮匝肌、咬肌、颞肌、上枕肌、下枕肌和上斜方肌、下斜方肌中的一种或多种。可以将至少单位剂量的嵌合梭菌神经毒素施用于额肌、皱眉肌、鼻肌、眼轮匝肌、咬肌、颞肌、上枕肌、下枕肌和上斜方肌、下斜方肌。在一个实施方案中:

[0655] (i)将单个单位剂量施用至以下的一块或多块:降眉间肌肌肉;皱眉肌肌肉(优选将单个单位剂量施用于面部第一侧(如左侧)的皱眉肌肌肉,以及将第二单位剂量施用于面部第二侧(如右侧)的皱眉肌肌肉);鼻肌肌肉;眼轮匝肌肌肉;咬肌肌肉;和下枕肌肌肉;和/或(优选和)

[0656] (iii)将多个单位剂量施用给以下一块或多块:颞肌肌肉;上枕肌肌肉;和斜方肌肌肉。多个单位剂量可以是2-8个单位剂量,例如2-5个单位剂量。

[0657] 头痛疼痛(例如偏头痛疼痛)或偏头痛治疗可包括施用:

[0658] (i)1个单位剂量至额肌肌肉(优选每块额肌肌肉1个单位剂量);

[0659] (ii)1个单位剂量至皱眉肌肌肉(优选每块皱眉肌肌肉1个单位剂量);

[0660] (iii)1个单位剂量至鼻肌肌肉(优选每块鼻肌肌肉1个单位剂量);

[0661] (iv)1个单位剂量至眼轮匝肌肌肉(优选每块眼轮匝肌肌肉1个单位剂量);

[0662] (v)1个单位剂量至咬肌肌肉(优选每块咬肌肌肉1个单位剂量);

[0663] (vi)4个单位剂量至颞肌肌肉(优选每块颞肌肌肉4个单位剂量);

[0664] (vii)2个单位剂量至上枕肌肌肉(优选每块上枕肌肌肉2个单位剂量);

[0665] (viii)1个单位剂量至下枕肌肌肉(优选每块下枕肌肌肉1个单位剂量);和/或

[0666] (viii)2个单位剂量至斜方肌肌肉(优选每块斜方肌肌肉2个单位剂量)。

[0667] 头痛疼痛(例如偏头痛疼痛)或偏头痛治疗可包括施用:

[0668] (i)1个单位剂量至额肌肌肉(优选每块额肌肌肉1个单位剂量);

[0669] (ii)1个单位剂量至皱眉肌肌肉(优选每块皱眉肌肌肉1个单位剂量);

[0670] (iii)1个单位剂量至鼻肌肌肉(优选每块鼻肌肌肉1个单位剂量);

[0671] (iv)1个单位剂量至眼轮匝肌肌肉(优选每块眼轮匝肌肌肉1个单位剂量);

[0672] (v)1个单位剂量至咬肌肌肉(优选每块咬肌肌肉1个单位剂量);

[0673] (vi)4个单位剂量至颞肌肌肉(优选每块颞肌肌肉4个单位剂量);

[0674] (vii)2个单位剂量至上枕肌肌肉(优选每块上枕肌肌肉2个单位剂量);

[0675] (viii)1个单位剂量至下枕肌肌肉(优选每块下枕肌肌肉1个单位剂量);和

[0676] (viii)2个单位剂量至斜方肌肌肉(优选每块斜方肌肌肉2个单位剂量)。

[0677] 头痛疼痛(例如偏头痛疼痛)或偏头痛治疗可包括施用:

[0678] (i)2个单位剂量至额肌肌肉(优选1个单位剂量至面部第一侧额肌肌肉,以及1个单位剂量至面部第二侧额肌肌肉);

[0679] (ii)2个单位剂量至皱眉肌肌肉(优选1个单位剂量至面部第一侧的皱眉肌肌肉,以及1个单位剂量至面部第二侧的皱眉肌肌肉);

[0680] (iii)2个单位剂量至鼻肌肌肉(优选1个单位剂量至面部第一侧的鼻肌肌肉,以及1个单位剂量至面部第二侧的鼻肌肌肉);

[0681] (iv)2个单位剂量至眼轮匝肌肌肉(优选1个单位剂量至面部第一侧的眼轮匝肌肌肉,以及1个单位剂量至面部第二侧的眼轮匝肌肌肉);

[0682] (v)2个单位剂量至咬肌肌肉(优选1个单位剂量至面部第一侧咬肌肌肉,以及1个单位剂量至面部第二侧额肌肌肉);

[0683] (vi)8个单位剂量至颞肌肌肉(优选4个单位剂量至头部第一侧的颞肌肌肉,以及4个单位剂量至头部第二侧的颞肌肌肉);

[0684] (vii)4个单位剂量至上枕肌肌肉(优选2个单位剂量至头部第一侧的上枕肌肌肉,以及2个单位剂量至头部第二侧的上枕肌肌肉);

[0685] (viii)2个单位剂量至下枕肌肌肉(优选1个单位剂量至头部第一侧的下枕肌肌肉,以及1个单位剂量至头部第二侧的下枕肌肌肉);和/或

[0686] (ix)4个单位剂量至斜方肌肌肉(优选2个单位剂量至颈部第一侧的斜方肌肌肉,以及2个单位剂量至颈部第二侧的斜方肌肌肉)。

[0687] 头痛疼痛(例如偏头痛疼痛)或偏头痛治疗可包括施用:

[0688] (i)2个单位剂量至额肌肌肉(优选1个单位剂量至面部第一侧额肌肌肉,以及1个单位剂量至面部第二侧额肌肌肉);

[0689] (ii)2个单位剂量至皱眉肌肌肉(优选1个单位剂量至面部第一侧的皱眉肌肌肉,以及1个单位剂量至面部第二侧的皱眉肌肌肉);

[0690] (iii)2个单位剂量至鼻肌肌肉(优选1个单位剂量至面部第一侧的鼻肌肌肉,以及1个单位剂量至面部第二侧的鼻肌肌肉);

[0691] (iv)2个单位剂量至眼轮匝肌肌肉(优选1个单位剂量至面部第一侧的眼轮匝肌肌肉,以及1个单位剂量至面部第二侧的眼轮匝肌肌肉);

[0692] (v)2个单位剂量至咬肌肌肉(优选1个单位剂量至面部第一侧咬肌肌肉,以及1个单位剂量至面部第二侧额肌肌肉);

[0693] (vi)8个单位剂量至颞肌肌肉(优选4个单位剂量至头部第一侧的颞肌肌肉,以及4个单位剂量至头部第二侧的颞肌肌肉);

[0694] (vii)4个单位剂量至上枕肌肌肉(优选2个单位剂量至头部第一侧的上枕肌肌肉,以及2个单位剂量至头部第二侧的上枕肌肌肉);

[0695] (viii)2个单位剂量至下枕肌肌肉(优选1个单位剂量至头部第一侧的下枕肌肌肉,以及1个单位剂量至头部第二侧的下枕肌肌肉);和

[0696] (ix)4个单位剂量至斜方肌肌肉(优选2个单位剂量至颈部第一侧的斜方肌肌肉,以及2个单位剂量至颈部第二侧的斜方肌肌肉)。

[0697] 嵌合梭菌神经毒素的施用可包括:

[0698] (i)注射1次至额肌肌肉(优选每块额肌肌内注射1次);

[0699] (ii)注射1次至皱眉肌肌肉(优选每块皱眉肌肌内注射1次);

[0700] (iii)注射1次至鼻肌肌肉(优选每块鼻肌肌内注射1次);

[0701] (iv)注射1次至眼轮匝肌肌肉(优选每块眼轮匝肌肌内注射1次);

[0702] (v)注射1次至咬肌肌肉(优选每块咬肌肌内注射1次);

[0703] (vi)注射4次至颞肌肌肉(优选每块颞肌肌内注射4次);

[0704] (vii)注射2次至上枕肌肌肉(优选每块上枕肌肌内注射2次);

[0705] (viii)注射1次至下枕肌肌肉(优选每块下枕肌肌内注射1次);和/或

[0706] (viii)注射2次至斜方肌肌肉(优选每块斜方肌肌内注射2次)。

[0707] 嵌合梭菌神经毒素的施用可包括：

[0708] (i)注射1次至额肌肌肉(优选每块额肌肌内注射1次)；

[0709] (ii)注射1次至皱眉肌肌肉(优选每块皱眉肌肌内注射1次)；

[0710] (iii)注射1次至鼻肌肌肉(优选每块鼻肌肌内注射1次)；

[0711] (iv)注射1次至眼轮匝肌肌肉(优选每块眼轮匝肌肌内注射1次)；

[0712] (v)注射1次至咬肌肌肉(优选每块咬肌肌内注射1次)；

[0713] (vi)注射4次至颞肌肌肉(优选每块颞肌肌内注射4次)；

[0714] (vii)注射2次至上枕肌肌肉(优选每块上枕肌肌内注射2次)；

[0715] (viii)注射1次至下枕肌肌肉(优选每块下枕肌肌内注射1次)；和

[0716] (viii)注射2次至斜方肌肌肉(优选每块斜方肌肌内注射2次)。

[0717] 嵌合梭菌神经毒素的施用可包括：

[0718] (i)注射2次至额肌肌肉(优选注射1次至面部第一侧的额肌肌肉，以及注射1次至面部第二侧的额肌肌肉)；

[0719] (ii)注射2次至皱眉肌肌肉(优选注射1次至面部第一侧的皱眉肌肌肉，以及注射1次至面部第二侧的皱眉肌肌肉)；

[0720] (iii)注射2次至鼻肌肌肉(优选注射1次至面部第一侧的鼻肌肌肉，以及注射1次至面部第二侧的鼻肌肌肉))；

[0721] (iv)注射2次至眼轮匝肌肌肉(优选注射1次至面部第一侧的眼轮匝肌肌肉，以及注射1次至面部第二侧的眼轮匝肌肌肉)；

[0722] (v)注射2次至咬肌肌肉(优选注射1次至面部第一侧的咬肌肌肉，以及注射1次至面部第二侧的咬肌肌肉)；

[0723] (vi)注射8次至颞肌肌肉(优选注射4次至头部第一侧的颞肌肌肉，以及注射4次至头部第二侧的颞肌肌肉)；

[0724] (vii)注射4次至上枕肌肌肉(优选注射2次至头部第一侧的上枕肌肌肉，以及注射2次至头部第二侧的上枕肌肌肉)；

[0725] (viii)注射2次至枕肌肌肉(优选注射1次至头部第一侧的枕肌肌肉，以及注射1次至头部第二侧的枕肌肌肉)；和/或

[0726] (ix)注射4次至斜方肌肌肉(优选注射2次至颈部第一侧的斜方肌肌肉，以及注射2次至颈部第二侧的斜方肌肌肉)。

[0727] 嵌合梭菌神经毒素的施用可包括：

[0728] (i)注射2次至额肌肌肉(优选注射1次至面部第一侧的额肌肌肉，以及注射1次至面部第二侧的额肌肌肉)；

[0729] (ii)注射2次至皱眉肌肌肉(优选注射1次至面部第一侧的皱眉肌肌肉，以及注射1次至面部第二侧的皱眉肌肌肉)；

[0730] (iii)注射2次至鼻肌肌肉(优选注射1次至面部第一侧的鼻肌肌肉，以及注射1次至面部第二侧的鼻肌肌肉))；

[0731] (iv)注射2次至眼轮匝肌肌肉(优选注射1次至面部第一侧的眼轮匝肌肌肉，以及注射1次至面部第二侧的眼轮匝肌肌肉)；

[0732] (v)注射2次至咬肌肌肉(优选注射1次至面部第一侧的咬肌肌肉，以及注射1次至

面部第二侧的咬肌肌肉)；

[0733] (vi)注射8次至颞肌肌肉(优选注射4次至头部第一侧的颞肌肌肉,以及注射4次至头部第二侧的颞肌肌肉)；

[0734] (vii)注射4次至上枕肌肌肉(优选注射2次至头部第一侧的上枕肌肌肉,以及注射2次至头部第二侧的上枕肌肌肉)；

[0735] (viii)注射2次至枕肌肌肉(优选注射1次至头部第一侧的枕肌肌肉,以及注射1次至头部第二侧的枕肌肌肉)；和

[0736] (ix)注射4次至斜方肌肌肉(优选注射2次至颈部第一侧的斜方肌肌肉,以及注射2次至颈部第二侧的斜方肌肌肉)。

[0737] 在本文所述的任何方面或实施方案中,嵌合梭菌神经毒素的施用可包括将嵌合梭菌神经毒素直接注射到肌肉中或间接注射到肌肉中。例如,当嵌合梭菌神经毒素向肌肉的注射是间接注射到肌肉中时,嵌合梭菌神经毒素可以在肌肉区域施用。在一个实施方案中,当嵌合梭菌神经毒素向肌肉的注射是直接注射到肌肉中时,嵌合梭菌神经毒素可以肌内施用至肌肉。在一个实施方案中,当嵌合梭菌神经毒素向肌肉的注射是间接注射到肌肉中时,嵌合梭菌神经毒素可以皮内施用。

[0738] 当病症是头痛(例如偏头痛疼痛)或偏头痛时,可以将至少单位剂量的嵌合梭菌神经毒素皮内施用于以下一种或多种:三叉神经眼区;三叉神经上颌区;三叉神经下颌区;和头后部。优选地,可以将至少单位剂量的嵌合梭菌神经毒素皮内施用于:三叉神经眼区;三叉神经上颌区;三叉神经下颌区;和头后部。

[0739] 在一个或多个所述区域的皮内施用可以将嵌合梭菌神经毒素靶向目标三叉神经(例如目标神经末梢)。三叉神经眼区的目标神经(例如目标神经末梢)可以是以下的一种或多种:眶上神经;滑车上神经;和滑车内神经(例如其神经末梢)。三叉神经上颌区的目标神经(例如目标神经末梢)可以是以下的一种或多种:颧颞神经和颧面神经(例如其神经末梢)。三叉神经下颌区的目标神经(例如目标神经末梢)可以是耳颞神经(例如其神经末梢)。头后部的目标神经(例如目标神经末梢)可以是以下的一种或多种:枕大神经和枕小神经(例如,其神经末梢)。

[0740] 在一个或多个所述区域的皮内施用可以将嵌合梭菌神经毒素靶向目标三叉神经(例如目标神经末梢)。三叉神经眼区的目标神经(例如目标神经末梢)可以是以下的一种或多种:眶上神经;滑车上神经;和滑车内神经(例如其神经末梢)。三叉神经上颌区的目标神经(例如目标神经末梢)可以是以下的一种或多种:颧颞神经;和眶内神经(intraorbital nerve)(例如其神经末梢)。三叉神经下颌区的目标神经(例如目标神经末梢)可以是以下的一种或多种:耳颞神经;和下颌神经(例如其神经末梢)。头后部的目标神经(例如目标神经末梢)可以是以下的一种或多种:枕大神经;枕小神经;和枕下神经(例如其神经末梢)。

[0741] 在一个实施方案中:

[0742] (i)在以下一个或多个区域皮内施用单个单位剂量:眶上神经(优选在面部第一侧(例如左侧)的眶上神经区施用单个单位剂量,并在面部第二侧(例如右侧)的眶上神经区施用第二单位剂量)；滑车上神经(优选在面部第一侧(例如左侧)的滑车上神经区施用单个单位剂量,并在面部第二侧(例如右侧)的滑车上神经区施用第二单位剂量)；滑车内神经(优选在面部第一侧(例如左侧)的滑车内神经区施用单个单位剂量,并在面部第二侧(例如右

侧)的滑车内神经区施用第二单位剂量);颧颞神经(优选在面部第一侧(例如左侧)的颧颞神经区施用单个单位剂量,并在面部第二侧(例如右侧)的颧颞神经区施用第二单位剂量);颧面神经(优选在面部第一侧(例如左侧)的颧面神经区施用单个单位剂量,并在面部第二侧(例如右侧)的颧面神经区施用第二单位剂量);枕小神经(优选在颈部第一侧(例如左侧)的枕小神经区施用单个单位剂量,并在颈部第二侧(例如右侧)的枕小神经区施用第二单位剂量);和/或(优选和)

[0743] (iii)在以下一个或多个区域皮内施用多个单位剂量:眶上神经(优选在面部第一侧(例如左侧)的眶上神经区施用单个单位剂量,并在面部第二侧(例如右侧)的眶上神经区施用第二单位剂量);滑车上神经(优选在面部第一侧(例如左侧)的滑车上神经区施用单个单位剂量,并在面部第二侧(例如右侧)的滑车上神经区施用第二单位剂量);滑车内神经(优选在面部第一侧(例如左侧)的滑车内神经区施用单个单位剂量,并在面部第二侧(例如右侧)的滑车内神经区施用第二单位剂量);颧颞神经(优选在面部第一侧(例如左侧)的颧颞神经区施用单个单位剂量,并在面部第二侧(例如右侧)的颧颞神经区施用第二单位剂量);颧面神经(优选在面部第一侧(例如左侧)的颧面神经区施用单个单位剂量,并在面部第二侧(例如右侧)的颧面神经区施用第二单位剂量);耳颞神经;枕大神经;枕小神经(优选在头部第一侧(例如左侧)的枕小神经区施用单个单位剂量,并在头部第二侧(例如右侧)的枕小神经区施用第二单位剂量)。多个单位剂量可以是2-8个单位剂量,例如2-5个单位剂量。

[0744] 优选的注射部位和注射次数如图6所示。在这种情况下,每个注射部位可以施用一个单位剂量的嵌合梭菌神经毒素。

[0745] 优选地,注射部位在指定神经的末端区域。

[0746] 头痛疼痛(例如偏头痛疼痛)或偏头痛的治疗可包括双侧皮内施用单位剂量的嵌合梭菌神经毒素。头痛疼痛(例如偏头痛疼痛)或偏头痛治疗可包括施用:

[0747] (i)1个单位剂量至面部第一侧的眶上神经区和/或1个单位剂量至面部第二侧的眶上神经区;

[0748] (ii)1个单位剂量至面部第一侧的滑车上神经区和/或1个单位剂量至面部第二侧的滑车上神经区;

[0749] (iii)1个单位剂量至面部第一侧的滑车内神经区和/或1个单位剂量至面部第二侧的滑车内神经区;

[0750] (iv)1个单位剂量至面部第一侧的颧颞神经区和/或1个单位剂量至面部第二侧颧颞神经区;

[0751] (v)1个单位剂量至面部第一侧的颧面神经区和/或1个单位剂量至面部第二侧的颧面神经区;

[0752] (vi)2个单位剂量至面部第一侧的耳颞神经区和/或2个单位剂量至面部第二侧的耳颞神经区;

[0753] (vii)2个单位剂量至颈部第一侧枕大神经区和/或2个单位剂量至颈部第二侧枕大神经区;和/或

[0754] (vii)1个单位剂量至颈部第一侧的枕小神经区和/或1个单位剂量至颈部第二侧的枕小神经区。

[0755] 头痛疼痛(例如偏头痛疼痛)或偏头痛的治疗可包括双侧施用单位剂量的嵌合梭菌神经毒素。头痛疼痛(例如偏头痛疼痛)或偏头痛治疗可包括施用:

[0756] (i)1个单位剂量至面部第一侧的眶上神经区和/或1个单位剂量至面部第二侧的眶上神经区;

[0757] (ii)1个单位剂量至面部第一侧的滑车上神经区和/或1个单位剂量至面部第二侧的滑车上神经区;

[0758] (iii)1个单位剂量至面部第一侧的滑车内神经区和/或1个单位剂量至面部第二侧的滑车内神经区;

[0759] (iv)1个单位剂量至面部第一侧的颧颞神经区和/或1个单位剂量至面部第二侧颧颞神经区;

[0760] (v)1个单位剂量至面部第一侧的颧面神经区和/或1个单位剂量至面部第二侧的颧面神经区;

[0761] (vi)2个单位剂量至面部第一侧的耳颞神经区和/或2个单位剂量至面部第二侧的耳颞神经区;

[0762] (vii)2个单位剂量至颈部第一侧的枕大神经区和/或2个单位剂量至颈部第二侧的枕大神经区;和/或

[0763] (vii)1个单位剂量至颈部第一侧的枕小神经区和/或1个单位剂量至颈部第二侧的枕小神经区。

[0764] 优选地,头痛疼痛(例如偏头痛疼痛)或偏头痛治疗可包括施用:

[0765] (i)1个单位剂量至面部第一侧的眶上神经区和1个单位剂量至面部第二侧的眶上神经区;

[0766] (ii)1个单位剂量至面部第一侧的滑车上神经区和1个单位剂量至面部第二侧的滑车上神经区;

[0767] (iii)1个单位剂量至面部第一侧的滑车内神经区和1个单位剂量至面部第二侧的滑车内神经区;

[0768] (iv)1个单位剂量至面部第一侧的颧颞神经区和1个单位剂量至面部第二侧颧颞神经区;

[0769] (v)1个单位剂量至面部第一侧的颧面神经区和1个单位剂量至面部第二侧的颧面神经区;

[0770] (vi)2个单位剂量至面部第一侧的耳颞神经区和2个单位剂量至面部第二侧的耳颞神经区;

[0771] (vii)2个单位剂量至颈部第一侧的枕大神经区和2个单位剂量至颈部第二侧枕大神经区;和/或

[0772] (vii)1个单位剂量至颈部第一侧的枕小神经区和1个单位剂量至颈部第二侧的枕小神经区。

[0773] 更优选地,头痛疼痛(例如偏头痛疼痛)或偏头痛治疗可包括施用:

[0774] (i)1个单位剂量至面部第一侧的眶上神经区和1个单位剂量至面部第二侧的眶上神经区;

[0775] (ii)1个单位剂量至面部第一侧的滑车上神经区和1个单位剂量至面部第二侧的

滑车上神经区;

[0776] (iii)1个单位剂量至面部第一侧的滑车内神经区和1个单位剂量至面部第二侧的滑车内神经区;

[0777] (iv)1个单位剂量至面部第一侧的颧颞神经区和1个单位剂量至面部第二侧颧颞神经区;

[0778] (v)1个单位剂量至面部第一侧的颧面神经区和1个单位剂量至面部第二侧的颧面神经区;

[0779] (vi)2个单位剂量至面部第一侧的耳颞神经区和2个单位剂量至面部第二侧的耳颞神经区;

[0780] (vii)2个单位剂量至颈部第一侧的枕大神经区和/或2个单位剂量至颈部第二侧的枕大神经区;和

[0781] (vii)1个单位剂量至颈部第一侧的枕小神经区和1个单位剂量至颈部第二侧的枕小神经区。

[0782] 头痛疼痛(例如偏头痛疼痛)或偏头痛的治疗可包括双侧皮内施用单位剂量的嵌合梭菌神经毒素。头痛疼痛(例如偏头痛疼痛)或偏头痛治疗可包括施用:

[0783] (i)2个单位剂量至面部第一侧的眶上神经区和/或2个单位剂量至面部第二侧的眶上神经区;

[0784] (ii)2个单位剂量至面部第一侧的滑车上神经区和/或2个单位剂量至面部第二侧的滑车上神经区;

[0785] (iii)2个单位剂量至面部第一侧的滑车内神经区和/或2个单位剂量至面部第二侧的滑车内神经区;

[0786] (iv)2个单位剂量至面部第一侧的颧颞神经区和/或2个单位剂量至面部第二侧颧颞神经区;

[0787] (v)2个单位剂量至面部第一侧的颧面神经区和/或2个单位剂量至面部第二侧的颧面神经区;

[0788] (vi)4个单位剂量至面部第一侧的耳颞神经区和/或4个单位剂量至面部第二侧的耳颞神经区;

[0789] (vii)4个单位剂量至颈部第一侧枕大神经区和/或4个单位剂量至颈部第二侧枕大神经区;和/或

[0790] (vii)2个单位剂量至颈部第一侧的枕小神经区和/或2个单位剂量至颈部第二侧的枕小神经区。

[0791] 头痛疼痛(例如偏头痛疼痛)或偏头痛的治疗可包括双侧施用单位剂量的嵌合梭菌神经毒素。头痛疼痛(例如偏头痛疼痛)或偏头痛治疗可包括施用:

[0792] (i)2个单位剂量至面部第一侧的眶上神经区和/或2个单位剂量至面部第二侧的眶上神经区;

[0793] (ii)2个单位剂量至面部第一侧的滑车上神经区和/或2个单位剂量至面部第二侧的滑车上神经区;

[0794] (iii)2个单位剂量至面部第一侧的滑车内神经区和/或2个单位剂量至面部第二侧的滑车内神经区;

[0795] (iv)2个单位剂量至面部第一侧的颧颞神经区和/或2个单位剂量至面部第二侧颧颞神经区;

[0796] (v)2个单位剂量至面部第一侧的颧面神经区和/或2个单位剂量至面部第二侧的颧面神经区;

[0797] (vi)4个单位剂量至面部第一侧的耳颞神经区和/或4个单位剂量至面部第二侧的耳颞神经区;

[0798] (vii)4个单位剂量至颈部第一侧枕大神经区和/或4个单位剂量至颈部第二侧枕大神经区;和/或

[0799] (vii)2个单位剂量至颈部第一侧的枕小神经区和/或2个单位剂量至颈部第二侧的枕小神经区。

[0800] 优选地,头痛疼痛(例如偏头痛疼痛)或偏头痛治疗可包括施用:

[0801] (i)2个单位剂量至面部第一侧的眶上神经区和2个单位剂量至面部第二侧的眶上神经区;

[0802] (ii)2个单位剂量至面部第一侧的滑车上神经区和2个单位剂量至面部第二侧的滑车上神经区;

[0803] (iii)2个单位剂量至面部第一侧的滑车内神经区和2个单位剂量至面部第二侧的滑车内神经区;

[0804] (iv)2个单位剂量至面部第一侧的颧颞神经区和2个单位剂量至面部第二侧颧颞神经区;

[0805] (v)2个单位剂量至面部第一侧的颧面神经区和2个单位剂量至面部第二侧的颧面神经区;

[0806] (vi)4个单位剂量至面部第一侧的耳颞神经区和4个单位剂量至面部第二侧的耳颞神经区;

[0807] (vii)4个单位剂量至颈部第一侧的枕大神经区和4个单位剂量至颈部第二侧枕大神经区;和/或

[0808] (vii)2个单位剂量至颈部第一侧的枕小神经区和2个单位剂量至颈部第二侧的枕小神经区。

[0809] 更优选地,头痛疼痛(例如偏头痛疼痛)或偏头痛治疗可包括施用:

[0810] (i)2个单位剂量至面部第一侧的眶上神经区和2个单位剂量至面部第二侧的眶上神经区;

[0811] (ii)2个单位剂量至面部第一侧的滑车上神经区和2个单位剂量至面部第二侧的滑车上神经区;

[0812] (iii)2个单位剂量至面部第一侧的滑车内神经区和2个单位剂量至面部第二侧的滑车内神经区;

[0813] (iv)2个单位剂量至面部第一侧的颧颞神经区和2个单位剂量至面部第二侧颧颞神经区;

[0814] (v)2个单位剂量至面部第一侧的颧面神经区和2个单位剂量至面部第二侧的颧面神经区;

[0815] (vi)4个单位剂量至面部第一侧的耳颞神经区和4个单位剂量至面部第二侧的耳

颞神经区;

[0816] (vii)4个单位剂量至颈部第一侧的枕大神经区和4个单位剂量至颈部第二侧的枕大神经区;和

[0817] (vii)2个单位剂量至颈部第一侧的枕小神经区和2个单位剂量至颈部第二侧的枕小神经区。

[0818] 当如前述实施方案中所述治疗头痛疼痛(例如偏头痛疼痛)或偏头痛时,优选每个注射部位施用一个单位剂量。因此,所述治疗可包括施用嵌合梭菌神经毒素至:

[0819] (i)面部第一侧的眶上神经区的1个注射部位和/或面部第二侧的眶上神经区的1个注射部位;

[0820] (ii)面部第一侧的滑车上神经区的1个注射部位和/或面部第二侧的滑车上神经区的1个注射部位;

[0821] (iii)面部第一侧的滑车内神经区的1个注射部位和/或面部第二侧的滑车内神经区的1个注射部位;

[0822] (iv)面部第一侧的颧颞神经区的1个注射部位和/或面部第二侧的颧颞神经区的1个注射部位;

[0823] (v)面部第一侧的颧面神经区的1个注射部位和/或面部第二侧的颧面神经区的1个注射部位;

[0824] (vi)面部第一侧的耳颞神经区的2个注射部位和/或面部第二侧的耳颞神经区的2个注射部位;

[0825] (vii)颈部第一侧的枕大神经区的2个注射部位和/或颈部第二侧的枕大神经区的2个注射部位;和/或

[0826] (vii)颈部第一侧的枕小神经区的1个注射部位和/或颈部第二侧的枕小神经区的1个注射部位。

[0827] 优选地,治疗可包括施用嵌合梭菌神经毒素至:

[0828] (i)面部第一侧的眶上神经区的1个注射部位和面部第二侧的眶上神经区的1个注射部位;

[0829] (ii)面部第一侧的滑车上神经区的1个注射部位和面部第二侧的滑车上神经区的1个注射部位;

[0830] (iii)面部第一侧的滑车内神经区的1个注射部位和面部第二侧的滑车内神经区的1个注射部位;

[0831] (iv)面部第一侧的颧颞神经区的1个注射部位和面部第二侧的颧颞神经区的1个注射部位;

[0832] (v)面部第一侧的颧面神经区的1个注射部位和面部第二侧的颧面神经区的1个注射部位;

[0833] (vi)面部第一侧的耳颞神经区的2个注射部位和面部第二侧的耳颞神经区的2个注射部位;

[0834] (vii)颈部第一侧的枕大神经区的2个注射部位和颈部第二侧的枕大神经区的2个注射部位;和/或

[0835] (vii)颈部第一侧的枕小神经区的1个注射部位和颈部第二侧的枕小神经区的1个

注射部位。

[0836] 更优选地,治疗可包括施用嵌合梭菌神经毒素至:

[0837] (i)面部第一侧的眶上神经区的1个注射部位和面部第二侧的眶上神经区的1个注射部位;

[0838] (ii)面部第一侧的滑车上神经区的1个注射部位和面部第二侧的滑车上神经区的1个注射部位;

[0839] (iii)面部第一侧的滑车内神经区的1个注射部位和面部第二侧的滑车内神经区的1个注射部位;

[0840] (iv)面部第一侧的颧颞神经区的1个注射部位和面部第二侧的颧颞神经区的1个注射部位;

[0841] (v)面部第一侧的颧面神经区的1个注射部位和面部第二侧的颧面神经区的1个注射部位;

[0842] (vi)面部第一侧的耳颞神经区的2个注射部位和面部第二侧的耳颞神经区的2个注射部位;

[0843] (vii)颈部第一侧的枕大神经区的2个注射部位和颈部第二侧的枕大神经区的2个注射部位;和

[0844] (vii)颈部第一侧的枕小神经区的1个注射部位和颈部第二侧的枕小神经区的1个注射部位。

[0845] 当如前述实施方案中所述治疗头痛疼痛(例如偏头痛疼痛)或偏头痛时,优选每个注射部位施用一个单位剂量。因此,所述治疗可包括施用嵌合梭菌神经毒素至:

[0846] (i)面部第一侧的眶上神经区的2个注射部位和/或面部第二侧的眶上神经区的2个注射部位;

[0847] (ii)面部第一侧的滑车上神经区的2个注射部位和/或面部第二侧的滑车上神经区的2个注射部位;

[0848] (iii)面部第一侧的滑车内神经区的2个注射部位和/或面部第二侧的滑车内神经区的2个注射部位;

[0849] (iv)面部第一侧的颧颞神经区的2个注射部位和/或面部第二侧的颧颞神经区的2个注射部位;

[0850] (v)面部第一侧的颧面神经区的2个注射部位和/或面部第二侧的颧面神经区的2个注射部位;

[0851] (vi)面部第一侧的耳颞神经区的4个注射部位和/或面部第二侧的耳颞神经区的4个注射部位;

[0852] (vii)颈部第一侧的枕大神经区的4个注射部位和/或颈部第二侧的枕大神经区的4个注射部位;和/或

[0853] (vii)颈部第一侧的枕小神经区的2个注射部位和/或颈部第二侧的枕小神经区的2个注射部位。

[0854] 优选地,治疗可包括施用嵌合梭菌神经毒素至:

[0855] (i)面部第一侧的眶上神经区的2个注射部位和面部第二侧的眶上神经区的2个注射部位;

[0856] (ii)面部第一侧的滑车上神经区的2个注射部位和面部第二侧的滑车上神经区的2个注射部位;

[0857] (iii)面部第一侧的滑车内神经区的2个注射部位和面部第二侧的滑车内神经区的2个注射部位;

[0858] (iv)面部第一侧的颧颞神经区的2个注射部位和面部第二侧的颧颞神经区的2个注射部位;

[0859] (v)面部第一侧的颧面神经区的2个注射部位和面部第二侧的颧面神经区的2个注射部位;

[0860] (vi)面部第一侧的耳颞神经区的4个注射部位和面部第二侧的耳颞神经区的4个注射部位;

[0861] (vii)颈部第一侧的枕大神经区的4个注射部位和颈部第二侧的枕大神经区的4个注射部位;和/或

[0862] (vii)颈部第一侧的枕小神经区的2个注射部位和颈部第二侧的枕小神经区的2个注射部位。

[0863] 更优选地,治疗可包括施用嵌合梭菌神经毒素至:

[0864] (i)面部第一侧的眶上神经区的2个注射部位和面部第二侧的眶上神经区的2个注射部位;

[0865] (ii)面部第一侧的滑车上神经区的2个注射部位和面部第二侧的滑车上神经区的2个注射部位;

[0866] (iii)面部第一侧的滑车内神经区的2个注射部位和面部第二侧的滑车内神经区的2个注射部位;

[0867] (iv)面部第一侧的颧颞神经区的2个注射部位和面部第二侧的颧颞神经区的2个注射部位;

[0868] (v)面部第一侧的颧面神经区的2个注射部位和面部第二侧的颧面神经区的2个注射部位;

[0869] (vi)面部第一侧的耳颞神经区的4个注射部位和面部第二侧的耳颞神经区的4个注射部位;

[0870] (vii)颈部第一侧的枕大神经区的4个注射部位和颈部第二侧的枕大神经区的4个注射部位;和

[0871] (vii)颈部第一侧的枕小神经区的2个注射部位和颈部第二侧的枕小神经区的2个注射部位。

[0872] 当如前述实施方案中所述治疗头痛疼痛(例如偏头痛)或偏头痛时,优选每个注射部位施用多于一个单位剂量(优选2个单位剂量)。因此,所述治疗可包括施用嵌合梭菌神经毒素至:

[0873] (i)面部第一侧的眶上神经区的1个注射部位和/或面部第二侧的眶上神经区的1个注射部位;

[0874] (ii)面部第一侧的滑车上神经区的1个注射部位和/或面部第二侧的滑车上神经区的1个注射部位;

[0875] (iii)面部第一侧的滑车内神经区的1个注射部位和/或面部第二侧的滑车内神经

区的1个注射部位;

[0876] (iv)面部第一侧的颧颞神经区的1个注射部位和/或面部第二侧的颧颞神经区的1个注射部位;

[0877] (v)面部第一侧的颧面神经区的1个注射部位和/或面部第二侧的颧面神经区的1个注射部位;

[0878] (vi)面部第一侧的耳颞神经区的2个注射部位和/或面部第二侧的耳颞神经区的2个注射部位;

[0879] (vii)颈部第一侧的枕大神经区的2个注射部位和/或颈部第二侧的枕大神经区的2个注射部位;和/或

[0880] (vii)颈部第一侧的枕小神经区的1个注射部位和/或颈部第二侧的枕小神经区的1个注射部位。

[0881] 优选地,治疗可包括施用嵌合梭菌神经毒素至:

[0882] (i)面部第一侧的眶上神经区的1个注射部位和面部第二侧的眶上神经区的1个注射部位;

[0883] (ii)面部第一侧的滑车上神经区的1个注射部位和面部第二侧的滑车上神经区的1个注射部位;

[0884] (iii)面部第一侧的滑车内神经区的1个注射部位和面部第二侧的滑车内神经区的1个注射部位;

[0885] (iv)面部第一侧的颧颞神经区的1个注射部位和面部第二侧的颧颞神经区的1个注射部位;

[0886] (v)面部第一侧的颧面神经区的1个注射部位和面部第二侧的颧面神经区的1个注射部位;

[0887] (vi)面部第一侧的耳颞神经区的2个注射部位和面部第二侧的耳颞神经区的2个注射部位;

[0888] (vii)颈部第一侧的枕大神经区的2个注射部位和颈部第二侧的枕大神经区的2个注射部位;和/或

[0889] (vii)颈部第一侧的枕小神经区的1个注射部位和颈部第二侧的枕小神经区的1个注射部位。

[0890] 更优选地,治疗可包括施用嵌合梭菌神经毒素至:

[0891] (i)面部第一侧的眶上神经区的1个注射部位和面部第二侧的眶上神经区的1个注射部位;

[0892] (ii)面部第一侧的滑车上神经区的1个注射部位和面部第二侧的滑车上神经区的1个注射部位;

[0893] (iii)面部第一侧的滑车内神经区的1个注射部位和面部第二侧的滑车内神经区的1个注射部位;

[0894] (iv)面部第一侧的颧颞神经区的1个注射部位和面部第二侧的颧颞神经区的1个注射部位;

[0895] (v)面部第一侧的颧面神经区的1个注射部位和面部第二侧的颧面神经区的1个注射部位;

[0896] (vi)面部第一侧的耳颞神经区的2个注射部位和面部第二侧的耳颞神经区的2个注射部位;

[0897] (vii)颈部第一侧的枕大神经区的2个注射部位和颈部第二侧的枕大神经区的2个注射部位;和

[0898] (vii)颈部第一侧的枕小神经区的1个注射部位和颈部第二侧的枕小神经区的1个注射部位。

[0899] 因此,当治疗头痛疼痛(例如偏头痛疼痛)或偏头痛时,可以施用1-50、5-45或10-38个单位剂量。优选施用最多35个单位剂量。优选地,每次治疗期间施用的总剂量可以是最多192,500pg的嵌合梭菌神经毒素。例如,施用的总剂量可以是最多180,000pg,优选最多177,000pg(更优选最多175,000pg)。最优选地,施用的总剂量可以是最多115,000pg或75,000pg,例如最多112,000pg或70,000pg。

[0900] 因此,当通过肌内注射治疗头痛疼痛(例如偏头痛疼痛)或偏头痛时,可以施用1-50、5-45或10-38个单位剂量。优选施用最多35个单位剂量。优选地,每次治疗期间施用的总剂量可以是最多192,500pg的嵌合梭菌神经毒素。例如,施用的总剂量可以是最多180,000pg,优选最多177,000pg(更优选最多175,000pg)。最优选地,施用的总剂量可以是最多115,000pg或75,000pg,例如最多112,000pg或70,000pg。

[0901] 因此,当通过皮内注射治疗头痛疼痛(例如偏头痛疼痛)或偏头痛时,可以施用1-35、5-25或10-20个单位剂量。优选施用最多20个单位剂量。优选地,每次治疗期间施用的总剂量可以是最多110,000pg的嵌合梭菌神经毒素。例如,施用的总剂量可以是最多105,000pg,优选最多102,000pg(更优选最多100,000pg)。

[0902] 当通过皮内注射治疗头痛疼痛(例如偏头痛疼痛)或偏头痛时,可以施用2-70、10-50或20-40个单位剂量。优选施用最多40个单位剂量。优选地,每次治疗期间施用的总剂量可以是最多220,000pg的嵌合梭菌神经毒素。例如,施用的总剂量可以是最多210,000pg,优选最多204,000pg(更优选最多200,000pg)。

[0903] 在一些实施方案中,可以通过肌内注射和皮内注射的混合进行头痛疼痛(例如偏头痛疼痛)或偏头痛的治疗。例如,可以将本发明的嵌合梭菌神经毒素皮内施用至受试者的颈部,并可以将本发明的嵌合梭菌神经毒素肌内施用至受试者的面部。优选地,可以将本发明的嵌合梭菌神经毒素皮内施用至受试者的面部,并可以将本发明的嵌合梭菌神经毒素肌内施用至受试者的颈部。例如除了施用于颈部和/或面部之外,嵌合梭菌神经毒素还可以施用于受试者的头部。

[0904] 当通过皮内注射或通过肌内注射治疗头痛疼痛(例如偏头痛疼痛)或偏头痛时,优选的单位剂量可以是1,000pg至5,500pg。单位剂量范围的上限可以是5,250、5,200、5,100、5,000、4,500、4,000、3,500、3,000、2,500或2,000pg的嵌合梭菌神经毒素,优选上限是5,100pg,更优选5,000pg。单位剂量范围的下限可以是1,100、1,200、1,250、1,300、1,350、1,400或1,450、1,500、2,000、2,500、3,000、3,500或4,000pg的嵌合梭菌神经毒素,优选下限是1,400pg,更优选1,500pg。所述范围的下限可以大于3,000pg。单位剂量可以是1,400pg至5,100pg(例如1,500pg至5,000pg)、2,000pg至5,100pg、3,000至5,100pg或3,000至4,000pg的嵌合梭菌神经毒素。单位剂量可包含大于3,000pg至最多5,500pg的嵌合梭菌神经毒素。嵌合梭菌神经毒素的单位剂量可以是2,000pg至4,500pg、2,000pg至3,000pg(例如2,

500pg)或3,500至4,500pg的嵌合梭菌神经毒素。优选地,单位剂量包含4,000pg的嵌合梭菌神经毒素。

[0905] 当通过皮内注射或通过肌内注射治疗头痛疼痛(例如偏头痛疼痛)或偏头痛时,优选的单位剂量可以是42单位至229单位。单位剂量范围的上限可以是225、220、215、210、205、200、190、180、170、160、150、125、100或83单位的嵌合梭菌神经毒素,优选上限是212单位,更优选208单位。单位剂量范围的下限可以是46、50、55、60、65、70、75、80或90、100、110、120、130、140、150、160或166单位的嵌合梭菌神经毒素,优选下限是58单位,更优选62单位。所述范围的下限可以大于125个单位。单位剂量可以是58单位至212单位(例如62单位至208单位)、83单位至212单位、125至212单位或125至166单位的嵌合梭菌神经毒素。单位剂量可包含大于125单位至最多229单位的嵌合梭菌神经毒素。嵌合梭菌神经毒素的单位剂量可以是83单位至188单位、83单位至125单位(例如104单位)或146单位至188单位的嵌合梭菌神经毒素。优选地,单位剂量包含166单位的嵌合梭菌神经毒素。单位剂量可包含47单位至258单位的嵌合梭菌神经毒素,例如94单位至211单位,94单位至141单位(例如117单位)或164至211单位的嵌合梭菌神经毒素。单位剂型可包含188单位的嵌合梭菌神经毒素。

[0906] 当通过肌内注射或皮内注射(优选肌内注射)治疗头痛疼痛(例如偏头痛疼痛)或偏头痛时,优选的单位剂量可以是2,500pg,并且总剂量可以是至多70,000pg。例如,优选的单位剂量可以是2,500pg,并且总剂量可以是70,000pg。优选的单位剂量可以是4,000pg,并且总剂量可以是至多112,000pg。例如,优选的单位剂量可以是4,000pg,并且总剂量可以是112,000pg。合适的单位剂量可以是5,000pg,并且总剂量可以是至多155,000pg。例如,合适的单位剂量可以是5,000pg,并且总剂量可以是155,000pg。

[0907] 当通过肌内注射或皮内注射(优选肌内注射)治疗头痛疼痛(例如偏头痛疼痛)或偏头痛时,优选的单位剂量可以是104单位,并且总剂量可以是至多2,912单位。例如,优选的单位剂量可以是104单位,并且总剂量可以是2,912单位。优选的单位剂量可以是166单位,并且总剂量可以至多是4,659单位。例如,优选的单位剂量可以是166单位,并且总剂量可以是4,659单位。合适的单位剂量可以是208单位,并且总剂量可以是至多6,448单位。例如,合适的单位剂量可以是208单位,并且总剂量可以是6,448单位。

[0908] 当通过肌内注射或皮内注射(优选肌内注射)治疗头痛疼痛(例如偏头痛疼痛)或偏头痛时,优选的单位剂量可以是117单位,并且总剂量可以是至多3,286单位。例如,优选的单位剂量可以是117单位,并且总剂量可以是3,286单位。优选的单位剂量可以是188单位,并且总剂量可以至多是5,258单位。例如,优选的单位剂量可以是188单位,并且总剂量可以是5,258单位。合适的单位剂量可以是235单位,并且总剂量可以是至多7,277单位。例如,合适的单位剂量可以是235单位,并且总剂量可以是7,277单位。

[0909] 当通过肌内注射治疗头痛疼痛(例如偏头痛疼痛)或偏头痛时,每次治疗期间施用的总剂量可以至多是8,007单位的嵌合梭菌神经毒素。例如,施用的总剂量可以是至多7,488单位,或至多7,363单位(例如至多7,280单位)。最优选地,施用的总剂量可以是至多4,784单位或3,120单位,例如至多4,659单位或2,912单位。每次治疗期间施用的总剂量可以是至多9,037单位的嵌合梭菌神经毒素。例如,施用的总剂量可以是至多8,451单位,或至多8,310单位(例如至多8,216单位)。最优选地,施用的总剂量可以是至多5,399单位或3,521单位,例如至多5,258单位或3,286单位。

[0910] 当通过皮内注射治疗头痛疼痛(例如偏头痛疼痛)或偏头痛时,每次治疗期间施用的总剂量可以至多是4,576单位的嵌合梭菌神经毒素。例如,施用的总剂量可以是至多4,368单位,或至多4,243单位(例如至多4,160单位)。每次治疗期间施用的总剂量可以是至多5,165单位的嵌合梭菌神经毒素。例如,施用的总剂量可以是至多4,929单位,或至多4,789单位(例如至多4,695单位)。每次治疗期间施用的总剂量可以是至多5,165单位的嵌合梭菌神经毒素。例如,施用的总剂量可以是至多4,929单位,或至多4,789单位(例如至多4,695单位)。

[0911] 在优选的实施方案中,当用嵌合梭菌神经毒素治疗疼痛(例如头痛或偏头痛疼痛)或偏头痛时,该治疗不诱导肌肉麻痹。例如,在一些实施方案中,嵌合梭菌神经毒素的单位剂量可以低于诱导肌肉麻痹所需的嵌合梭菌神经毒素的单位剂量。特别地,在一些实施方案中,在特定部位(例如注射部位)施用的嵌合梭菌神经毒素的单位剂量可以低于(例如在该部位和/或肌肉)诱导肌肉麻痹所需的嵌合梭菌神经毒素的单位剂量。

[0912] 上述头痛疼痛优选为偏头痛疼痛。所述偏头痛可以是阵发性偏头痛疼痛或慢性偏头痛疼痛,例如由阵发性偏头痛引起或与之相关的疼痛,或由慢性偏头痛引起或与之相关的疼痛。

[0913] 本发明的嵌合梭菌神经毒素优选作为治疗方案的一部分(优选在不同日期,例如连续治疗之间至少间隔1天)迭代施用(例如至多5、10、15或20次)。迭代施用(iterative administration)是指施用至少两次,例如至少5、10、15或20次。因此,在一个实施方案中,本发明的嵌合梭菌神经毒素可以施用两次或多次以治疗受试者的病症(优选疼痛)。这对于慢性病况的治疗尤其重要,例如慢性疼痛,通常需要持续治疗。在一个实施方案中,本发明的嵌合梭菌神经毒素可以每周施用,每月施用两次,每月施用,每两个月施用,每六个月施用或每年施用,优选至少每年施用两次或每年施用。在一个实施方案中,本发明的嵌合梭菌神经毒素在10年、5年、2年或1年的时间内施用两次或更多次。优选地,本发明的嵌合梭菌神经毒素在1年内施用两次或多次。治疗可持续至少6个月、1年、2年、3年、5年、10年、15年、20年、25年或30年。

[0914] 在一些实施方案中,在根据本发明的嵌合梭菌神经毒素的第一次施用(例如,第一治疗期)之后,受试者可以接受嵌合梭菌神经毒素的第二次施用(例如,第二治疗期)。第一次施用和第二次施用之间的时间间隔可以是至少5、6、7、8、9或10个月。例如,第一次施用和第二次施用之间的时间间隔可以是5-10个月、5-9个月、5-8个月、6-10个月、6-9个月或6-8个月。

[0915] 优选嵌合梭菌神经毒素不与除了轻链和重链之外的其他治疗剂或诊断剂(例如核酸、蛋白质、肽或小分子治疗剂或诊断剂)一起施用。例如,在一个实施方案中,嵌合梭菌神经毒素不与其他镇痛剂一起施用。在一个实施方案中,本发明的嵌合梭菌神经毒素不与共价结合的治疗剂一起施用。在一个实施方案中,本发明的嵌合梭菌神经毒素不与非共价结合的治疗剂一起施用。

[0916] 嵌合梭菌神经毒素优选用于治疗疼痛,并且可以用于治疗患有一种或多种类型疼痛的受试者。

[0917] 本文所用的术语“疼痛”是指与实际或潜在的组织损伤相关或类似的任何不愉快的感觉和情绪体验。任何相关的身体病症对于临床医生可能是或可能不是明显的。

[0918] 疼痛可能与介质(例如神经递质)从神经元的释放有关。神经元优选是本发明的嵌合梭菌神经毒素结合的神经元。例如,介质可以是与疼痛传递相关的任何介质。介质可以是神经肽如P物质、CGRP或血管活性肠肽(VIP)。

[0919] 介质可以是炎性介质或非炎性介质。介质可以是以下的一种或多种:CGRP、神经激肽(例如速激肽、P物质、神经激肽A、神经激肽B、血红素激肽(hemokinin)和/或endokinin)、促肾上腺皮质激素(ACTH)、糖皮质激素、血管加压素、催产素、儿茶酚胺、阿片类物质(例如阿片肽和/或脑阿片类药物)、血管紧张素II、内啡肽、脑啡肽、血管活性肠肽(VIP)、类二十烷酸(例如前列腺素如前列腺素E2(PGE2)和/或白三烯)、组织激肽原(例如缓激肽))、组胺、血清素、钾、前列环素(PGI2)、白三烯B4(LTB4)、神经生长因子(NGF)、质子、ATP、腺苷、5-羟色胺(5-HT)、组胺、谷氨酸、去甲肾上腺素(NE)、一氧化氮(NO)、γ-氨基丁酸(GABA)、甘氨酸、乙酰胆碱、大麻素、组织坏死因子α(TNF-α)、细胞因子(例如白细胞介素(IL)-6、IL-1和/或IL-8)、血小板激活因子(PAF)、神经营养生长因子(NGF)、谷氨酸、天冬氨酸、垂体腺苷酸环化酶激活肽(PACAP)和蛋白水解酶。

[0920] 介质可以是降钙素基因相关肽(CGRP)、胰淀素、垂体腺苷酸环化酶激活肽(PACAP)、催产素、神经肽Y(NPY)、P物质、血管紧张素、促肾上腺皮质激素释放激素(CRH)、瘦素、脂联素、食欲素和/或黑色素浓缩激素(MCH)。

[0921] 介质可以是选自以下的一种或多种:CGRP、P物质和谷氨酸。

[0922] 介质可以是以下的一种或多种:神经肽(例如P物质、CGRP或VIP)、一氧化氮、谷氨酸和天冬氨酸。

[0923] 当疼痛是头痛疼痛(优选偏头痛疼痛)时,介质可以是以下的一种或多种:CGRP、VIP、PACAP和促炎细胞因子(例如IL-6、IL-8和/或TNF-α)。

[0924] 优选地,介质可以是CGRP、P物质和/或替代的神经激肽。

[0925] 最优选地,介质是CGRP。CGRP可以是α-CGRP或β-CGRP,优选α-CGRP。

[0926] 疼痛可与疼痛介质(例如疼痛神经递质)从包含Aδ神经纤维或C神经纤维的神经元的释放有关。疼痛介质可以是从包含Aδ神经纤维或C神经纤维的神经元释放/分泌的任何疼痛介质。疼痛介质可以是神经肽,例如P物质、CGRP或血管活性肠肽(VIP)。

[0927] 疼痛介质可以是炎性介质或非炎性介质。疼痛介质可以是以下的一种或多种:CGRP、神经激肽(例如速激肽、P物质、神经激肽A、神经激肽B、血红素激肽(hemokinin)和/或endokinin)、促肾上腺皮质激素(ACTH)、糖皮质激素、血管加压素、催产素、儿茶酚胺、阿片类物质(例如阿片肽和/或脑阿片类药物)、血管紧张素II、内啡肽、脑啡肽、血管活性肠肽(VIP)、类二十烷酸(例如前列腺素如前列腺素E2(PGE2)和/或白三烯)、组织激肽原(例如缓激肽))、组胺、血清素、钾、前列环素(PGI2)、白三烯B4(LTB4)、神经生长因子(NGF)、质子、ATP、腺苷、5-羟色胺(5-HT)、组胺、谷氨酸、去甲肾上腺素(NE)、一氧化氮(NO)、γ-氨基丁酸(GABA)、甘氨酸、乙酰胆碱、大麻素、组织坏死因子α(TNF-α)、细胞因子(例如白细胞介素(IL)-6、IL-1和/或IL-8)、血小板激活因子(PAF)、神经营养生长因子(NGF)、谷氨酸、天冬氨酸、垂体腺苷酸环化酶激活肽(PACAP)和蛋白水解酶。

[0928] 疼痛介质可以是降钙素基因相关肽(CGRP)、胰淀素、垂体腺苷酸环化酶激活肽(PACAP)、催产素、神经肽Y(NPY)、P物质、血管紧张素、促肾上腺皮质激素释放激素(CRH)、瘦素、脂联素、食欲素和/或黑色素浓缩激素(MCH)。

[0929] 从包含Aδ神经纤维的神经元释放的疼痛介质可以是选自以下的一种或多种:CGRP、P物质和谷氨酸。

[0930] 从包含C神经纤维的神经元释放的疼痛介质可以是以下的一种或多种:神经肽(例如P物质、CGRP或VIP)、一氧化氮、谷氨酸和天冬氨酸。

[0931] 谷氨酸可以与慢性疼痛和/或神经性疼痛的引发有关。

[0932] 当疼痛是头痛疼痛(优选偏头痛疼痛)时,疼痛介质可以是以下的一种或多种:CGRP、VIP、PACAP和促炎细胞因子(例如IL-6、IL-8和/或TNF-α)。

[0933] 优选地,当神经元包含Aδ神经纤维或C神经纤维时,疼痛介质可以是CGRP,优选地,当神经元包含C纤维时,疼痛介质是CGRP。当神经元包含C纤维时,疼痛介质可以是P物质和/或替代的神经激肽。

[0934] 最优选地,疼痛介质是CGRP。CGRP可以是α-CGRP或β-CGRP,优选α-CGRP。

[0935] 因此,本发明的嵌合梭菌神经毒素可以抑制CGRP从包含Aδ神经纤维或C神经纤维的感觉神经元释放。

[0936] 当疼痛介质是CGRP时,疼痛可以是CGRP相关的疼痛。

[0937] 本文所用的术语"CGRP相关的疼痛"是指与CGRP从神经元释放及其任何作用相关的疼痛。CGRP诱导的疼痛可以是CGRP依赖性疼痛。在一个实施方案中,CGRP相关的疼痛是由神经元的CGRP释放及其任何作用诱导的CGRP诱导的疼痛。

[0938] CGRP相关疼痛的实例包括偏头痛和瘙痒。

[0939] 在一个实施方案中,本发明的治疗用途或方法不包括治疗与除CGRP以外的任何疼痛介质相关的疼痛。本发明的治疗用途或方法可以排除治疗与以下一种或多种相关的疼痛:神经激肽(例如速激肽、P物质、神经激肽A、神经激肽B、血红素激肽(hemokinin)和/或endokinin)、促肾上腺皮质激素(ACTH)、糖皮质激素、血管加压素、催产素、儿茶酚胺、阿片类物质(例如阿片肽和/或脑阿片类药物)、血管紧张素II、内啡肽、脑啡肽、血管活性肠肽(VIP)、类二十烷酸(例如前列腺素如前列腺素E2(PGE2)和/或白三烯)、组织激肽原(例如缓激肽))、组胺、血清素、钾、前列环素(PGI2)、白三烯B4(LTB4)、神经生长因子(NGF)、质子、ATP、腺苷、5-羟色胺(5-HT)、组胺、谷氨酸、去甲肾上腺素(NE)、一氧化氮(NO)、γ-氨基丁酸(GABA)、甘氨酸、乙酰胆碱、大麻素、组织坏死因子α(TNF-α)、细胞因子(例如白细胞介素(IL)-6、IL-1和/或IL-8)、血小板激活因子(PAF)、神经营养生长因子(NGF)、谷氨酸、天冬氨酸、垂体腺苷酸环化酶激活肽(PACAP)和蛋白水解酶。

[0940] 疼痛可以是慢性的或急性的。"急性疼痛"是突然发作的持续时间较短的疼痛。例如,一种类型的急性疼痛是在皮肤或其他浅表组织损伤时感觉到的皮肤疼痛,例如由割伤或烧伤引起的。皮肤伤害性感受器终止于皮肤正下方,并且由于神经末梢高度集中,产生持续时间较短的明确的局部疼痛。"慢性疼痛"是除急性疼痛以外的疼痛。

[0941] 因此,疼痛可以是慢性或急性疼痛。疼痛可以是选自以下四类疼痛中的一种或多种:伤害性疼痛;神经性疼痛;混合性疼痛;以及未知来源的疼痛。伤害性疼痛可以由对伤害性感受器(疼痛受体)的已知的有害刺激引起,并且可以是躯体的或内脏的疼痛。神经性疼痛可以是由神经系统中的原发性损害或功能障碍引发或引起的疼痛。混合性疼痛可以是伤害性疼痛和神经性疼痛的组合。

[0942] 疼痛(例如慢性疼痛)可以是选自以下的一种或多种:神经性疼痛、炎性疼痛、头痛

疼痛、躯体疼痛、内脏疼痛、牵涉性疼痛、异常性疼痛、混合性疼痛和术后疼痛。然而,优选地,疼痛不是术后疼痛。

[0943] 在一个实施方案中,疼痛不是内脏疼痛。在一个实施方案中,病症不是内脏疼痛病症。

[0944] 躯体疼痛可以是选自以下的一种或多种:头痛疼痛(例如创伤后头痛、头部受伤头痛或创伤性脑损伤后头痛)、关节炎疼痛(例如骨关节炎疼痛和/或类风湿性关节炎疼痛)、运动疼痛、退行性椎间盘疾病疼痛、腕管压迫疼痛、软组织损伤疼痛、颞下颌关节疼痛、肌肉骨骼疼痛、由血管病症(例如雷诺氏综合征、血栓闭塞性脉管炎、外周静脉疾病、外周动脉疾病、静脉曲张、静脉血栓、凝血障碍或淋巴水肿)引起或与之相关的躯体疼痛、面部疼痛、由三叉神经自主神经性头痛引起或与之相关的躯体疼痛;由三叉神经痛引起或与之相关的躯体疼痛;和骨痛(例如癌症引起的骨痛,例如CGRP相关的癌症引起的骨痛)。

[0945] 疼痛优选为头痛疼痛。更优选地,疼痛是偏头痛疼痛。

[0946] 内脏疼痛可以是选自以下的一种或多种:子宫内膜异位症疼痛、胰腺炎疼痛、胃肠道疼痛和由血管病症引起或与之相关的内脏疼痛。

[0947] 炎性疼痛可以是选自慢性疼痛、伤口愈合疼痛、瘙痒疼痛和烧伤疼痛中的一种或多种。

[0948] 神经性疼痛可以是选自以下的一种或多种:带状疱疹后神经痛、糖尿病疼痛、慢性神经性疼痛和莫顿神经瘤疼痛。

[0949] 下面更详细地描述了可以用本发明的嵌合梭菌神经毒素治疗的疼痛和病况。

[0950] 本发明的嵌合梭菌神经毒素可用于治疗由任何下列神经性疼痛病况引起的或与之相关的疼痛。“神经性疼痛”是指来自外周神经系统、中枢神经系统或两者的异常感觉输入,导致不适。神经性疼痛的症状可包括持续性、自发性疼痛,以及异常性疼痛(对通常不是疼痛的刺激的疼痛反应)、痛觉过敏(对通常仅引起轻度不适的疼痛刺激如针刺的强烈反应)或痛觉过度(其中短暂的不适变成长期的严重疼痛)。神经性疼痛可由以下任一者引起:

[0951] 1.创伤性损伤,例如神经压迫损伤(例如神经挤压、神经拉伸、神经卡压或不完全神经横断);脊髓损伤(例如脊髓半切);肢体截肢;挫伤;炎症(例如脊髓炎症);或外科手术。

[0952] 2.缺血事件,包括例如中风和心脏病发作。

[0953] 3.感染性因子。

[0954] 4.接触有毒物质,包括例如药物、酒精、重金属(例如铅、砷、汞)、工业制剂(例如溶剂、胶水烟雾)或一氧化二氮。

[0955] 5.疾病,包括例如炎性病症、赘生性肿瘤、获得性免疫缺陷综合征(AIDS)、莱姆病、麻风病、代谢性疾病、外周神经病症如神经瘤、单发神经病或多发神经病。

[0956] 神经性疼痛的类型包括以下:

[0957] 1.神经痛。

[0958] 神经痛是一种沿着一个或多个特定神经的路线放射的疼痛,通常在神经结构中没有任何明显的病理变化。神经痛的原因多种多样。化学刺激、炎症、创伤(包括手术)、附近结构(例如肿瘤)的压迫和感染都可导致神经痛。然而,在许多情况下,原因是未知的或不可识别的。神经痛在老年人中是最常见的,但任何年龄都可能发生。神经痛包括但不限于三叉神经痛、带状疱疹后神经痛、带状疱疹后神经痛、舌咽神经痛、坐骨神经痛和非典型面部疼痛。

[0959] 神经痛是指一种或多种神经分布区域的疼痛。实例是三叉神经痛、非典型面部疼痛和带状疱疹后神经痛(由带状疱疹或疱疹引起)。受影响的神经负责感测从颌到前额的面部区域中的触摸、温度和压力。该病症通常引起剧烈疼痛的短暂发作,通常少于两分钟并且仅在面部的一侧。疼痛可以以多种方式描述,例如“刺痛”、“锐痛”、“类似闪电”、“灼烧”以及甚至“发痒”。在TN的非典型形式中,疼痛也可表现为严重的或仅仅是疼痛并持续较长时间。与TN相关的疼痛被认为是可经历的最剧烈疼痛之一。

[0960] 简单的刺激如吃饭、说话、洗脸或任何轻触或感觉都可以引发发作(甚至微风的感觉)。发作可以以群集的形式或以孤立的形式发生。

[0961] 症状包括任何部位的尖锐刺痛或持续灼痛,通常在身体表面或附近,每次发作都在同一位置;沿着特定神经路径的疼痛;由于疼痛而导致受影响的身体部位的功能受损,或由于伴随的运动神经损伤而导致肌肉无力;皮肤敏感性增加或受影响皮肤区域麻木(感觉类似于注射普鲁卡因等局部麻醉剂);以及任何触摸或压力都会被解释为疼痛。运动也可能会疼痛。

[0962] 三叉神经痛是最常见的神经痛形式。它影响面部的主要感觉神经,即三叉神经(“三叉神经”字面意思是“三个起源”,指神经分成3个分支)。这种情况涉及面部一侧突然、短暂的剧烈疼痛,沿着该侧三叉神经支配的区域。疼痛发作可能严重到足以引起面部表情,这通常被称为疼痛性抽动(痛性抽搐(tic douloureux))。有时,三叉神经痛的原因是血管或小肿瘤压迫神经。诸如多发性硬化症(一种影响脑部和脊髓的炎性疾病)、某些形式的关节炎、糖尿病(高血糖)等病症也可能导致三叉神经痛,但原因并不总是能确定。在这种情况下,诸如咀嚼、说话、吞咽或接触面部区域的某些运动可能会触发剧烈疼痛的痉挛。

[0963] 相关但相当不常见的神经痛影响舌-咽神经,该神经为咽喉提供感觉。这种神经痛的症状是位于咽喉处的短暂的休克样疼痛发作。

[0964] 带状疱疹等感染后可能会出现神经痛,带状疱疹是由水痘带状疱疹病毒(一种疱疹病毒)引起的。这种神经痛在带状疱疹已经愈合后产生持续的灼痛。疼痛因受影响区域的移动或接触而恶化。并非所有诊断为带状疱疹的患者都会经历带状疱疹后神经痛,这可能比带状疱疹更加疼痛。疼痛和敏感可持续数月或甚至数年。疼痛通常表现为对任何触摸(尤其是轻微触摸)难以忍受的敏感性。带状疱疹后神经痛不限制于面部;它可以发生在身体上的任何部位,但通常发生在带状疱疹的位置。由于患病期间的疼痛和社会孤立,抑郁症并不罕见。

[0965] 带状疱疹后神经痛在最初的疱疹感染症状消失后可能会使人虚弱。其他可能引起神经痛的感染性疾病是梅毒和莱姆病。

[0966] 糖尿病是神经痛的另一个常见原因。每20名美国成年人中几乎就有1个受这种非常普遍的医学问题的影响。糖尿病会损害为神经提供循环的微小动脉,导致神经纤维功能障碍,有时甚至导致神经丧失。糖尿病几乎可产生任何神经痛,包括三叉神经痛、腕管综合征(手和腕部的疼痛和麻木)和感觉异常性股痛症(由于股外侧皮肤神经损伤引起的大腿麻木和疼痛)。严格控制血糖可以预防糖尿病神经损伤,并可以加速患有神经痛的受试者的康复。

[0967] 可能与神经痛相关的其他医学病况是慢性肾功能不全和卟啉症——一种遗传性疾病,其中身体不能排除身体中血液正常分解后产生的某些物质。某些药物也可能引起这

种问题。

[0968] 2.传入神经阻滞。

[0969] 传入神经阻滞表示来自身体一部分的感觉输入的丧失,并且可能是由外周感觉纤维或来自中枢神经系统的神经的中断引起。传入神经阻滞疼痛综合征包括但不限于脑部或脊髓损伤、中风后疼痛、幻痛、截瘫、臂丛神经撕脱伤、腰椎神经根病。

[0970] 3.复杂性区域疼痛综合征(CRPS)

[0971] CRPS是由交感神经维持性疼痛引起的慢性疼痛综合征,并且以两种形式呈现。CRPS1目前代替术语“反射性交感神经营养不良综合征”。它是一种慢性神经病症,最常发生在轻微或严重受伤后的手臂或腿部。CRPS1与剧烈疼痛;指甲、骨骼和皮肤的变化;以及受影响肢体的触觉敏感性增加有关。CRPS2代替了术语灼痛(causalgia),是由于神经损伤所致。CRPS包括但不限于I型CRPS(反射性交感神经营养不良)和II型CRPS(灼痛)。

[0972] 4.神经病变(neuropathy)。

[0973] 神经病变是神经中的功能性或病理性改变,并且在临床上以感觉或运动神经元异常为特征。

[0974] 中枢神经病变是中枢神经系统的功能性或病理性改变。

[0975] 外周神经病变是一种或多种外周神经的功能性或病理性改变。外周神经将信息从中枢神经系统(脑部和脊髓)传递到肌肉和其他器官,并从皮肤、关节和其他器官传递回脑部。当这些神经无法在脑部和脊髓之间传递信息时,就会发生外周神经病变,从而导致疼痛、感觉丧失或无法控制肌肉。在某些情况下,控制血管、肠道和其他器官的神经衰竭会导致血压异常、消化问题和其他基本身体过程的丧失。神经病变的危险因素包括糖尿病、酗酒和接触某些化学品和药物。一些人具有神经病变的遗传性倾向。对神经的长时间压迫是发生神经损伤的另一个风险。压力性损伤可能是由于长时间不动(例如长时间的外科手术或长期患病)或石膏、夹板、支架、拐杖或其他装置压迫神经造成的。多发神经病变意味着一种广泛的过程,其通常同等地影响身体的两侧。症状取决于哪种类型的神经受到影响。三种主要类型的神经是感觉神经、运动神经和自主神经。神经病变可以影响所有三种类型的神经中的任何一种或其组合。症状还取决于该病况是影响全身还是仅影响一种神经(如来自损伤)。慢性炎性多发神经病变的原因是异常的免疫应答。特异性抗原、免疫过程和触发因子是可变的,并且在许多情况下是未知的。它可能与其他病况如HIV、炎性肠病、红斑狼疮、慢性活动性肝炎和血细胞异常有关。

[0976] 外周神经病变可涉及单个神经或神经群的功能性或病理性改变(单发神经病变)或影响多个神经的功能性或病理性改变(多发神经病变)。

[0977] 外周神经病变可包括以下:

[0978] 遗传性病症

[0979] 腓骨肌萎缩症(Charcot-Marie-Tooth disease)

[0980] 弗里德赖希共济失调(Friedreich’s ataxia)

[0981] 全身性或代谢性病症

[0982] 糖尿病(糖尿病性神经病变)

[0983] 膳食性缺乏(尤其是维生素B-12)

[0984] 过量饮酒(酒精性神经病变)

[0985] 尿毒症(肾衰竭)

[0986] 癌症

[0987] 感染性或炎性病况

[0988] AIDS

[0989] 肝炎

[0990] 科罗拉多蜱热

[0991] 白喉

[0992] 格林-巴利综合征

[0993] 未发展为AIDS的HIV感染

[0994] 麻风病

[0995] 莱姆病

[0996] 结节性多动脉炎

[0997] 类风湿性关节炎

[0998] 结节病

[0999] 干燥综合征

[1000] 梅毒

[1001] 系统性红斑狼疮

[1002] 淀粉样蛋白

[1003] 接触有毒化合物

[1004] 嗅胶或其他有毒化合物

[1005] 一氧化二氮

[1006] 工业试剂-尤其是溶剂

[1007] 重金属(铅、砷、汞等)

[1008] 镇痛性肾病等药物继发性神经病变

[1009] 其他原因

[1010] 缺血(氧气减少/血流减少)

[1011] 长期暴露于低温

[1012] a.多发神经病变

[1013] 多发神经病变是一种外周神经病变,其涉及由多个外周神经的损伤或破坏引起的区域运动或感觉的丧失。多发神经病变性疼痛包括但不限于脊髓灰质炎后综合征、乳房切除术后综合征、糖尿病性神经病变、酒精性神经病变、淀粉样蛋白、毒素、AIDS、甲状腺功能减退症、尿毒症、维生素缺乏、化疗引起的疼痛、2',3'-双脱氧胞苷(ddC)治疗、格林-巴利综合征或法布瑞氏症(Fabry's disease)。

[1014] b.单发神经病变

[1015] 单发神经病变是外周神经病变,其涉及由单个外周神经或神经群的损伤或破坏引起的区域的运动或感觉丧失。单发神经病变最通常是由损伤或创伤造成的局部区域损伤引起的,尽管偶尔全身性病症可引起孤立的神经损伤(如多发性单神经炎)。常见的原因是直接创伤、长期压迫神经以及因附近身体结构肿胀或损伤而压迫神经。损伤包括神经髓鞘(覆盖物)或部分神经细胞(轴突)的破坏。这种损伤会减慢或阻止神经冲动的传导。单发神经病

变可涉及身体的任何部分。单发神经病性疼痛包括但不限于坐骨神经功能障碍、腓总神经功能障碍、桡神经功能障碍、尺神经功能障碍、颅单发神经病变VI、颅单发神经病变VII、颅单发神经病变III(压迫型)、颅单发神经病变III(糖尿病型)、腋神经功能障碍、腕管综合征、股神经功能障碍、胫神经功能障碍、贝尔麻痹、胸廓出口综合征、腕管综合征和第六(外展)神经麻痹。

[1016] c.全身性外周神经病变

[1017] 全身性外周神经病变是对称的,通常是由于影响整个外周神经系统的各种系统性疾病和疾病过程所致。它们被进一步细分为几个类别:

[1018] i.远端轴突病变是神经元某些代谢或毒性紊乱的结果。它们可以由糖尿病等代谢性疾病、肾衰竭、营养不良等缺乏综合症和酗酒或毒素或药物的作用引起的。远端轴突病变(又名逆死性神经病变(dying back neuropathy))是一种外周神经病变,由外周神经系统(PNS)神经元的某些代谢或毒性紊乱引起。它是神经对代谢或毒性紊乱的最常见反应,并且因此可由糖尿病等代谢性疾病、肾衰竭、营养不良等缺乏综合症和酗酒或毒素或药物的作用引起。远端轴突病的最常见原因是糖尿病,且最常见的远端轴突病变是糖尿病性神经病变。

[1019] ii.髓鞘性神经病变(myelinopathy)是由于对髓鞘的原发性攻击引起的冲动传导的急性衰竭。最常见的原因是急性炎症性脱髓鞘性多发神经病变(AIDP;又名格林-巴利综合征),而其他原因包括慢性炎症性脱髓鞘综合征(CIDP)、遗传代谢性病症(例如脑白质营养不良)或毒素。髓鞘性神经病变是由于髓鞘或髓鞘形成施旺细胞的原发性破坏,其使轴突保持完整,但引起冲动传导的急性衰竭。这种脱髓鞘减慢或完全阻断通过神经的电脉冲传导。最常见的原因是急性炎症性脱髓鞘性多发神经病变(AIDP,更广为人知的名称为格林-巴利综合征),而其他原因包括慢性炎症性脱髓鞘性多发神经病变(CIDP)、遗传代谢性病症(例如脑白质营养不良或腓骨肌萎缩症)或毒素。

[1020] iii.神经元病变是外周神经系统(PNS)神经元破坏的结果。它们可由运动神经元疾病、感觉神经元病变(例如带状疱疹)、毒素或自主功能障碍引起。神经毒素如化疗剂长春新碱可引起神经元病变。神经元病变是由于外周神经系统(PNS)的神经元损伤导致的功能障碍,导致外周神经病变。其可由运动神经元疾病、感觉神经元病变(例如带状疱疹)、毒性物质或自主功能障碍引起。患有神经元病变的人可以不同的方式出现,具体取决于病因、影响神经细胞的方式以及受影响最严重的神经细胞类型。

[1021] iv.局灶性卡压性神经病变(例如腕管综合征)。

[1022] 本发明的嵌合梭菌神经毒素可用于治疗由任何下列炎性病况引起的或与之相关的疼痛。

[1023] A.关节炎病症

[1024] 关节炎病症包括例如类风湿性关节炎;幼年类风湿性关节炎;系统性红斑狼疮(SLE);痛风性关节炎;硬皮病;骨关节炎;银屑病关节炎;强直性脊柱炎;赖特综合征(Reiter's syndrome)(反应性关节炎);成人斯蒂尔病;病毒感染引起的关节炎;细菌感染引起的关节炎,例如淋球菌性关节炎和非淋菌性细菌性关节炎(化脓性关节炎);三级莱姆病(Tertiary Lyme disease);结核性关节炎;以及由真菌感染引起的关节炎,例如芽生菌病。

[1025] B.自身免疫性疾病

[1026] 自身免疫性疾病包括例如格林-巴利综合征、桥本甲状腺炎、恶性贫血、艾迪森氏病、I型糖尿病、系统性红斑狼疮、皮肌炎、干燥综合征、红斑狼疮、多发性硬化症、重症肌无力、赖特综合征(Reiter's syndrome)和格雷夫斯病。

[1027] C.结缔组织病症

[1028] 结缔组织疾病包括例如脊柱关节炎、皮肌炎和纤维肌痛。

[1029] D.损伤

[1030] 由损伤引起的炎症,包括例如组织或关节的挤压、穿刺、拉伸,可能引起慢性炎性疼痛。

[1031] E.感染

[1032] 由感染引起的炎症,包括例如结核病或间质性角膜炎,可能引起慢性炎性疼痛。

[1033] F.神经炎

[1034] 神经炎是影响神经或神经群的炎性过程。症状取决于所涉及的神经,但可包括疼痛、感觉异常、麻痹或感觉减退(麻木)。

[1035] 实例包括:

[1036] a.臂神经炎(Brachial neuritis)

[1037] b.球后神经病变,一种影响位于眼球正后方的视神经部分的炎症过程。

[1038] c.视神经病变,一种影响视神经的炎症过程,导致受影响眼睛的视力突然下降。视神经炎的原因未知。视神经(连接眼睛和脑部的神经)的突然炎症导致髓鞘肿胀和破坏。炎症有时可能是病毒感染的结果,或者它可能是由自身免疫疾病如多发性硬化症引起的。风险因素与可能的原因有关。

[1039] d.前庭神经炎,一种病毒感染,引起影响前庭神经的炎症过程。

[1040] G.关节炎症

[1041] 关节的炎症,例如由滑囊炎或肌腱炎引起的炎症,可能会引起慢性炎性疼痛。

[1042] H.晒伤和/或紫外线引起的损伤

[1043] 本发明的嵌合梭菌神经毒素可用于治疗由任何下列头痛病况引起的或与之相关的疼痛。头痛(医学上称为头痛(cephalgia))是一种头部轻度至重度疼痛的病况;有时颈部或上背部疼痛也可能被解释为头痛。它可能表明潜在的局部或全身疾病或本身就是一种病症。

[1044] A.肌性/肌源性头痛

[1045] 肌肉/肌源性头痛似乎涉及面部和颈部肌肉的收紧或紧张;它们可以辐射到前额。紧张性头痛是肌源性头痛的最常见形式。

[1046] 紧张性头痛是一种涉及头部、头皮或颈部疼痛或不适的病况,通常与这些区域的肌肉紧张性有关。紧张性头痛由颈部和头皮肌肉的收缩引起。这种肌肉收缩的一个原因是对压力、抑郁或焦虑的反应。使头部长时间保持在一个位置而不移动的任何活动都会引起头痛。这样的活动包括打字或使用计算机、手工精细工作以及使用显微镜。睡在寒冷的房间或睡觉时颈部位置不正常也可能引发此类头痛。紧张型头痛包括但不限于阵发性紧张性头痛和慢性紧张性头痛。

[1047] B.血管性头痛

[1048] 最常见的血管性头痛类型是偏头痛。其他类型的血管性头痛包括引起反复发作的强烈疼痛的丛集性头痛(cluster headaches)和由高血压引起的头痛。

[1049] 1.偏头痛

[1050] 偏头痛是一种通常涉及复发性头痛的异质性病症。偏头痛与其他头痛不同,因为它们伴有其他症状,例如恶心、呕吐或对光敏感。对于大多数人来说,只有头部的一侧会感到抽痛。在具有不同潜在病理生理学和遗传机制的受试者亚组中,可能会观察到临床特征如先兆症状类型、前驱症状的存在或眩晕等相关症状。偏头痛包括但不限于无先兆偏头痛(普通偏头痛)、有先兆偏头痛(典型偏头痛)、经期偏头痛、偏头痛等位症(头痛(acephalic headache))、复杂性偏头痛、腹部偏头痛和混合性紧张性偏头痛。

[1051] 2.丛集性头痛

[1052] 丛集性头痛影响头部的一侧(单侧),并且可能与眼泪和鼻腔阻塞有关。它们成簇出现,每天同一时间重复发生,持续数周,然后缓解。

[1053] D.高血压头痛

[1054] E.牵引头痛和炎性头痛

[1055] 牵引性头痛和炎性头痛通常是其他疾病的症状,包括中风和鼻窦感染。

[1056] F.激素性头痛

[1057] G.反跳性头痛

[1058] 反跳性头痛,也称为药物过度使用性头痛,是由于过于频繁地服用药物来缓解头痛而发生的。反跳性头痛每天都会频繁发生,而且会非常痛苦。

[1059] H.慢性鼻窦炎头痛

[1060] 鼻窦炎是鼻窦的细菌性、真菌性、病毒性、过敏性或自身免疫性炎症。慢性鼻窦炎是普通感冒最常见的并发症之一。症状包括:鼻塞;面部疼痛;头痛;发烧;全身不适;浓稠的绿色或黄色分泌物;俯身时面部“发胀”感恶化。在少数情况下,慢性上颌窦炎也可能是由牙齿感染引起的细菌传播引起的。慢性增生性嗜酸粒细胞性鼻窦炎是慢性鼻窦炎的一种非感染性形式。

[1061] I.器质性头痛

[1062] J.阵发性头痛

[1063] 阵发性头痛是与癫痫发作活动相关的头痛。

[1064] 本发明的嵌合梭菌神经毒素可用于治疗由任何以下躯体疼痛病况引起的或以其他方式与其相关的疼痛。躯体疼痛源自韧带、肌腱、骨骼、血管,甚至神经本身。它是用躯体伤害性感受器检测到的。这些区域缺乏疼痛感受器,会产生比皮肤疼痛持续时间更长的钝痛、定位不明确的疼痛;实例包括扭伤和骨折。其他实例包括以下内容。

[1065] A.肌肉张力过大

[1066] 过度的肌肉张力可由例如扭伤或拉伤引起。

[1067] B.重复性运动障碍

[1068] 重复性运动障碍可能是由于过度使用手、手腕、肘部、肩膀、颈部、背部、臀部、膝盖、脚、腿或脚踝造成的。

[1069] C.肌肉病症

[1070] 引起躯体疼痛的肌肉病症包括例如多发性肌炎、皮肌炎、狼疮、纤维肌痛、风湿性

多肌痛和横纹肌溶解症。

[1071] D.肌痛

[1072] 肌痛是肌肉疼痛并且是许多疾病和病症的症状。肌痛最常见的原因是肌肉或肌肉群的过度使用或过度伸展。没有外伤史的肌痛通常是由于病毒感染。长期肌痛可能表明存在代谢性肌病、某些营养缺乏或慢性疲劳综合症。

[1073] E.感染

[1074] 感染可引起躯体疼痛。此类感染的实例包括例如肌肉脓肿、旋毛虫病、流感、莱姆病、疟疾、落基山斑疹热、禽流感、普通感冒、社区获得性肺炎、脑膜炎、猴痘、严重急性呼吸系统综合症、中毒性休克综合症、旋毛虫病、伤寒和上呼吸道感染。

[1075] F.药物

[1076] 药物可引起躯体疼痛。此类药物包括例如可卡因、用于降低胆固醇的他汀类药物(例如阿托伐他汀、辛伐他汀和洛伐他汀)以及用于降低血压的ACE抑制剂(例如依那普利和卡托普利)。

[1077] 本发明的嵌合梭菌神经毒素可用于治疗由任何以下内脏疼痛病况引起的或以其他方式与其相关的疼痛。内脏疼痛源于身体的内脏或器官。内脏伤害性感受器位于身体器官和内腔中。这些区域的伤害性感受器更加稀缺,产生的疼痛通常比躯体疼痛更剧烈、持续时间更长。内脏疼痛极难定位,并且内脏组织的一些损伤表现出“牵涉”疼痛,其中感觉局限于与损伤部位完全无关的区域。内脏疼痛的实例包括以下。

[1078] A.功能性内脏疼痛

[1079] 功能性内脏疼痛包括例如肠易激综合征和慢性功能性腹痛(CFAP)、功能性便秘和功能性消化不良、非心源性胸痛(NCCP)和慢性腹痛。

[1080] B.慢性胃肠炎症

[1081] 慢性胃肠炎症包括例如胃炎、炎性肠病例如克罗恩病、溃疡性结肠炎、显微镜下结肠炎、憩室炎和胃肠炎;间质性膀胱炎;肠道缺血;胆囊炎;阑尾炎;胃食管反流;溃疡、肾结石、尿路感染、胰腺炎和疝气。

[1082] C.自身免疫性疼痛

[1083] 自身免疫疼痛包括例如结节病和血管炎。

[1084] D.器质性内脏疼痛

[1085] 器质性内脏疼痛包括例如由肠道的创伤性、炎性或退行性病变引起的疼痛或由肿瘤撞击感觉神经支配而产生的疼痛。

[1086] E.治疗引起的内脏疼痛

[1087] 治疗引起的内脏疼痛包括例如化学疗法伴随的疼痛或放射疗法伴随的疼痛。

[1088] 本发明的嵌合梭菌神经毒素可用于治疗由任何以下提及的疼痛病况引起的或以其他方式与其相关的疼痛。

[1089] 牵涉性疼痛是由局部疼痛引起的,该区域与疼痛刺激部位分开。通常,当神经在其起源处或其附近被压缩或损伤时,会出现牵涉性疼痛。在这种情况下,疼痛的感觉通常在神经所支配的区域中感觉到,即使损伤起源于其他地方。常见的实例出现在椎间盘突出中,其中源自脊髓的神经根被相邻的椎间盘材料压缩。虽然疼痛可能是由受损的椎间盘本身引起的,但受压神经所支配的区域(例如大腿、膝盖或脚)也会感到疼痛。如果没有发生永久性神

经损伤,减轻神经根的压力可能会减轻牵涉性疼痛。心肌局部缺血(流向心肌组织一部分的血流损失)可能是最为人所知的牵涉性疼痛的实例;这种感觉可能出现在上胸部,表现为一种受限的感觉,或者表现为左肩、手臂甚至手部的疼痛。

[1090] 本发明的嵌合梭菌神经毒素可用于治疗术后疼痛。然而,优选本发明的疼痛不是术后疼痛。

[1091] 术后(例如,手术后)疼痛是由外科手术引起的不愉快的感觉。术后疼痛可能是由切口、手术本身、伤口闭合以及手术期间施加的任何力对组织造成的损伤引起的。手术后疼痛(例如,术后疼痛)也可源于伴随手术的因素。例如,受试者可能由于其在手术台上的定位方式而遭受背痛,或者可能由于胸部区域中的切口而导致胸痛。全身麻醉后也可能发生咽喉痛,因为插入呼吸管可能引起刺激。然而,最常见的是由手术切口切入皮肤和肌肉引起的术后疼痛。术后疼痛还可包括由术后瘢痕引起或与术后瘢痕相关的疼痛(例如,术后瘢痕疼痛)。

[1092] 例如,手术过程(或更具体地,手术切口)代表引起疼痛的“有害刺激”。有害刺激(可引起组织损伤的刺激)可激活疼痛介质从感受伤害的传入终端和从感觉终端的释放(例如从其释放CGRP)。然后将有害信息从外周神经系统转导到中枢神经系统,个体在中枢神经系统中感觉到疼痛。

[1093] 术后疼痛可由炎症和神经组织损伤的组合引起。例如,响应组织损伤而激活的肥大细胞脱粒可导致包括蛋白酶、细胞因子、血清素和细胞外间隙的多种物质的释放。这些物质可以使初级传入神经元敏化(在较低阈值下激活)以产生疼痛超敏反应。由于组织受到广泛的神经支配,身体的任何区域都容易受到手术造成的神经损伤。

[1094] 提及手术是指涉及治疗受试者的损伤或疾病的医疗程序,包括使身体的一部分经受切口(任选地去除或修复身体的受损部分)。尽管侵入性水平(例如,所需的手术切口的水平)在手术类型之间可能不同,但是意图涵盖具有一旦手术完成就引起受试者疼痛的侵入性水平的手术。

[1095] 手术可以包括切开皮肤和/或筋膜和/或肌肉。优选地,手术包括切开皮肤。

[1096] 手术不限于可由医生进行的手术,还包括例如牙科手术。手术的非限制性实例包括阑尾切除术、乳房活检、乳房增大或减小、整容术、胆囊切除术、冠状动脉搭桥术、清创术(例如伤口、烧伤或感染的清创术)、皮肤移植、器官移植和扁桃体切除术。

[1097] 优选地,“术后”可指在手术后(例如手术后)至多一天开始的时间段。换言之,术语“术后”可指从手术后不超过一天开始的时间段。例如,术语“术后”可指手术后1-20小时开始的时间点;任选地手术后2-15小时;任选地手术后5-10小时。这样的时间可以表示从时序界面(chronological interface)开始的时间段,在该时序界面处,施用于受试者的手术麻醉剂的镇痛效果减弱(例如逐渐减小),从而受试者开始感觉到疼痛。

[1098] 此外,术语“术后(post-operative)”可与术语“手术后(post-surgical)”互换使用,因为“术”在本文中以“手术”的意义使用。

[1099] 类似地,术语“术后疼痛”可指在手术后(例如,手术后)至多一天开始的时间段内感觉到(或更具体地,开始感觉到)的疼痛。换言之,术语“术后疼痛”可指受试者在手术后不超过一天的时间段内感觉到的疼痛。例如,术语“术后疼痛”可指在手术后1-20小时开始的时间段内感觉到的疼痛;任选地手术后2-15小时;任选地手术后5-10小时。

[1100] 所述时间段可以是1-50周;例如手术后5-45周、10-40周或10-35周。

[1101] 这与术语“围手术期(peri-operative)”形成对比,术语“围手术期”可以指,例如,在受试者进行手术时或在其周围的时间段(例如,当受试者在手术室中时),合适地,在手术前至少1小时开始和/或在手术后不到1小时结束的时间段。

[1102] 本发明涉及宽范围的疼痛病况,例如慢性疼痛病况。在一些实施方案中,本发明的嵌合梭菌神经毒素用于治疗癌性和/或非癌性疼痛。

[1103] 优选地,本发明的嵌合梭菌神经毒素用于治疗膀胱疼痛综合征(例如膀胱疼痛)、幻肢疼痛或偏头痛疼痛。膀胱疼痛综合征(例如膀胱疼痛)可由间质性膀胱炎引起或与间质性膀胱炎相关。

[1104] 在特别优选的实施方案中,疼痛是膀胱疼痛,例如由间质性膀胱炎引起或与间质性膀胱炎相关。

[1105] 治疗疼痛优选地意指减轻疼痛。换言之,在一个实施方案中,施用本发明的嵌合梭菌神经毒素减轻了受试者的疼痛。

[1106] 更详细地,提及“减轻(reduced)”或“减轻(reducing)”(就疼痛而言)优选是指与施用之前(施用前)受试者感知的疼痛水平相比,施用本发明的嵌合梭菌神经毒素之后(施用后)受试者感知的疼痛水平较低。例如,相对于施用前,施用后感知的疼痛水平可以降低至少15%、25%、35%、45%、55%、65%、75%、85%或95%。例如,施用后感知的疼痛水平可以减少至少75%;优选至少85%;更优选至少95%。

[1107] 本领域技术人员已知多种评估疼痛知觉(pain perception)的方法。例如,机械异常性疼痛(静态或动态)的评估通常用于人类疼痛研究,如Pogatzki-Zahn等人(Pain Rep.2017Mar;2(2):e588)所述,通过引用并入本文。

[1108] 用于评估受试者中的疼痛知觉的合适的(尽管非限制性)方法包括以下:数值评级量表(NRS)得分;尽管技术人员知晓可以附加地或替代地使用其他方法,例如感觉阈值、疼痛知觉阈值、静态机械异常性疼痛、动态机械异常性疼痛、时间求和、压力疼痛阈值、条件性疼痛调节和温度阈值。

[1109] 疼痛知觉测量的其他非限制性实例包括:每个预定时间点的SF-36评分相对于基线的变化;研究期间服用的救援药物的量以及首次服用救援药物的时间。这些可被认为是“探索性”终点或疼痛知觉评估措施。

[1110] 因此,在一个优选的实施方案中,在施用本发明的嵌合梭菌神经毒素后,可以通过以下一项或多项评估疼痛知觉:(a)数值评级量表(NRS);(b)刺激诱发的NRS;(c)疼痛部位的温度;(d)疼痛区域的大小;(e)镇痛作用起效的时间;(f)峰值镇痛效果;(g)达到镇痛效果峰值的时间;(h)镇痛效果的持续时间;(i)SF-36生活质量评估。

[1111] 本领域技术人员知晓这种用于评估疼痛知觉的方法。为方便起见,下文提供了数值评级评分和生活质量问卷简表36的进一步描述。

[1112] 数值评级量表(NRS):通常,根据本发明的疼痛知觉使用数值评级量表(NRS)。NRS是评估受试者疼痛知觉的11分制量表。要求受试者给出最适合其疼痛强度的0-10之间的数字。0表示“根本没有疼痛”,而上限10表示“可能的最严重疼痛”。

[1113] NRS可用于评估疼痛的多个方面,包括自发性平均疼痛、自发性最严重疼痛和自发性当前疼痛。通过要求受试者选择最能描述受试者在一段时间(例如至少6小时、12小时、24

小时或至少48小时)内的平均疼痛(例如感知疼痛)的数字来评估自发性性平均疼痛。通过要求受试者选择最能描述受试者在指定时间段(例如至少在先前的6小时、12小时、24小时或之前48小时)内最严重疼痛的数字来评估自发性最严重疼痛。通过要求受试者选择最能描述受试者在评估时疼痛程度的数字来评估自发性当前疼痛。

[1114] NRS还可用于评估受试者响应于多种不同刺激的疼痛知觉。为了评估响应于刺激的疼痛知觉,受试者将受到施加于疼痛区域的多种性质的刺激。将询问受试者在施用前和刺激后的当前NRS评分。

[1115] 所用刺激的实例包括:(i)轻触(其可通过在施用如本文所述的von Frey纤毛后测量径向辐条上疼痛区域的表面上的疼痛来评估);(ii)压力(压力疼痛阈值),其可通过要求受试者在使用压力测痛计施加增加的压力时给出NRS评分来评估;和(iii)温度(其可通过使用施用于疼痛区域的热电极询问受试者暖、冷和热刺激的NRS评分来评估)。

[1116] 优选地,与施用前受试者的NRS得分相比,施用本发明的嵌合梭菌神经毒素降低了施用后受试者的NRS得分(例如从评级≥7降低到评级≤6)。

[1117] 生活质量问卷简表36(SF-36):SF-36生活质量问卷可用于评估受试者的疼痛知觉。SF-36是一项36项受试者报告的受试者健康调查。SF-36由八个量表得分组成(活力、身体功能、身体疼痛、一般健康认知、身体角色功能、情感角色功能、社会角色功能和心理健康)。假设每个问题具有相等的权重,则将每个量表直接转换成0-100等级。SF-36中记录的得分越高,缺陷越少。

[1118] 在治疗疼痛的临床试验中通常测试的相关参数是本领域已知的,并且可以由本领域普通技术人员容易地选择。此类参数的实例包括但不限于NRS;刺激诱发的NRS;疼痛部位的温度;疼痛区域的大小;镇痛作用起效的时间;峰值镇痛效果;达到镇痛效果峰值的时间;镇痛效果的持续时间;和/或本文所述的SF-36生活质量。用于评估这些参数的方法也是本领域已知的,并且可以由普通技术人员使用常规方法和程序来进行。

[1119] 优选地,与施用前受试者的SF-36得分相比,施用本发明的嵌合梭菌神经毒素增加了施用后受试者的SF-36得分(例如从≤50的得分到≥50的得分)。

[1120] 本发明还可以(例如,附加地或者可替代地)涉及任何感觉障碍的治疗,所述感觉障碍可以通过嵌合梭菌神经毒素治疗,所述嵌合梭菌神经毒素分别结合至包含Aδ神经纤维或C神经纤维的神经元,并抑制介质从其释放。不希望受理论束缚,据信所述病症可以与疼痛类似地治疗,如本文所述。因此,上述关于治疗疼痛的所有实施方案在治疗感觉障碍的情况下可以同样有效。所述介质可以是参与感觉的任何介质(例如感觉介质),在一些情况下,所述介质可以是本文所述的疼痛介质。所述介质可以是神经递质。抑制所述介质从神经元的释放可以是部分或完全抑制,优选完全抑制。例如,嵌合梭菌神经毒素可以抑制从所述神经元释放的至少80%、90%、95%或99%的介质。优选地,嵌合梭菌神经毒素抑制从神经元释放的100%的介质。嵌合梭菌神经毒素可抑制多种介质从神经元的释放。感觉障碍可以是感觉调节障碍(例如感觉反应过度)和/或异常感觉处理障碍(例如纤维肌痛)。

[1121] 因此,在一个方面,本发明提供了用于通过抑制介质从包含Aδ神经纤维或C神经纤维的神经元释放来治疗感觉障碍的嵌合梭菌神经毒素,其中所述嵌合梭菌神经毒素分别结合至包含Aδ神经纤维或C神经纤维的神经元,并且其中所述嵌合梭菌神经毒素包含肉毒杆菌神经毒素A(BoNT/A)轻链和转位结构域(H_N结构域)以及BoNT/B受体结合结构域(H_C结构

域)。

[1122] 在一个相关的方面,本发明提供了通过抑制介质从包含Aδ神经纤维或C神经纤维的神经元释放来治疗感觉障碍的方法,所述方法包括向受试者施用所述嵌合梭菌神经毒素,其中所述嵌合梭菌神经毒素分别结合至包含Aδ神经纤维或C神经纤维的神经元,并且其中所述嵌合梭菌神经毒素包含肉毒杆菌神经毒素A(BoNT/A)轻链和转位结构域(H_N结构域)以及BoNT/B受体结合结构域(H_C结构域)。

[1123] 在另一个相关的方面,本发明提供了嵌合梭菌神经毒素在制备用于通过抑制介质从包含Aδ神经纤维或C神经纤维的神经元中释放来治疗感觉障碍的药物中的用途,其中所述嵌合梭菌神经毒素分别结合至包含Aδ神经纤维或C神经纤维的神经元,并且其中所述嵌合梭菌神经毒素包含肉毒杆菌神经毒素A(BoNT/A)轻链和转位结构域(H_N结构域)以及BoNT/B受体结合结构域(H_C结构域)。

[1124] 在另一个方面,本发明提供了一种试剂盒,其包含:

[1125] (a)根据本发明的单位剂型;和

[1126] (b)使用其在治疗疼痛中的说明书;以及

[1127] (c)任选的稀释剂。

[1128] 条款:

[1129] 1.一种嵌合梭菌神经毒素,其用于通过抑制疼痛介质从包含Aδ神经纤维或C神经纤维的神经元释放来治疗疼痛,其中所述嵌合梭菌神经毒素分别结合至包含Aδ神经纤维或C神经纤维的神经元,并且其中所述嵌合梭菌神经毒素包含肉毒杆菌神经毒素A(BoNT/A)轻链和转位结构域(H_N结构域)以及BoNT/B受体结合结构域(H_C结构域)。

[1130] 2.根据条款1所述用于所述用途的嵌合梭菌神经毒素,其中疼痛介质是选自以下的一种或多种:降钙素基因相关肽(CGRP);P物质;和神经激肽。

[1131] 3.根据条款1或2所述用于所述用途的嵌合梭菌神经毒素,其中疼痛介质是CGRP并且疼痛是CGRP相关疼痛。

[1132] 4.根据条款3所述用于所述用途的嵌合梭菌神经毒素,其中CGRP相关疼痛是CGRP相关头痛疼痛。

[1133] 5.根据条款3或4所述用于所述用途的嵌合梭菌神经毒素,其中CGRP相关疼痛是CGRP相关偏头痛疼痛。

[1134] 6.根据条款3-5中任一项所述用于所述用途的嵌合梭菌神经毒素,其中CGRP相关疼痛是:

[1135] (a)CGRP相关躯体疼痛选自:头痛疼痛(例如外伤后头痛、头部受伤头痛或外伤性脑损伤后头痛)、关节炎疼痛(例如骨关节炎疼痛和/或类风湿性关节炎疼痛)、运动疼痛、退行性椎间盘疾病疼痛、腕管压迫疼痛、软组织损伤疼痛、颞下颌关节疼痛、肌肉骨骼疼痛、由血管病症(例如雷诺氏综合征、血栓闭塞性脉管炎、外周静脉疾病、外周动脉疾病、静脉曲张、静脉血栓、凝血障碍或淋巴水肿)引起或与之相关的CGRP相关躯体疼痛、面部疼痛、由三叉神经自主神经性头痛引起或与之相关的CGRP相关躯体疼痛;由三叉神经痛引起或与之相关的CGRP相关躯体疼痛;和CGRP相关的癌症引起的疼痛(例如CGRP相关的癌症引起的骨痛);

[1136] (b)CGRP相关内脏疼痛选自:子宫内膜异位症疼痛、胰腺炎疼痛、胃肠道疼痛和由

血管病症引起或与之相关的CGRP相关内脏疼痛;

[1137] (c)CGRP相关炎性疼痛选自:慢性疼痛、伤口愈合疼痛、瘙痒疼痛和烧伤疼痛;和/或

[1138] (d)CGRP相关神经性疼痛选自:带状疱疹后神经痛、糖尿病疼痛、慢性神经性疼痛和莫顿神经瘤疼痛。

[1139] 7.根据前述条款中任一项所述用于所述用途的嵌合梭菌神经毒素,其中神经元是三叉神经节。

[1140] 8.根据前述条款中任一项所述用于所述用途的嵌合梭菌神经毒素,其中嵌合梭菌神经毒素施用于面部、颈部和/或颅骨。

[1141] 9.根据前述条款中任一项所述用于所述用途的嵌合梭菌神经毒素,其中嵌合梭菌神经毒素皮内施用。

[1142] 1.10.根据前述条款中任一项所述用于所述用途的嵌合梭菌神经毒素,其中嵌合梭菌神经毒素通过在每次治疗期间至多10个注射部位皮内注射施用。

[1143] 11.根据条款1-8中任一项所述用于所述用途的嵌合梭菌神经毒素,其中嵌合梭菌神经毒素肌内施用。

[1144] 12.根据条款1-8或11中任一项所述用于所述用途的嵌合梭菌神经毒素,其中嵌合梭菌神经毒素施用于受试者的一块或多块肌肉,所述肌肉选自:额肌(frontalis)、皱眉肌(corrugator)(例如皱眉肌(corrugator supercilii))、降眉间肌(例如降眉间肌-鼻肌(procerus nasalis))、枕肌、颞肌、斜方肌、咬肌、鼻肌、眼轮匝肌、颈椎旁肌、颞筋膜、耳上肌、耳前肌、耳后肌、胸锁乳突肌、颈阔肌、前鼻孔扩张肌、后鼻孔扩张肌、降鼻中隔肌、颏肌、口轮匝肌、颧肌、笑肌、颊肌、枕额肌、提上唇肌、降下唇肌、降口角肌、甲状舌骨肌、肩胛舌骨肌、胸骨舌骨肌、颈夹肌、头夹肌、颈半棘肌、头半棘肌、肩胛提肌、二腹肌和斜角肌;优选地,其中嵌合梭菌神经毒素施用于受试者的一块或多块肌肉,所述肌肉选自:额肌(frontalis)、皱眉肌(corrugator)、降眉间肌(例如降眉间肌-鼻肌(procerus nasalis))、枕肌、颞肌、斜方肌、咬肌、鼻肌、眼轮匝肌、颈椎旁肌、颞筋膜、耳上肌、耳前肌、耳后肌、胸锁乳突肌、颈阔肌、前鼻孔扩张肌、后鼻孔扩张肌、降鼻中隔肌、颏肌、口轮匝肌、颧肌、笑肌、颊肌、枕额肌、提上唇肌、降下唇肌、降口角肌、甲状舌骨肌、肩胛舌骨肌、胸骨舌骨肌、颈夹肌、肩胛提肌、二腹肌和斜角肌。

[1145] 13.根据条款1-8或11-12中任一项所述用于所述用途的嵌合梭菌神经毒素,其中嵌合梭菌神经毒素通过在每次治疗期间至多10个注射部位肌内注射施用。

[1146] 14.根据条款1-8中任一项所述用于所述用途的嵌合梭菌神经毒素,其中嵌合梭菌神经毒素通过神经内、神经周围或通过神经节周围施用来施用。

[1147] 15.根据条款1-8或14中任一项所述用于所述用途的嵌合梭菌神经毒素,其中嵌合梭菌神经毒素被施用至三叉神经、半月神经节、中间神经、舌咽神经、迷走神经和/或经由枕神经施用至上颈根。

[1148] 16.根据条款1-8中任一项所述用于所述用途的嵌合梭菌神经毒素,其中嵌合梭菌神经毒素通过血管周围施用来施用。

[1149] 17.根据前述条款中任一项所述用于所述用途的嵌合梭菌神经毒素,其中嵌合梭菌神经毒素以5pg至17,000pg嵌合梭菌神经毒素的单位剂量施用。

[1150] 18.根据前述条款中任一项所述用于所述用途的嵌合梭菌神经毒素,其中嵌合梭菌神经毒素以500pg至17,000pg的单位剂量施用。

[1151] 19.根据前述条款中任一项所述用于所述用途的嵌合梭菌神经毒素,其中嵌合梭菌神经毒素以1,000pg至17,000pg的单位剂量施用。

[1152] 20.根据前述条款中任一项所述用于所述用途的嵌合梭菌神经毒素,其中每次治疗期间施用的总剂量为最多255,000pg的嵌合梭菌神经毒素,例如3,640-255,000pg。

[1153] 21.根据前述条款中任一项所述用于所述用途的嵌合梭菌神经毒素,其中嵌合梭菌神经毒素以3,640pg至17,000pg的单位剂量施用。

[1154] 22.根据前述条款中任一项所述用于所述用途的嵌合梭菌神经毒素,其中嵌合梭菌神经毒素具有大于7的安全比(优选至少10的安全比),其中所述安全比计算为:以pg/小鼠测量的-10%体重变化所需的毒素剂量除以以pg/小鼠测量的DAS EC_{50},其中EC_{50}=产生DAS得分为2所需的剂量。

[1155] 23.根据前述条款中任一项所述用于所述用途的嵌合梭菌神经毒素,其中所述H_N结构域的C-末端氨基酸残基对应于分隔BoNT/A的H_N和H_C结构域的3_{10}螺旋的第一个氨基酸残基,并且其中所述H_C结构域的N-末端氨基酸残基对应于分隔BoNT/B的H_N和H_C结构域的3_{10}螺旋的第二个氨基酸残基。

[1156] 24.根据前述条款中任一项所述用于所述用途的嵌合梭菌神经毒素,其中嵌合梭菌神经毒素包含与SEQ ID NO:1具有至少70%序列同一性的多肽序列。

[1157] 25.根据前述条款中任一项所述用于所述用途的嵌合梭菌神经毒素,其中所述BoNT/B H_C结构域包含一个或多个选自以下的取代突变:E1191M;S1199Y;V1118M;Y1183M;E1191I;E1191Q;E1191T;S1199F;S1199L;S1201V及其组合,优选地其中BoNT/B H_C结构域包含在E1191M和S1199Y处的取代突变。

[1158] 与本发明的各种治疗用途相关的实施方案旨在同等地应用于本发明的方法、组合物(例如单位剂型)和试剂盒,反之亦然。

[1159] 序列同源性

[1160] 可以使用多种序列比对方法中的任一种来确定同一性百分比,包括但不限于全局方法、局部方法和混合方法,例如区段方法。确定百分比同一性的方案是本领域技术人员能力范围内的常规程序。全局方法将序列从分子的开始到末端进行比对,并通过将单个残基对的得分相加并施加空位罚分来确定最佳比对。非限制性方法包括,例如CLUSTAL W,参见例如Julie D.Thompson等人,CLUSTAL W:Improving the Sensitivity of Progressive Multiple Sequence Alignment Through Sequence Weighting,Position-Specific Gap Penalties和Weight Matrix Choice,22(22)Nucleic Acids Research 4673-4680(1994);以及迭代改进(iterative refinement),参见例如,Osamu Gotoh,Significant Improvement in Accuracy of Multiple Protein.Sequence Alignments by Iterative Refinement as Assessed by Reference to Structural Alignments,264(4)J.MoI.Biol.823-838(1996)。局部方法通过鉴定所有输入序列共有的一个或多个保守基序来比对序列。非限制性方法包括,例如火柴盒(Match-box),参见例如,Eric Depiereux and Ernest Feytmans,Match-Box:A Fundamentally New Algorithm for the Simultaneous Alignment of Several Protein Sequences,8(5)CABIOS 501 -509(1992);吉布斯采样,

参见例如C.E.Lawrence等人,Detecting Subtle Sequence Signals:A Gibbs Sampling Strategy for Multiple Alignment,262(5131)Science 208-214(1993);Align-M,参见例如,Ivo Van WaIIe等人,Align-M-A New Algorithm for Multiple Alignment of Highly Divergent Sequences,20(9)Bioinformatics:1428-1435(2004)。

[1161] 因此,通过常规方法测定序列同一性百分比。参见例如,Altschul等人,Bull.Math.Bio.48:603-16,1986和Henikoff and Henikoff,Proc.Natl.Acad.Sci.USA 89:10915-19,1992。简言之,如下所示,使用空位开放罚分10、空位延伸罚分1,以及Henikoff和Henikoff的“blosum 62”评分矩阵(同上)比对两个氨基酸序列,以优化比对得分(氨基酸由标准单字母代码表示);优选地,该方法用于将序列与本文的主题序列(例如SEQ ID NO:7)进行比对,以定义本文所述的氨基酸位置编号。

[1162] 两个或更多个核酸或氨基酸序列之间的“序列同一性百分比”是序列共享的相同位置的数目的函数。因此,同一性%可计算为相同核苷酸/氨基酸的数目除以核苷酸/氨基酸的总数,再乘以100。序列同一性%的计算还可以考虑需要引入以优化两个或更多个序列的比对的空位的数目以及每个空位的长度。可以使用本领域技术人员熟悉的特定数学算法如BLAST进行序列比较并确定两个或多个序列之间的百分比同一性。

[1163] 用于确定序列同一性的比对得分

```
   A  R  N  D  C  Q  E  G  H  I  L  K  M  F  P  S  T  W  Y  V
A  4
R -1  5
N -2  0  6
D -2 -2  1  6
C  0 -3 -3 -3  9
Q -1  1  0  0 -3  5
E -1  0  0  2 -4  2  5
G  0 -2  0 -1 -3 -2 -2  6
H -2  0  1 -1 -3  0  0 -2  8
I -1 -3 -3 -3 -1 -3 -3 -4 -3  4
L -1 -2 -3 -4 -1 -2 -3 -4 -3  2  4
K -1  2  0 -1 -3  1  1 -2 -1 -3 -2  5
M -1 -1 -2 -3 -1  0 -2 -3 -2  1  2 -1  5
F -2 -3 -3 -3 -2 -3 -3 -3 -1  0  0 -3  0  6
P -1 -2 -2 -1 -3 -1 -1 -2 -2 -3 -3 -1 -2 -4  7
S  1 -1  1  0 -1  0  0  0 -1 -2 -2  0 -1 -2 -1  4
T  0 -1  0 -1 -1 -1 -1 -2 -2 -1 -1 -1 -1 -2 -1  1  5
W -3 -3 -4 -4 -2 -2 -3 -2 -2 -3 -2 -3 -1  1 -4 -3 -2 11
Y -2 -2 -2 -3 -2 -1 -2 -3  2 -1 -1 -2 -1  3 -3 -2 -2  2  7
V  0 -3 -3 -3 -1 -2 -2 -3 -3  3  1 -2  1 -1 -2 -2  0 -3 -1  4
```

[1164] 然后,百分比同一性计算如下:

[1165]
$$\frac{\text{相同匹配的总数}}{\text{[较长序列的长度加上引入较长序列以比对两个序列的空位数目]}} \times 100$$

[1166] 基本上同源的多肽的特征在于具有一个或多个氨基酸取代、缺失或添加。这些改变优选是不重要的,即保守氨基酸取代(参见下文);以及不会显著影响多肽的折叠或活性的其他取代;小的缺失,通常为1至约30个氨基酸;和小的氨基末端或羧基末端延伸,例如氨基末端甲硫氨酸残基、至多约20-25个残基的小接头肽、或亲和标签。

[1167] 保守氨基酸取代

[1168] 基本氨基酸:精氨酸

[1169] 赖氨酸

[1170] 组氨酸

[1171] 酸性氨基酸:谷氨酸

[1172] 天冬氨酸

[1173] 极性氨基酸:谷氨酰胺

[1174] 天冬酰胺

[1175] 疏水性氨基酸:亮氨酸

[1176] 异亮氨酸

[1177] 缬氨酸

[1178] 芳香族氨基酸:苯丙氨酸

[1179] 色氨酸

[1180] 酪氨酸

[1181] 小氨基酸:甘氨酸

[1182] 丙氨酸

[1183] 丝氨酸

[1184] 苏氨酸

[1185] 甲硫氨酸

[1186] 除了20种标准氨基酸外,非标准氨基酸(如4-羟基脯氨酸、6-N-甲基赖氨酸、2-氨基异丁酸、异缬氨酸和α-甲基丝氨酸)可替代本发明多肽的氨基酸残基。有限数量的非保守氨基酸、未被遗传密码编码的氨基酸和非天然氨基酸可以取代多肽氨基酸残基。本发明的多肽还可以包含非天然存在的氨基酸残基。

[1187] 非天然存在的氨基酸包括但不限于反式3-甲基脯氨酸、2,4-甲酰脯氨酸(2,4-methano-proline)、顺式4-羟基脯氨酸、反式4-羟基脯氨酸、N-甲基甘氨酸、别苏氨酸、甲基苏氨酸、羟乙基半胱氨酸、羟乙基高半胱氨酸、硝基谷氨酰胺、高谷氨酰胺、哌啶酸、叔亮氨酸、正缬氨酸、2-氮杂苯丙氨酸、3-氮杂苯丙氨酸、4-氮杂苯丙氨酸和4-氟苯丙氨酸。本领域已知几种将非天然存在的氨基酸残基掺入蛋白质的方法。例如,可使用体外系统,其中使用化学氨基酰化抑制子tRNA来抑制无义突变。合成氨基酸和氨酰化tRNA的方法是本领域已知的。含有无义突变的质粒的转录和翻译是在无细胞系统中进行的,该无细胞系统包含大肠杆菌S30提取物以及市售酶和其他试剂。蛋白质通过色谱法纯化。参见例如,Robertson等人,J.Am.Chem.Soc.113:2722,1991;Ellman等人,Methods Enzymol.202:301,1991;Chung等人,Science 259:806-9,1993;以及Chung等人,Proc.Natl.Acad.Sci.USA 90:10145-9,1993)。在第二种方法中,通过显微注射突变的mRNA和化学氨基酰化的抑制子tRNA在非洲爪蟾卵母细胞中进行翻译(Turcatti等人,J.Biol.Chem.271:19991-8,1996)。在第三种方法中,在不存在待替代的天然氨基酸(例如苯丙氨酸)和存在所需的非天然存在的氨基酸(例如2-氮杂苯丙氨酸、3-氮杂苯丙氨酸、4-氮杂苯丙氨酸或4-氟苯丙氨酸)的情况下培养大肠杆菌细胞。将非天然存在的氨基酸代替其天然对应物掺入多肽中。参见Koide等人,Biochem.33:7470-6,1994。天然存在的氨基酸残基可以通过体外化学修饰转化为非天然存在的种类。化学修饰可与定点诱变组合以进一步扩大取代的范围(Wynn and Richards,

Protein Sci.2:395-403,1993)。

[1188] 有限数量的非保守氨基酸、未被遗传密码编码的氨基酸、非天然存在的氨基酸和非天然氨基酸可取代本发明多肽的氨基酸残基。

[1189] 本发明的多肽中的必需氨基酸可以根据本领域已知的方法鉴定,例如定诱突变或丙氨酸扫描诱变(Cunningham and Wells,Science 244:1081-5,1989)。生物相互作用的位点还可以通过结构的物理分析来确定,如通过诸如核磁共振、结晶学、电子衍射或光亲和标记等技术结合推定的接触位点氨基酸的突变来确定。参见例如de Vos等人,Science 255:306-12,1992;Smith等人,J.Mol.Biol.224:899-904,1992;Wlodaver等人,FEBS Lett.309:59-64,1992。必需氨基酸的同一性也可以通过与本发明的多肽的相关组分(例如易位或蛋白酶组分)的同源性分析来推断。

[1190] 可以使用已知的诱变和筛选方法进行多种氨基酸取代,并进行测试,例如Reidhaar-Olson and Sauer(Science 241:53-7,1988)或Bowie and Sauer(Proc.Natl.Acad.Sci.USA 86:2152-6,1989)公开的那些方法。简而言之,这些作者公开了用于同时随机化多肽中的两个或多个位置,选择功能性多肽,然后对诱变的多肽测序以确定每个位置上允许的取代范围的方法。可使用的其他方法包括噬菌体展示(例如,Lowman等人,Biochem.30:10832-7,1991;Ladner等人,美国专利号5,223,409;Huse,WIPO公开WO 92/06204)和区域定点诱变(Derbyshire等人,Gene 46:145,1986;Ner et al.,DNA 7:127,1988)。

[1191] 除非另外定义,否则本文使用的所有技术和科学术语具有与本公开所属领域的普通技术人员通常理解的相同含义。Singleton等人,DICTIONARY OF MICROBIOLOGY AND MOLECULAR BIOLOGY,第20版,John Wiley and Sons,New York(1994),和Hale&Marham,THE HARPER COLLINSDICTIONARY OF BIOLOGY,Harper Perennial,NY(1991)为本领域技术人员提供了本公开中使用的许多术语的通用词典。

[1192] 本公开不限于本文公开的示例性方法和材料,并且与本文描述的那些方法和材料类似或等同的任何方法和材料都可用于实践或测试本公开的实施方案。数值范围包括定义该范围的数字。除非另有说明,否则任何核酸序列以5'至3'方向从左到右书写;氨基酸序列分别以氨基到羧基的方向从左到右书写。

[1193] 本文提供的标题不是对本公开的各个方面或实施方案的限制。

[1194] 本文使用氨基酸的名称、三字母缩写或单字母缩写来指代氨基酸。如本文所用,术语"蛋白质"包括蛋白质、多肽和肽。如本文所用,术语"氨基酸序列"与术语"多肽"和/或术语"蛋白质"同义。在一些情况下,术语"氨基酸序列"与术语"肽"同义。在一些情况下,术语"氨基酸序列"与术语"酶"同义。术语"蛋白质"和"多肽"在本文中可互换使用。在本公开和权利要求中,可以使用氨基酸残基的常规单字母和三字母代码。根据IUPACIUB生物化学命名联合委员会(JCBN)定义氨基酸的3字母代码。还应当理解,由于遗传密码子的简并性,多肽可以由一个以上的核苷酸序列编码。

[1195] 在整个说明书中可以出现术语的其他定义。在更详细地描述示范性实施方案之前,应当了解,本公开不限于所描述的特定实施方案,且因此可变化。还应理解,本文所用的术语仅出于描述特定实施方案的目的,而不旨在进行限制,因为本公开的范围将仅由所附权利要求书界定。

[1196] 在提供数值范围的情况下,应当理解,除非上下文另外明确指出,否则在该范围的上限与下限之间的每一居中值(至下限单位的十分之一)也被具体公开。任何指定范围内的规定值或居中值与该指定范围内的任何其他指定值或居中值之间的每个较小范围涵盖在本公开内。这些较小范围的上限和下限可独立地包括或排除在该范围内,并且每个范围(其中较小范围内包括上下限之一、均不包括、或包括二者)也涵盖在本公开内,以所述范围内的任何明确排除的限值为准。当所述范围包括一个或两个界限时,排除这些所包括的界限中的一个或两个的范围也包括在本公开中。

[1197] 必须注意,除非上下文明确地另外指明,本说明书和所附权利要求中所用的单数形式"一种(a、an)"和"该(the)"包括复数指代物。因此,例如,提及"一种嵌合梭菌神经毒素"包括多种这样的候选药剂,提及"该嵌合梭菌神经毒素"包括提及一种或多种嵌合梭菌神经毒素和本领域技术人员已知的其等价物,等等。

[1198] 本文所讨论的出版物仅因在本申请的提交日期之前公开其内容而提供。本文中的任何内容都不应被解释为承认这些出版物构成所附权利要求的现有技术。

附图说明

[1199] 现在将参考以下附图和实施例,仅通过实施例的方式描述本发明的实施方案。本文提交的许多附图的以彩色形式可以更好地理解。附图的彩色版本是所提交的申请的一部分,因此保留在随后的程序中呈现附图的彩色图像的权利。

[1200] 图1显示了未处理的aDRG神经元的代表性单一图像(n=3),该神经元用能够识别切割的SNAP25(绿色)的抗体和用于核染色的DAPI进行染色。底部图像表示两个通道的合并。

[1201] 图2显示了aDRG神经元的代表性单一图像(n=3),该神经元用1nM rBoNT/A处理24小时并用能够识别切割的SNAP25(绿色)的抗体、针对aDRG标志物NF200、CGRP、P2X3、TrkA(红色)的抗体和用于核染色的DAPI进行染色。箭头指示共同定位更明显或不存在的特定区域。底部图像表示两个通道的合并。

[1202] 图3显示了aDRG神经元的代表性单一图像(n=3),该神经元用1nM mrBoNT/AB处理24小时并用能够识别切割的SNAP25(绿色)的抗体、针对aDRG标志物NF200、CGRP、P2X3、TrkA(红色)的抗体和用于核染色的DAPI进行染色。箭头指示共同定位更明显或不存在的特定区域。底部图像表示两个通道的合并。

[1203] 图4显示了在用KCl刺激之前用BoNT处理24小时的aDRG神经元的%CGRP释放。通过EIA测定CGRP释放。从刺激释放中减去基础CGRP释放,并将结果归一化为无BoNT对照细胞。将非线性曲线拟合于各个实验剂量响应(可变斜率-四个参数,其中曲线的底部被限制为20%)以确定IC_{50}。显示了与用mrBoNT/AB(n=3)或rBoNT/A(n=3)处理的KCl刺激的aDRG拟合的代表性曲线,显示了平均值±SEM。各实验一式三份进行。

[1204] 图5显示了用1nM BoNT处理24小时的aDRG的最大%CGRP释放抑制。单因素ANOVA与事后Tukey检验用于多重比较:**-p≤0.01.mrBoNT/AB(n=3)和rBoNT/A(n=3)。各实验一式三份进行(平均值±SEM)。

[1205] 图6显示了优选的皮内注射部位的位置。该表指示了给定目标神经末梢的面部每侧的注射次数以及该目标神经末梢的注射总次数。

[1206] 图7显示了用以下物质处理的细胞的基于CGRP释放(pg/ml)的浓度响应曲线:(A)rBoNT/A;或(B)mrBoNT/AB。每个平均值(▲)表示来自三个不同板的6个(或对于rBoNT/A为5个)样品的数据+/-标准偏差。还包括所有单个数据点(+)。

[1207] 图8显示了在体外大鼠原代TG神经元中用mrBoNT/AB(□;12.14)或rBoNT/A(o;9.67)处理24小时后,SNAP25切割的pEC_{50}值。数据是n=3次实验的均值±sem。****p<0.0001(Student's非配对t检验)。

[1208] 图9显示了在体外来自hiPSCs的感觉神经元中用mrBoNT/AB(pEC_{50}=12.85)或rBoNT/A(pEC_{50}=10.73)处理24小时后SNAP25切割的浓度-响应曲线值。

[1209] 图10显示了在通过肌内(IM)或皮内(ID)注射施用300pg/kg mrBoNT/AB的大鼠中,脑干(左)或三叉神经运动核(右)中的脊髓三叉神经感觉核对于切割的SNAP25染色呈阳性的面积比例(%)。*p<0.05;Mann-Whitney检验。

[1210] 图11显示了在通过肌内(IM)或皮内(ID)注射施用300pg/kg mrBoNT/AB的大鼠中,颈脊髓背角(感觉)(左)或腹角(运动)(右)中切割的SNAP25的量。如实施例12中所解释的,通过评分系统("c-SNAP25 IHC评分")表示该量。

[1211] 图12显示了在通过肌内(IM)或皮内(ID)注射施用300pg/kg mrBoNT/AB或通过IM注射施用肉毒杆菌毒素(Botox)的大鼠中,三叉神经节轴突中切割的SNAP25的量。基于实施例12中解释的评分系统分配"c-SNAP25 IHC评分"。**p<0.01;Kruskal-Wallis检验,随后进行Dunn多重比较检验。

[1212] 序列表

[1213] 当在下列SEQ ID NO中的任一项中指示起始Met氨基酸残基时,所述残基是任选的。

[1214] SEQ ID NO:1-嵌合梭菌神经毒素1(mrBoNT/AB)的多肽序列

[1215] SEQ ID NO:2-嵌合梭菌神经毒素2的多肽序列

[1216] SEQ ID NO:3-嵌合梭菌神经毒素3的多肽序列

[1217] SEQ ID NO:4-嵌合梭菌神经毒素4的多肽序列

[1218] SEQ ID NO:5-嵌合梭菌神经毒素5的多肽序列

[1219] SEQ ID NO:6-天然BoNT/A(rBoNT/A)的多肽序列

[1220] SEQ ID NO:7-BoNT/B的多肽序列

[1221] SEQ ID NO:8-BoNT/C的多肽序列

[1222] SEQ ID NO:9-BoNT/D的多肽序列

[1223] SEQ ID NO:10-BoNT/E的多肽序列

[1224] SEQ ID NO:11-BoNT/F的多肽序列

[1225] SEQ ID NO:12-BoNT/G的多肽序列

[1226] SEQ ID NO:13-BoNT/X的多肽序列

[1227] SEQ ID NO:14-TeNT的多肽序列

[1228] SEQ ID NO:15-C-末端L-链片段

[1229] SEQ ID NO:16-C-末端L-链片段2

[1230] SEQ ID NO:17-双链L-链1

[1231] SEQ ID NO:18-双链L-链2

[1232] SEQ ID NO:19-双链H-链

[1233] SEQ ID NO:1-嵌合梭菌神经毒素1(mrBoNT/AB)的多肽序列

[1234] MPFVNKQFNYKDPVNGVDIAYIKIPNAGQMQPVKAFKIHNKIWVIPERDTFTNPEEGDLNPPPEAKQVP
VSYYDSTYLSTDNEKDNYLKGVTKLFERIYSTDLGRMLLTSIVRGIPFWGGSTIDTELKVIDTNCINVIQPDGSYRS
EELNLVIIGPSADIIQFECKSFGHEVLNLTRNGYGSTQYIRFSPDFTFGFEESLEVDTNPLLGAGKFATDPAVTLAH
ELIHAGHRLYGIAINPNRVFKVNTNAYYEMSGLEVSFEELRTFGGHDAKFIDSLQENEFRLYYYNKFKDIASTLNKA
KSIVGTTASLQYMKNVFKEKYLLSEDTSGKFSVDKLKFDKLYKMLTEIYTEDNFVKFFKVLNRKTYLNFDKAVFKIN
IVPKVNYTIYDGFNLRNTNLAANFNGQNTEINNMNFTKLKNFTGLFEFYKLLCVRGIITSKTKSLDKGYNKALNDLC
IKVNNWDLFFSPSEDNFTNDLNKGEEITSDTNIEAAEENISLDLIQQYYLTFNFDNEPENISIENLSSDIIGQLELM
PNIERFPNGKKYELDKYTMFHYLRAQEFEHGKSRIALTNSVNEALLNPSRVYTFFSSDYVKKVNKATEAAMFLGWVE
QLVYDFTDETSEVSTTDKIADITIIIPYIGPALNIGNMLYKDDFVGALIFSGAVILLEFIPEIAIPVLGTFALVSYI
ANKVLTVQTIDNALSKRNEKWDEVYKYIVTNWLAKVNTQIDLIRKKMKEALENQAEATKAIINYQYNQYTEEEKNNI
NFNIDDLSSKLNESINKAMININKFLNQCSVSYLMNSMIPYGVKRLEDFDASLKDALLKYIYDNRGTLIGQVDRLKD
KVNNTLSTDIPFQLSKYVDNQRLLSTFTEYIKNILNNIILNLRYKDNNLIDLSGYGAKVEVYDGVELNDKNQFKLTS
SANSKIRVTQNQNIIFNSVFLDFSVSFWIRIPKYKNDGIQNYIHNEYTIINCMKNNSGWKISIRGNRIIWTLIDING
KTKSVFFEYNIREDISEYINRWFFVTITNNLNNAKIYINGKLESNTDIKDIREVIANGEIIFKLDGDIDRTQFIWMK
YFSIFNTELSQSNIEERYKIQSYSEYLKDFWGNPLMYNKEYYMFNAGNKNSYIKLKKDSPVGEILTRSKYNQNSKYI
NYRDLYIGEKFIIRRKSNSQSINDDIVRKEDYIYLDFFNLNQEWRVYTYKYFKKEEMKLFLAPIYDSDEFYNTIQIK
EYDEQPTYSCQLLFKKDEESTDEIGLIGIHRFYESGIVFEEYKDYFCISKWYLKEVKRKPYNLKLGCNWQFIPKDEG
WTE

[1235] SEQ ID NO:2-嵌合梭菌神经毒素2多肽序列

[1236] MPFVNKQFNYKDPVNGVDIAYIKIPNAGQMQPVKAFKIHNKIWVIPERDTFTNPEEGDLNPPPEAKQVP
VSYYDSTYLSTDNEKDNYLKGVTKLFERIYSTDLGRMLLTSIVRGIPFWGGSTIDTELKVIDTNCINVIQPDGSYRS
EELNLVIIGPSADIIQFECKSFGHEVLNLTRNGYGSTQYIRFSPDFTFGFEESLEVDTNPLLGAGKFATDPAVTLAH
ELIHAGHRLYGIAINPNRVFKVNTNAYYEMSGLEVSFEELRTFGGHDAKFIDSLQENEFRLYYYNKFKDIASTLNKA
KSIVGTTASLQYMKNVFKEKYLLSEDTSGKFSVDKLKFDKLYKMLTEIYTEDNFVKFFKVLNRKTYLNFDKAVFKIN
IVPKVNYTIYDGFNLRNTNLAANFNGQNTEINNMNFTKLKNFTGLFEFYKLLCVRGIITSKTKSLDKGYNKALNDLC
IKVNNWDLFFSPSEDNFTNDLNKGEEITSDTNIEAAEENISLDLIQQYYLTFNFDNEPENISIENLSSDIIGQLELM
PNIERFPNGKKYELDKYTMFHYLRAQEFEHGKSRIALTNSVNEALLNPSRVYTFFSSDYVKKVNKATEAAMFLGWVE
QLVYDFTDETSEVSTTDKIADITIIIPYIGPALNIGNMLYKDDFVGALIFSGAVILLEFIPEIAIPVLGTFALVSYI
ANKVLTVQTIDNALSKRNEKWDEVYKYIVTNWLAKVNTQIDLIRKKMKEALENQAEATKAIINYQYNQYTEEEKNNI
NFNIDDLSSKLNESINKAMININKFLNQCSVSYLMNSMIPYGVKRLEDFDASLKDALLKYIYDNRGTLIGQVDRLKD
KVNNTLSTDIPFQLSKYVDNQRLLSTFTEYIKSEILNNIILNLRYKDNNLIDLSGYGAKVEVYDGVELNDKNQFKLT
SSANSKIRVTQNQNIIFNSVFLDFSVSFWIRIPKYKNDGIQNYIHNEYTIINCMKNNSGWKISIRGNRIIWTLIDIN
GKTKSVFFEYNIREDISEYINRWFFVTITNNLNNAKIYINGKLESNTDIKDIREVIANGEIIFKLDGDIDRTQFIWM
KYFSIFNTELSQSNIEERYKIQSYSEYLKDFWGNPLMYNKEYYMFNAGNKNSYIKLKKDSPVGEILTRSKYNQNSKY
INYRDLYIGEKFIIRRKSNSQSINDDIVRKEDYIYLDFFNLNQEWRVYTYKYFKKEEMKLFLAPIYDSDEFYNTIQI
KEYDEQPTYSCQLLFKKDEESTDEIGLIGIHRFYESGIVFEEYKDYFCISKWYLKEVKRKPYNLKLGCNWQFIPKDE
GWTEHHHHHHHHHH

[1237] SEQ ID NO:3-嵌合梭菌神经毒素3多肽序列

[1238] MPFVNKQFNYKDPVNGVDIAYIKIPNAGQMQPVKAFKIHNKIWVIPERDTFTNPEEGDLNPPPEAKQVP
VSYYDSTYLSTDNEKDNYLKGVTKLFERIYSTDLGRMLLTSIVRGIPFWGGSTIDTELKVIDTNCINVIQPDGSYRS
EELNLVIIGPSADIIQFECKSFGHEVLNLTRNGYGSTQYIRFSPDFTFGFEESLEVDTNPLLGAGKFATDPAVTLAH
ELIHAGHRLYGIAINPNRVFKVNTNAYYEMSGLEVSFEELRTFGGHDAKFIDSLQENEFRLYYYNKFKDIASTLNKA
KSIVGTTASLQYMKNVFKEKYLLSEDTSGKFSVDKLKFDKLYKMLTEIYTEDNFVKFFKVLNRKTYLNFDKAVFKIN
IVPKVNYTIYDGFNLRNTNLAANFNGQNTEINNMNFTKLKNFTGLFEFYKLLCVRGIITSKTKSLDKGYNKALNDLC
IKVNNWDLFFSPSEDNFTNDLNKGEEITSDTNIEAAEENISLDLIQQYYLTFNFDNEPENISIENLSSDIIGQLELM
PNIERFPNGKKYELDKYTMFHYLRAQEFEHGKSRIALTNSVNEALLNPSRVYTFFSSDYVKKVNKATEAAMFLGWVE
QLVYDFTDETSEVSTTDKIADITIIIPYIGPALNIGNMLYKDDFVGALIFSGAVILLEFIPEIAIPVLGTFALVSYI
ANKVLTVQTIDNALSKRNEKWDEVYKYIVTNWLAKVNTQIDLIRKKMKEALENQAEATKAIINYQYNQYTEEEKNNI
NFNIDDLSSKLNESINKAMININKFLNQCSVSYLMNSMIPYGVKRLEDFDASLKDALLKYIYDNRGTLIGQVDRLKD
KVNNTLSTDIPFQLSKYVDNQRLLSTFTEYIKNIIELGGGGSELSEILNNIILNLRYKDNNLIDLSGYGAKVEVYDG
VELNDKNQFKLTSSANSKIRVTQNQNIIFNSVFLDFSVSFWIRIPKYKNDGIQNYIHNEYTIINCMKNNSGWKISIR
GNRIIWTLIDINGKTKSVFFEYNIREDISEYINRWFFVTITNNLNNAKIYINGKLESNTDIKDIREVIANGEIIFKL
DGDIDRTQFIWMKYFSIFNTELSQSNIEERYKIQSYSEYLKDFWGNPLMYNKEYYMFNAGNKNSYIKLKKDSPVGEI
LTRSKYNQNSKYINYRDLYIGEKFIIRRKSNSQSINDDIVRKEDYIYLDFFNLNQEWRVYTYKYFKKEEMKLFLAPI
YDSDEFYNTIQIKEYDEQPTYSCQLLFKKDEESTDEIGLIGIHRFYESGIVFEEYKDYFCISKWYLKEVKRKPYNLK
LGCNWQFIPKDEGWTEHHHHHHHHHH

[1239] SEQ ID NO:4-嵌合梭菌神经毒素4多肽序列

[1240] MPFVNKQFNYKDPVNGVDIAYIKIPNAGQMQPVKAFKIHNKIWVIPERDTFTNPEEGDLNPPPEAKQVP
VSYYDSTYLSTDNEKDNYLKGVTKLFERIYSTDLGRMLLTSIVRGIPFWGGSTIDTELKVIDTNCINVIQPDGSYRS
EELNLVIIGPSADIIQFECKSFGHEVLNLTRNGYGSTQYIRFSPDFTFGFEESLEVDTNPLLGAGKFATDPAVTLAH
ELIHAGHRLYGIAINPNRVFKVNTNAYYEMSGLEVSFEELRTFGGHDAKFIDSLQENEFRLYYYNKFKDIASTLNKA
KSIVGTTASLQYMKNVFKEKYLLSEDTSGKFSVDKLKFDKLYKMLTEIYTEDNFVKFFKVLNRKTYLNFDKAVFKIN
IVPKVNYTIYDGFNLRNTNLAANFNGQNTEINNMNFTKLKNFTGLFEFYKLLCVRGIITSKTKSLDKGYNKALNDLC
IKVNNWDLFFSPSEDNFTNDLNKGEEITSDTNIEAAEENISLDLIQQYYLTFNFDNEPENISIENLSSDIIGQLELM
PNIERFPNGKKYELDKYTMFHYLRAQEFEHGKSRIALTNSVNEALLNPSRVYTFFSSDYVKKVNKATEAAMFLGWVE
QLVYDFTDETSEVSTTDKIADITIIIPYIGPALNIGNMLYKDDFVGALIFSGAVILLEFIPEIAIPVLGTFALVSYI
ANKVLTVQTIDNALSKRNEKWDEVYKYIVTNWLAKVNTQIDLIRKKMKEALENQAEATKAIINYQYNQYTEEEKNNI
NFNIDDLSSKLNESINKAMININKFLNQCSVSYLMNSMIPYGVKRLEDFDASLKDALLKYIYDNRGTLIGQVDRLKD
KVNNTLSTDIPFQLSKYVDNQRLLSTFTEYIKNILNNIILNLRYKDNNLIDLSGYGAKVEVYDGVELNDKNQFKLTS
SANSKIRVTQNQNIIFNSVFLDFSVSFWIRIPKYKNDGIQNYIHNEYTIINCMKNNSGWKISIRGNRIIWTLIDING
KTKSVFFEYNIREDISEYINRWFFVTITNNLNNAKIYINGKLESNTDIKDIREVIANGEIIFKLDGDIDRTQFIWMK
YFSIFNTELSQSNIEERYKIQSYSEYLKDFWGNPLMYNKEYYMFNAGNKNSYIKLKKDSPVGEILTRSKYNQNSKYI
NYRDLYIGEKFIIRRKSNSQSINDDIVRKEDYIYLDFFNLNQEWRVYTYKYFKKEEMKLFLAPIYDSDEFYNTIQIK
EYDEQPTYSCQLLFKKDEESTDEIGLIGIHRFYESGIVFEEYKDYFCISKWYLKEVKRKPYNLKLGCNWQFIPKDEG
WTEHHHHHHHHHH

[1241] SEQ ID NO:5-嵌合梭菌神经毒素5多肽序列

[1242] MPFVNKQFNYKDPVNGVDIAYIKIPNAGQMQPVKAFKIHNKIWVIPERDTFTNPEEGDLNPPPEAKQVP
VSYYDSTYLSTDNEKDNYLKGVTKLFERIYSTDLGRMLLTSIVRGIPFWGGSTIDTELKVIDTNCINVIQPDGSYRS
EELNLVIIGPSADIIQFECKSFGHEVLNLTRNGYGSTQYIRFSPDFTFGFEESLEVDTNPLLGAGKFATDPAVTLAH
ELIHAGHRLYGIAINPNRVFKVNTNAYYEMSGLEVSFEELRTFGGHDAKFIDSLQENEFRLYYYNKFKDIASTLNKA
KSIVGTTASLQYMKNVFKEKYLLSEDTSGKFSVDKLKFDKLYKMLTEIYTEDNFVKFFKVLNRKTYLNFDKAVFKIN
IVPKVNYTIYDGFNLRNTNLAANFNGQNTEINNMNFTKLKNFTGLFEFYKLLCVRGIITSKTKSLDKGYNKALNDLC
IKVNNWDLFFSPSEDNFTNDLNKGEEITSDTNIEAAEENISLDLIQQYYLTFNFDNEPENISIENLSSDIIGQLELM
PNIERFPNGKKYELDKYTMFHYLRAQEFEHGKSRIALTNSVNEALLNPSRVYTFFSSDYVKKVNKATEAAMFLGWVE
QLVYDFTDETSEVSTTDKIADITIIIPYIGPALNIGNMLYKDDFVGALIFSGAVILLEFIPEIAIPVLGTFALVSYI
ANKVLTVQTIDNALSKRNEKWDEVYKYIVTNWLAKVNTQIDLIRKKMKEALENQAEATKAIINYQYNQYTEEEKNNI
NFNIDDLSSKLNESINKAMININKFLNQCSVSYLMNSMIPYGVKRLEDFDASLKDALLKYIYDNRGTLIGQVDRLKD
KVNNTLSTDIPFQLSKYVDNQRLLSTFTEYIKNILNNIILNLRYKDNNLIDLSGYGAKVEVYDGVELNDKNQFKLTS
SANSKIRVTQNQNIIFNSVFLDFSVSFWIRIPKYKNDGIQNYIHNEYTIINCMKNNSGWKISIRGNRIIWTLIDING
KTKSVFFEYNIREDISEYINRWFFVTITNNLNNAKIYINGKLESNTDIKDIREVIANGEIIFKLDGDIDRTQFIWMK
YFSIFNTELSQSNIEERYKIQSYSEYLKDFWGNPLMYNKEYYMFNAGNKNSYIKLKKDSPVGEILTRSKYNQNSKYI
NYRDLYIGEKFIIRRKSNSQSINDDIVRKEDYIYLDFFNLNQEWRVYTYKYFKKEEEKLFLAPISDSDEFYNTIQIK
EYDEQPTYSCQLLFKKDEESTDEIGLIGIHRFYESGIVFEEYKDYFCISKWYLKEVKRKPYNLKLGCNWQFIPKDEG
WTE

[1243] SEQ ID NO:6-天然BoNT/A(rBoNT/A)的多肽序列

[1244] MPFVNKQFNYKDPVNGVDIAYIKIPNAGQMQPVKAFKIHNKIWVIPERDTFTNPEEGDLNPPPEAKQVP
VSYYDSTYLSTDNEKDNYLKGVTKLFERIYSTDLGRMLLTSIVRGIPFWGGSTIDTELKVIDTNCINVIQPDGSYRS
EELNLVIIGPSADIIQFECKSFGHEVLNLTRNGYGSTQYIRFSPDFTFGFEESLEVDTNPLLGAGKFATDPAVTLAH
ELIHAGHRLYGIAINPNRVFKVNTNAYYEMSGLEVSFEELRTFGGHDAKFIDSLQENEFRLYYYNKFKDIASTLNKA
KSIVGTTASLQYMKNVFKEKYLLSEDTSGKFSVDKLKFDKLYKMLTEIYTEDNFVKFFKVLNRKTYLNFDKAVFKIN
IVPKVNYTIYDGFNLRNTNLAANFNGQNTEINNMNFTKLKNFTGLFEFYKLLCVRGIITSKTKSLDKGYNKALNDLC
IKVNNWDLFFSPSEDNFTNDLNKGEEITSDTNIEAAEENISLDLIQQYYLTFNFDNEPENISIENLSSDIIGQLELM
PNIERFPNGKKYELDKYTMFHYLRAQEFEHGKSRIALTNSVNEALLNPSRVYTFFSSDYVKKVNKATEAAMFLGWVE
QLVYDFTDETSEVSTTDKIADITIIIPYIGPALNIGNMLYKDDFVGALIFSGAVILLEFIPEIAIPVLGTFALVSYI
ANKVLTVQTIDNALSKRNEKWDEVYKYIVTNWLAKVNTQIDLIRKKMKEALENQAEATKAIINYQYNQYTEEEKNNI
NFNIDDLSSKLNESINKAMININKFLNQCSVSYLMNSMIPYGVKRLEDFDASLKDALLKYIYDNRGTLIGQVDRLKD
KVNNTLSTDIPFQLSKYVDNQRLLSTFTEYIKNIINTSILNLRYESNHLIDLSRYASKINIGSKVNFDPIDKNQIQL
FNLESSKIEVILKNAIVYNSMYENFSTSFWIRIPKYFNSISLNNEYTIINCMENNSGWKVSLNYGEIIWTLQDTQEI
KQRVVFKYSQMINISDYINRWIFVTITNNRLNNSKIYINGRLIDQKPISNLGNIHASNNIMFKLDGCRDTHRYIWIK
YFNLFDKELNEKEIKDLYDNQSNSGILKDFWGDYLQYDKPYYMLNLYDPNKYVDVNNVGIRGYMYLKGPRGSVMTTN
IYLNSSLYRGTKFIIKKYASGNKDNIVRNNDRVYINVVVKNKEYRLATNASQAGVEKILSALEIPDVGNLSQVVVMK
SKNDQGITNKCKMNLQDNNGNDIGFIGFHQFNNIAKLVASNWYNRQIERSSRTLGCSWEFIPVDDGWGERPL

[1245] SEQ ID NO:7-BoNT/B的多肽序列

[1246] MPVTINNFNYNDPIDNNNIIMMEPPFARGTGRYYKAFKITDRIWIIPERYTFGYKPEDFNKSSGIFNRD
VCEYYDPDYLNTNDKKNIFLQTMIKLFNRIKSKPLGEKLLEMIINGIPYLGDRRVPLEEFNTNIASVTVNKLISNPG

EVERKKGIFANLIIFGPGPVLNENETIDIGIQNHFASREGFGGIMQMKFCPEYVSVFNNVQENKGASIFNRRGYFSD
PALILMHELIHVLHGLYGIKVDDLPIVPNEKKFFMQSTDAIQAEELYTFGGQDPSIITPSTDKSIYDKVLQNFRGIV
DRLNKVLVCISDPNININIYKNKFKDKYKFVEDSEGKYSIDVESFDKLYKSLMFGFTETNIAENYKIKTRASYFSDS
LPPVKIKNLLDNEIYTIEEGFNISDKDMEKEYRGQNKAINKQAYEEISKEHLAVYKIQMCKSVKAPGICIDVDNEDL
FFIADKNSFSDDLSKNERIEYNTQSNYIENDFPINELILDTDLISKIELPSENTESLTDFNVDVPVYEKQPAIKKIF
TDENTIFQYLYSQTFPLDIRDISLTSSFDDALLFSNKVYSFFSMDYIKTANKVVEAGLFAGWVKQIVNDFVIEANKS
NTMDKIADISLIVPYIGLALNVGNETAKGNFENAFEIAGASILLEFIPELLIPVVGAFLLESYIDNKNKIIKTIDNA
LTKRNEKWSDMYGLIVAQWLSTVNTQFYTIKEGMYKALNYQAQALEEIIKYRYNIYSEKEKSNINIDFNDINSKLNE
GINQAIDNINNFINGCSVSYLMKKMIPLAVEKLLDFDNTLKKNLLNYIDENKLYLIGSAEYEKSKVNKYLKTIMPFD
LSIYTNDTILIEMFNKYNSEILNNIILNLRYKDNNLIDLSGYGAKVEVYDGVELNDKNQFKLTSSANSKIRVTQNQN
IIFNSVFLDFSVSFWIRIPKYKNDGIQNYIHNEYTIINCMKNNSGWKISIRGNRIIWTLIDINGKTKSVFFEYNIRE
DISEYINRWFFVTITNNLNNAKIYINGKLESNTDIKDIREVIANGEIIFKLDGDIDRTQFIWMKYFSIFNTELSQSN
IEERYKIQSYSEYLKDFWGNPLMYNKEYYMFNAGNKNSYIKLKKDSPVGEILTRSKYNQNSKYINYRDLYIGEKFII
RRKSNSQSINDDIVRKEDYIYLDFFNLNQEWRVYTYKYFKKEEEKLFLAPISDSDEFYNTIQIKEYDEQPTYSCQLL
FKKDEESTDEIGLIGIHRFYESGIVFEEYKDYFCISKWYLKEVKRKPYNLKLGCNWQFIPKDEGWTE

[1247] SEQ ID NO:8-BoNT/C的多肽序列

[1248] MPITINNFNYSDPVDNKNILYLDTHLNTLANEPEKAFRITGNIWVIPDRFSRNSNPNLNKPPRVTSPKS
GYYDPNYLSTDSDKDPFLKEIIKLFKRINSREIGEELIYRLSTDIPFPGNNNTPINTFDFDVDFNSVDVKTRQGNNW
VKTGSINPSVIITGPRENIIDPETSTFKLTNNTFAAQEGFGALSIISISPRFMLTYSNATNDVGEGRFSKSEFCMDP
ILILMHELNHAMHNLYGIAIPNDQTISSVTSNIFYSQYNVKLEYAEIYAFGGPTIDLIPKSARKYFEEKALDYYRSI
AKRLNSITTANPSSFNKYIGEYKQKLIRKYRFVVESSGEVTVNRNKFVELYNELTQIFTEFNYAKIYNVQNRKIYLS
NVYTPVTANILDDNVYDIQNGFNIPKSNLNVLFMGQNLSRNPALRKVNPENMLYLFTKFCHKAIDGRSLYNKTLDCR
ELLVKNTDLPFIGDISDVKTDIFLRKDINEETEVIYYPDNVSVDQVILSKNTSEHGQLDLLYPSIDSESEILPGENQ
VFYDNRTQNVDYLNSYYYLESQKLSDNVEDFTFTRSIEEALDNSAKVYTYFPTLANKVNAGVQGGLFLMWANDVVED
FTTNILRKDTLDKISDVSAIIPYIGPALNISNSVRRGNFTEAFAVTGVTILLEAFPEFTIPALGAFVIYSKVQERNE
IIKTIDNCLEQRIKRWKDSYEWMMGTWLSRIITQFNNISYQMYDSLNYQAGAIKAKIDLEYKKYSGSDKENIKSQVE
NLKNSLDVKISEAMNNINKFIRECSVTYLFKNMLPKVIDELNEFDRNTKAKLINLIDSHNIILVGEVDKLKAKVNNS
FQNTIPFNIFSYTNNSLLKDIINEYFNNINDSKILSLQNRKNTLVDTSGYNAEVSEEGDVQLNPIFPFDFKLGSSGE
DRGKVIVTQNENIVYNSMYESFSISFWIRINKWVSNLPGYTIIDSVKNNSGWSIGIISNFLVFTLKQNEDSEQSINF
SYDISNNAPGYNKWFFVTVTNNMMGNMKIYINGKLIDTIKVKELTGINFSKTITFEINKIPDTGLITSDSDNINMWI
RDFYIFAKELDGKDINILFNSLQYTNVVKDYWGNDLRYNKEYYMVNIDYLNRYMYANSRQIVFNTRRNNNDFNEGYK
IIIKRIRGNTNDTRVRGGDILYFDMTINNKAYNLFMKNETMYADNHSTEDIYAIGLREQTKDINDNIIFQIQPMNNT
YYYASQIFKSNFNGENISGICSIGTYRFRLGGDWYRHNYLVPTVKQGNYASLLESTSTHWGFVPVSE

[1249] SEQ ID NO:9-BoNT/D的多肽序列

[1250] MTWPVKDFNYSDPVNDNDILYLRIPQNKLITTPVKAFMITQNIWVIPERFSSDTNPSLSKPPRPTSKYQ
SYYDPSYLSTDEQKDTFLKGIIKLFKRINERDIGKKLINYLVVGSPFMGDSSTPEDTFDFTRHTTNIAVEKFENGSW
KVTNIITPSVLIFGPLPNILDYTASLTLQGQQSNPSFEGFGTLSILKVAPEFLLTFSDVTSNQSSAVLGKSIFCMDP
VIALMHELTHSLHQLYGINIPSDKRIRPQVSEGFFSQDGPNVQFEELYTFGGLDVEIIPQIERSQLREKALGHYKDI
AKRLNNINKTIPSSWISNIDKYKKIFSEKYNFDKDNTGNFVVNIDKFNSLYSDLTNVMSEVVYSSQYNVKNRTHYFS

RHYLPVFANILDDNIYTIRDGFNLTNKGFNIENSGQNIERNPALQKLSSESVVDLFTKVCLRLTKNSRDDSTCIKVK
NNRLPYVADKDSISQEIFENKIITDETNVQNYSDKFSLDESILDGQVPINPEIVDPLLPNVNMEPLNLPGEEIVFYD
DITKYVDYLNSYYYLESQKLSNNVENITLTTSVEEALGYSNKIYTFLPSLAEKVNKGVQAGLFLNWANEVVEDFTTN
IMKKDTLDKISDVSVIIPYIGPALNIGNSALRGNFNQAFATAGVAFLLEGFPEFTIPALGVFTFYSSIQEREKIIKT
IENCLEQRVKRWKDSYQWMVSNWLSRITTQFNHINYQMYDSLSYQADAIKAKIDLEYKKYSGSDKENIKSQVENLKN
SLDVKISEAMNNINKFIRECSVTYLFKNMLPKVIDELNKFDLRTKTELINLIDSHNIILVGEVDRLKAKVNESFENT
MPFNIFSYTNNSLLKDIINEYFNSINDSKILSLQNKKNALVDTSGYNAEVRVGDNVQLNTIYTNDFKLSSSGDKIIV
NLNNNILYSAIYENSSVSFWIKISKDLTNSHNEYTIINSIEQNSGWKLCIRNGNIEWILQDVNRKYKSLIFDYSESL
SHTGYTNKWFFVTITNNIMGYMKLYINGELKQSQKIEDLDEVKLDKTIVFGIDENIDENQMLWIRDFNIFSKELSNE
DINIVYEGQILRNVIKDYWGNPLKFDTEYYIINDNYIDRYIAPESNVLVLVQYPDRSKLYTGNPITIKSVSDKNPYS
RILNGDNIILHMLYNSRKYMIIRDTDTIYATQGGECSQNCVYALKLQSNLGNYGIGIFSIKNIVSKNKYCSQIFSSF
RENTMLLADIYKPWRFSFKNAYTPVAVTNYETKLLSTSSFWKFISRDPGWVE

[1251] SEQ ID NO:10-BoNT/E的多肽序列

[1252] MPKINSFNYNDPVNDRTILYIKPGGCQEFYKSFNIMKNIWIIPERNVIGTTPQDFHPPTSLKNGDSSYY
DPNYLQSDEEKDRFLKIVTKIFNRINNNLSGGILLEELSKANPYLGNDNTPDNQFHIGDASAVEIKFSNGSQDILLP
NVIIMGAEPDLFETNSSNISLRNNYMPSNHRFGSIAIVTFSPEYSFRFNDNCMNEFIQDPALTLMHELIHSLHGLYG
AKGITTKYTITQKQNPLITNIRGTNIEEFLTFGGTDLNIITSAQSNDIYTNLLADYKKIASKLSKVQVSNPLLNPYK
DVFEAKYGLDKDASGIYSVNINKFNDIFKKLYSFTEFDLRTKFQVKCRQTYIGQYKYFKLSNLLNDSIYNISEGYNI
NNLKVNFRGQNANLNPRIITPITGRGLVKKIIRFCKNIVSVKGIRKSICIEINNGELFFVASENSYNDDNINTPKEI
DDTVTSNNNYENDLDQVILNFNSESAPGLSDEKLNLTIQNDAYIPKYDSNGTSDIEQHDVNELNVFFYLDAQKVPEG
ENNVNLTSSIDTALLEQPKIYTFFSSEFINNVNKPVQAALFVSWIQQVLVDFTTEANQKSTVDKIADISIVVPYIGL
ALNIGNEAQKGNFKDALELLGAGILLEFEPELLIPTILVFTIKSFLGSSDNKNKVIKAINNALKERDEKWKEVYSFI
VSNWMTKINTQFNKRKEQMYQALQNQVNAIKTIIESKYNSYTLEEKNELTNKYDIKQIENELNQKVSIAMNNIDRFL
TESSISYLMKIINEVKINKLREYDENVKTYLLNYIIQHGSILGESQQELNSMVTDTLNNSIPFKLSSYTDDKILISY
FNKFFKRIKSSSVLNMRYKNDKYVDTSGYDSNININGDVYKYPTNKNQFGIYNDKLSEVNISQNDYIIYDNKYKNFS
ISFWVRIPNYDNKIVNVNNEYTIINCMRDNNSGWKVSLNHNEIIWTFEDNRGINQKLAFNYGNANGISDYINKWIFV
TITNDRLGDSKLYINGNLIDQKSILNLGNIHVSDNILFKIVNCSYTRYIGIRYFNIFDKELDETEIQTLYSNEPNTN
ILKDFWGNYLLYDKEYYLLNVLKPNNFIDRRKDSTLSINNIRSTILLANRLYSGIKVKIQRVNNSSTNDNLVRKNDQ
VYINFVASKTHLFPLYADTATTNKEKTIKISSSGNRFNQVVVMNSVGNCTMNFKNNNGNNIGLLGFKADTVVASTWY
YTHMRDHTNSNGCFWNFISEEHGWQEK

[1253] SEQ ID NO:11-BoNT/F的多肽序列

[1254] MPVVINSFNYNDPVNDDTILYMQIPYEEKSKKYYKAFEIMRNVWIIPERNTIGTDPSDFDPPASLENGS
SAYYDPNYLTTDAEKDRYLKTTIKLFKRINSNPAGEVLLQEISYAKPYLGNEHTPINEFHPVTRTTSVNIKSSTNVK
SSIILNLLVLGAGPDIFENSSYPVRKLMDSGGVYDPSNDGFGSINIVTFSPEYEYTFNDISGGYNSSTESFIADPAI
SLAHELIHALHGLYGARGVTYKETIKVKQAPLMIAEKPIRLEEFLTFGGQDLNIITSAMKEKIYNNLLANYEKIATR
LSRVNSAPPEYDINEYKDYFQWKYGLDKNADGSYTVNENKFNEIYKKLYSFTEIDLANKFKVKCRNTYFIKYGFLKV
PNLLDDDIYTVSEGFNIGNLAVNNRGQNIKLNPKIIDSIPDKGLVEKIVKFCKSVIPRKGTKAPPRLCIRVNNRELF
FVASESSYNENDINTPKEIDDTTNLNNNYRNNLDEVILDYNSETIPQISNQTLNTLVQDDSYVPRYDSNGTSEIEEH
NVVDLNVFFYLHAQKVPEGETNISLTSSIDTALSEESQVYTFFSSEFINTINKPVHAALFISWINQVIRDFTTEATQ

KSTFDKIADISLVVPYVGLALNIGNEVQKENFKEAFELLGAGILLEFVPELLIPTILVFTIKSFIGSSENKNKIIKA
INNSLMERETKWKEIYSWIVSNWLTRINTQFNKRKEQMYQALQNQVDAIKTVIEYKYNNYTSDERNRLESEYNINNI
REELNKKVSLAMENIERFITESSIFYLMKLINEAKVSKLREYDEGVKEYLLDYISEHRSILGNSVQELNDLVTSTLN
NSIPFELSSYTNDKILILYFNKLYKKIKDNSILDMRYENNKFIDISGYGSNISINGDVYIYSTNRNQFGIYSSKPSE
VNIAQNNDIIYNGRYQNFSISFWVRIPKYFNKVNLNNEYTIIDCIRNNNSGWKISLNYNKIIWTLQDTAGNNQKLVF
NYTQMISISDYINKWIFVTITNNRLGNSRIYINGNLIDEKSISNLGDIHVSDNILFKIVGCNDTRYVGIRYFKVFDT
ELGKTEIETLYSDEPDPSILKDFWGNYLLYNKRYYLLNLLRTDKSITQNSNFLNINQQRGVYQKPNIFSNTRLYTGV
EVIIRKNGSTDISNTDNFVRKNDLAYINVVDRDVEYRLYADISIAKPEKIIKLIRTSNSNNSLGQIIVMDSIGNNCT
MNFQNNNGGNIGLLGFHSNNLVASSWYYNNIRKNTSSNGCFWSFISKEHGWQEN

[1255] SEQ ID NO:12-BoNT/G的多肽序列

[1256] MPVNIKNFNYNDPINNDDIIMMEPFNDPGPGTYYKAFRIIDRIWIVPERFTYGFQPDQFNASTGVFSKD
VYEYYDPTYLKTDAEKDKFLKTMIKLFNRINSKPSGQRLLDMIVDAIPYLGNASTPPDKFAANVANVSINKKIIQPG
AEDQIKGLMTNLIIFGPGPVLSDNFTDSMIMNGHSPISEGFGARMMIRFCPSCLNVFNNVQENKDTSIFSRRAYFAD
PALTLMHELIHVLHGLYGIKISNLPITPNTKEFFMQHSDPVQAEELYTFGGHDPSVISPSTDMNIYNKALQNFQDIA
NRLNIVSSAQGSGIDISLYKQIYKNKYDFVEDPNGKYSVDKDKFDKLYKALMFGFTETNLAGEYGIKTRYSYFSEYL
PPIKTEKLLDNTIYTQNEGFNIASKNLKTEFNGQNKAVNKEAYEEISLEHLVIYRIAMCKPVMYKNTGKSEQCIIVN
NEDLFFIANKDSFSKDLAKAETIAYNTQNNTIENNFSIDQLILDNDLSSGIDLPNENTEPFTNFDDIDIPVYIKQSA
LKKIFVDGDSLFEYLHAQTFPSNIENLQLTNSLNDALRNNNKVYTFFSTNLVEKANTVVGASLFVNWVKGVIDDFTS
ESTQKSTIDKVSDVSIIIPYIGPALNVGNETAKENFKNAFEIGGAAILMEFIPELIVPIVGFFTLESYVGNKGHIIM
TISNALKKRDQKWTDMYGLIVSQWLSTVNTQFYTIKERMYNALNNQSQAIEKIIEDQYNRYSEEDKMNINIDFNDID
FKLNQSINLAINNIDDFINQCSISYLMNRMIPLAVKKLKDFDDNLKRDLLEYIDTNELYLLDEVNILKSKVNRHLKD
SIPFDLSLYTKDTILIQVFNNYISNISSNAILSLSYRGGRLIDSSGYGATMNVGSDVIFNDIGNGQFKLNNSENSNI
TAHQSKFVVYDSMFDNFSINFWVRTPKYNNNDIQTYLQNEYTIISCIKNDSGWKVSIKGNRIIWTLIDVNAKSKSIF
FEYSIKDNISDYINKWFSITITNDRLGNANIYINGSLKKSEKILNLDRINSSNDIDFKLINCTDTTKFVWIKDFNIF
GRELNATEVSSLYWIQSSTNTLKDFWGNPLRYDTQYYLFNQGMQNIYIKYFSKASMGETAPRTNFNNAAINYQNLYL
GLRFIIKKASNSRNINNDNIVREGDYIYLNIDNISDESYRVYVLVNSKEIQTQLFLAPINDDPTFYDVLQIKKYYEK
TTYNCQILCEKDTKTFGLFGIGKFVKDYGYVWDTYDNYFCISQWYLRRISENINKLRLGCNWQFIPVDEGWTE

[1257] SEQ ID NO:13-BoNT/X的多肽序列

[1258] MKLEINKFNYNDPIDGINVITMRPPRHSDKINKGKGPFKAFQVIKNIWIVPERYNFTNNTNDLNIPSEP
IMEADAIYNPNYLNTPSEKDEFLQGVIKVLERIKSKPEGEKLLELISSSIPLPLVSNGALTLSDNETIAYQENNNIV
SNLQANLVIYGPGPDIANNATYGLYSTPISNGEGTLSEVSFSPFYLKPFDESYGNYRSLVNIVNKFVKREFAPDPAS
TLMHELVHVTHNLYGISNRNFYYNFDTGKIETSRQQNSLIFEELLTFGGIDSKAISSLIIKKIIETAKNNYTTLISE
RLNTVTVENDLLKYIKNKIPVQGRLGNFKLDTAEFEKKLNTILFVLNESNLAQRFSILVRKHYLKERPIDPIYVNIL
DDNSYSTLEGFNISSQGSNDFQGQLLESSYFEKIESNALRAFIKICPRNGLLYNAIYRNSKNYLNNIDLEDKKTTSK
TNVSYPCSLLNGCIEVENKDLFLISNKDSLNDINLSEEKIKPETTVFFKDKLPPQDITLSNYDFTEANSIPSISQQN
ILERNEELYEPIRNSLFEIKTIYVDKLTTFHFLEAQNIDESIDSSKIRVELTDSVDEALSNPNKVYSPFKNMSNTIN
SIETGITSTYIFYQWLRSIVKDFSDETGKIDVIDKSSDTLAIVPYIGPLLNIGNDIRHGDFVGAIELAGITALLEYV
PEFTIPILVGLEVIGGELAREQVEAIVNNALDKRDQKWAEVYNITKAQWWGTIHLQINTRLAHTYKALSRQANAIKM
NMEFQLANYKGNIDDKAKIKNAISETEILLNKSVEQAMKNTEKFMIKLSNSYLTKEMIPKVQDNLKNFDLETKKTLD

KFIKEKEDILGTNLSSSLRRKVSIRLNKNIAFDINDIPFSEFDDLINQYKNEIEDYEVLNLGAEDGKIKDLSGTTSD
INIGSDIELADGRENKAIKIKGSENSTIKIAMNKYLRFSATDNFSISFWIKHPKPTNLLNNGIEYTLVENFNQRGWK
ISIQDSKLIWYLRDHNNSIKIVTPDYIAFNGWNLITITNNRSKGSIVYVNGSKIEEKDISSIWNTEVDDPIIFRLKN
NRDTQAFTLLDQFSIYRKELNQNEVVKLYNYYFNSNYIRDIWGNPLQYNKKYYLQTQDKPGKGLIREYWSSFGYDYV
ILSDSKTITFPNNIRYGALYNGSKVLIKNSKKLDGLVRNKDFIQLEIDGYNMGISADRFNEDTNYIGTTYGTTHDLT
TDFEIIQRQEKYRNYCQLKTPYNIFHKSGLMSTETSKPTFHDYRDWVYSSAWYFQNYENLNLRKHTKTNWYFIPKDE
GWDED

[1259] SEQ ID NO:14-TeNT的多肽序列

[1260] MPITINNFRYSDPVNNDTIIMMEPPYCKGLDIYYKAFKITDRIWIVPERYEFGTKPEDFNPPSSLIEGA
SEYYDPNYLRTDSDKDRFLQTMVKLFNRIKNNVAGEALLDKIINAIPYLGNSYSLLDKFDTNSNSVSFNLLEQDPSG
ATTKSAMLTNLIIFGPGPVLNKNEVRGIVLRVDNKNYFPCRDGFGSIMQMAFCPEYVPTFDNVIENITSLTIGKSKY
FQDPALLLMHELIHVLHGLYGMQVSSHEIIPSKQEIYMQHTYPISAEELFTFGGQDANLISIDIKNDLYEKTLNDYK
AIANKLSQVTSCNDPNIDIDSYKQIYQQKYQFDKDSNGQYIVNEDKFQILYNSIMYGFTEIELGKKFNIKTRLSYFS
MNHDPVKIPNLLDDTIYNDTEGFNIESKDLKSEYKGQNMRVNTNAFRNVDGSGLVSKLIGLCKKIIPPTNIRENLYN
RTASLTDLGGELCIKIKNEDLTFIAEKNSFSEEPFQDEIVSYNTKNKPLNFNYSLDKIIVDYNLQSKITLPNDRTTP
VTKGIPYAPEYKSNAASTIEIHNIDDNTIYQYLYAQKSPTTLQRITMTNSVDDALINSTKIYSYFPSVISKVNQGAQ
GILFLQWVRDIIDDFTNESSQKTTIDKISDVSTIVPYIGPALNIVKQGYEGNFIGALETTGVVLLLEYIPEITLPVI
AALSIAESSTQKEKIIKTIDNFLEKRYEKWIEVYKLVKAKWLGTVNTQFQKRSYQMYRSLEYQVDAIKKIIDYEYKI
YSGPDKEQIADEINNLKNKLEEKANKAMININIFMRESSRSFLVNQMINEAKKQLLEFDTQSKNILMQYIKANSKFI
GITELKKLESKINKVFSTPIPFSYSKNLDCWVDNEEDIDVILKKSTILNLDINNDIISDISGFNSSVITYPDAQLVP
GINGKAIHLVNNESSEVIVHKAMDIEYNDMFNNFTVSFWLRVPKVSASHLEQYGTNEYSIISSMKKHSLSIGSGWSV
SLKGNNLIWTLKDSAGEVRQITFRDLPDKFNAYLANKWVFITITNDRLSSANLYINGVLMGSAEITGLGAIREDNNI
TLKLDRCNNNNQYVSIDKFRIFCKALNPKEIEKLYTSYLSITFLRDFWGNPLRYDTEYYLIPVASSSKDVQLKNITD
YMYLTNAPSYTNGKLNIYYRRLYNGLKFIIKRYTPNNEIDSFVKSGDFIKLYVSYNNNEHIVGYPKDGNAFNNLDRI
LRVGYNAPGIPLYKKMEAVKLRDLKTYSVQLKLYDDKNASLGLVGTHNGQIGNDPNRDILIASNWYFNHLKDKILGC
DWYFVPTDEGWTND

[1261] SEQ ID NO:15-C-末端L-链片段

[1262] TKSLDKGYNK

[1263] SEQ ID NO:16-C-末端L-链片段2

[1264] SLDKGYNK

[1265] SEQ ID NO:17-双链L-链1

[1266] PFVNKQFNYKDPVNGVDIAYIKIPNAGQMQPVKAFKIHNKIWVIPERDTFTNPEEGDLNPPPEAKQVPV
SYYDSTYLSTDNEKDNYLKGVTKLFERIYSTDLGRMLLTSIVRGIPFWGGSTIDTELKVIDTNCINVIQPDGSYRSE
ELNLVIIGPSADIIQFECKSFGHEVLNLTRNGYGSTQYIRFSPDFTFGFEESLEVDTNPLLGAGKFATDPAVTLAHE
LIHAGHRLYGIAINPNRVFKVNTNAYYEMSGLEVSFEELRTFGGHDAKFIDSLQENEFRLYYYNKFKDIASTLNKAK
SIVGTTASLQYMKNVFKEKYLLSEDTSGKFSVDKLKFDKLYKMLTEIYTEDNFVKFFKVLNRKTYLNFDKAVFKINI
VPKVNYTIYDGFNLRNTNLAANFNGQNTEINNMNFTKLKNFTGLFEFYKLLCVRGIITSK

[1267] SEQ ID NO:18-双链L-链2

[1268] PFVNKQFNYKDPVNGVDIAYIKIPNAGQMQPVKAFKIHNKIWVIPERDTFTNPEEGDLNPPPEAKQVPV

SYYDSTYLSTDNEKDNYLKGVTKLFERIYSTDLGRMLLTSIVRGIPFWGGSTIDTELKVIDTNCINVIQPDGSYRSE
ELNLVIIGPSADIIQFECKSFGHEVLNLTRNGYGSTQYIRFSPDFTFGFEESLEVDTNPLLGAGKFATDPAVTLAHE
LIHAGHRLYGIAINPNRVFKVNTNAYYEMSGLEVSFEELRTFGGHDAKFIDSLQENEFRLYYYNKFKDIASTLNKAK
SIVGTTASLQYMKNVFKEKYLLSEDTSGKFSVDKLKFDKLYKMLTEIYTEDNFVKFFKVLNRKTYLNFDKAVFKINI
VPKVNYTIYDGFNLRNTNLAANFNGQNTEINNMNFTKLKNFTGLFEFYKLLCVRGIITSKTK

[1269] SEQ ID NO:19-双链H-链

[1270] ALNDLCIKVNNWDLFFSPSEDNFTNDLNKGEEITSDTNIEAAEENISLDLIQQYYLTFNFDNEPENISI
ENLSSDIIGQLELMPNIERFPNGKKYELDKYTMFHYLRAQEFEHGKSRIALTNSVNEALLNPSRVYTFFSSDYVKKV
NKATEAAMFLGWVEQLVYDFTDETSEVSTTDKIADITIIIPYIGPALNIGNMLYKDDFVGALIFSGAVILLEFIPEI
AIPVLGTFALVSYIANKVLTVQTIDNALSKRNEKWDEVYKYIVTNWLAKVNTQIDLIRKKMKEALENQAEATKAIIN
YQYNQYTEEEKNNINFNIDDLSSKLNESINKAMININKFLNQCSVSYLMNSMIPYGVKRLEDFDASLKDALLKYIYD
NRGTLIGQVDRLKDKVNNTLSTDIPFQLSKYVDNQRLLSTFTEYIKNILNNIILNLRYKDNNLIDLSGYGAKVEVYD
GVELNDKNQFKLTSSANSKIRVTQNQNIIFNSVFLDFSVSFWIRIPKYKNDGIQNYIHNEYTIINCMKNNSGWKISI
RGNRIIWTLIDINGKTKSVFFEYNIREDISEYINRWFFVTITNNLNNAKIYINGKLESNTDIKDIREVIANGEIIFK
LDGDIDRTQFIWMKYFSIFNTELSQSNIEERYKIQSYSEYLKDFWGNPLMYNKEYYMFNAGNKNSYIKLKKDSPVGE
ILTRSKYNQNSKYINYRDLYIGEKFIIRRKSNSQSINDDIVRKEDYIYLDFFNLNQEWRVYTYKYFKKEEMKLFLAP
IYDSDEFYNTIQIKEYDEQPTYSCQLLFKKDEESTDEIGLIGIHRFYESGIVFEEYKDYFCISKWYLKEVKRKPYNL
KLGCNWQFIPKDEGWTE

[1271] 实施例

[1272] 实施例1

[1273] 嵌合梭菌神经毒素BoNT/AB靶向与BoNT/A不同类型的神经元

[1274] 设计一项研究以确定由各种梭菌神经毒素中毒的神经元亚型。采用成年大鼠背根神经节(aDRG)体外模型。在该模型的表征过程中,发现了不同的神经元亚型。这些亚型反映了Usoskin,D.,A.Furlan,S.Islam,H.Abdo,P.Lonnerberg,D.Lou,J.Hjerling-Leffler,J.Haeggstrom,O.Kharchenko,P.V.Kharchenko,S.Linnarsson and P.Ernfors(2015)“通过大规模单细胞RNA测序对感觉神经元类型进行无偏分类(Unbiased classification of sensory neuron types by large-scale single-cell RNA sequencing)”Nat Neurosci 18(1):145-153中描述的特征。

[1275] 材料和方法

[1276] aDRG培养物

[1277] 在玻璃盖玻片上生成aDRG神经元。简言之,从2-3月龄SD(Sprague Dawley)大鼠身上解剖成年大鼠DRG组织。使用木瓜蛋白酶消化解剖的组织,然后使用分散酶/胶原酶消化,并将其铺在聚-D-赖氨酸和层粘连蛋白涂覆的玻璃盖玻片上。使用抗有丝分裂剂抑制培养物中神经胶质细胞类型的增殖。从DIV 7起,确定神经元培养物可用于测定。

[1278] 免疫荧光

[1279] 用1nM天然重组BoNT/A(“rBoNT/A”-SEQ ID NO:6[转变为双链形式])或BoNT/AB嵌合体(“mrBoNT/AB”-SEQ ID NO:1[转变为双链形式])在DIV7-8处理aRDG神经元24小时。对照样品未经处理。处理后,将神经元在培养基中洗涤两次并在4% PFA中固定30分钟。然后使用1×PBS中的0.1% TritonX-100透化神经元15分钟,然后在10%驴血清中封闭30分钟。

如下表所示进行一抗和二抗染色。

[1280] 表1.一抗和二抗染色。

[1281]

Ab抗-	公司	货号	物种	单/多抗	$1^{st}/2^{nd}$	稀释
DEAN10	Ipsen	N/A	兔	多抗	1^{st}	1:400
C.SNAP25	Origene	AM31671SU-N	小鼠	单抗	1^{st}	1:100
NF200(N52)	Sigma	N0142-.2ML	小鼠	单抗	1^{st}	1:200
NF200	Abcam	ab8135	兔	多抗	1^{st}	1:200
CGRP	Stratech	311-CGRP-PPS	小鼠	单抗	1^{st}	1:150
CGRP	Sigma	C8198	兔	多抗	1^{st}	1:150
P2X3	Stratech	GP10108-NEU	豚鼠	多抗	1^{st}	1:150
TrkA	Fisher	15710644	山羊	多抗	1^{st}	1:100
小鼠Alexa Fluor594	Fischer	15960296	驴	多抗	2^{nd}	5×1^{st}
小鼠Alexa Fluor488	Fischer	16051752	驴	多抗	2^{nd}	5×1^{st}
兔Alexa Fluor594	Fischer	15910767	驴	多抗	2^{nd}	$5X1^{st}$
兔Alexa Fluor488	Thermo	A32790	驴	多抗	2^{nd}	$5X1^{st}$
豚鼠Alexa Fluor594	Thermo	A-11076	山羊	多抗	2^{nd}	$5X1^{st}$
山羊Alexa Fluor594	Fischer	16331974	驴	多抗	2^{nd}	5×1^{st}

[1282] 然后用含有1μg/ml DAPI的Prolong Diamond封固剂将盖玻片封固在载玻片上,用于核染色。

[1283] 成像和共定位分析

[1284] 使用LSM800 Zeiss共焦显微镜以40×的放大倍数拍摄图像。每种特定样品和抗体组合至少拍摄3张图像,并进行3次生物学重复。通过使用两种不同的检测SNAP25的切割同种型的抗体测定中毒神经元。所使用的aDRG亚型标志物是aDRG培养物的特征。这些是NF类的NF200、PEP神经元的CGRP、NP神经元的P2X3、NP和PEP神经元的TrkA。通过合并两种目的颜色(通常绿色(488)用于去切割的SNAP25,红色(594)用于特异性神经元标志物)进行共定位。

[1285] 结果

[1286] 图1-3中呈现的所有图像显示了切割的SNAP25信号和特异性标志物的单色图像的一个代表性实例(每个生物实验至少拍摄3张图像),以及添加蓝色核染色的两个通道的彩色合并。当存在箭头时,指示特异性标志物/切割的SNAP25共定位的目的区域。图1的未处理对照显示了用于检测切割的SNAP25的抗体给出的少量背景。在分析经处理的样品时考虑了这种背景染色。

[1287] 天然重组BoNT/A靶向Aβ纤维

[1288] 图2显示了当aDRG神经元与rBoNT/A(SEQ ID NO:6[转化为双链形式])接触时获得的结果。特别是,Aβ纤维(NF200)和切割的SNAP25之间存在明显的共定位。在所有分析的图像中,切割的SNAP25和NF200的表达都很高。对于Aδ和C纤维(NP,PEP),没有观察到共定位。表达特异性标志物(CGRP、P2X3、TrkA)的神经元不表达高水平的切割的SNAP25。在图像中,可以看到其中表达切割的SNAP25的情况,然而,信号量并不高于图1所示的背景信号,并且明显低于由表达高水平切割的SNAP25的神经元给出的信号。总之,发现aDRG神经元中

rBoNT/A的中毒发生在Aβ纤维(NF200)中。

[1289] 嵌合BoNT/AB靶向Aδ纤维和C纤维

[1290] 图3显示了当aDRG神经元与mrBoNT/AB(SEQ ID NO:1[转化为双链形式])接触时获得的结果。特别地,在Aβ纤维(NF200)和切割的SNAP25之间没有共定位。用箭头突出显示的神经元部分显示NF200的高表达而不存在切割的SNAP25。对于Aδ和C纤维(NP,PEP),观察到共定位。特别地,在表达CGRP和TrkA的神经元中观察到强共定位。总之,aDRG神经元中mrBoNT/AB的中毒发生在Aδ(PEP)和C纤维(NP)中。这与rBoNT/A所见的(图2)相反。

[1291] 结论

[1292] 对aDRG原代神经元的免疫荧光研究能够确定rBoNT/A和mrBoNT/AB中毒的神经元的亚型。研究显示,与rBoNT/A相比,mrBoNT/AB所中毒的神经元亚型之间有明显的差异。rBoNT/A在Aβ纤维中切割SNAP25,而mrBoNT/AB在Aδ和C纤维中切割SNAP25。考虑到它们在伤害性感受中的作用,这最后两个亚群代表特别重要的疼痛靶标。下表总结了这些差异。

[1293] 表2.不同毒素靶向的aDRG纤维类型的示意性总结。

[1294]

分子	纤维类型
rBoNT/A	Aβ
mrBoNT/AB	Aδ
mrBoNT/AB	C

[1295] 总之,数据显示BoNT/AB嵌合体靶向参与伤害性感受的细胞,因此可能表现出镇痛作用,例如与rBoNT/A相比改善的镇痛作用。

[1296] 实施例2

[1297] 嵌合梭菌神经毒素BoNT/AB有效抑制降钙素基因相关肽(CGRP)从Aδ和C纤维中的释放

[1298] 根据实施例1中呈现的发现,进行实验以通过确定BoNT/AB嵌合体靶向Aδ和C纤维是否能够抑制降钙素基因相关肽(CGRP)释放来确认其作为镇痛剂的作用。CGRP是主要在来自背根和三叉神经节的C和Aδ感觉纤维亚组中发现的神经肽。最近的研究表明CGRP参与外周敏化和增强的疼痛、神经炎症和神经性疼痛的发展。为了支持这一点,阻断CGRP功能已被证明可以缓解偏头痛。

[1299] 材料和方法

[1300] aDRG培养物

[1301] 按照实施例1中给出的方法的稍微改进的形式,将aDRG神经元接种在96孔半体积平板上。

[1302] CGRP释放测定

[1303] 在DIV7-14,用10nM-1pM的梭菌神经毒素的Log10稀释液处理aDRG神经元24小时。所用毒素:rBoNT/A(SEQ ID NO:6[转化为双链形式])和mrBoNT/AB(SEQ ID NO:1[转化成双链形式])。对照样品未经处理。处理后,将神经元在HBS(110mM氯化钠、3mM氯化钾、2mM氯化钙、1mM氯化镁、10mM HEPES、20mM葡萄糖,pH 7.2)中洗涤两次并放回37℃的培养箱中1小时。然后将细胞板转移到预热的加热块中并用HBS再洗涤一次。移除HBS并用50μL HBS+0.03%BSA替换。5分钟后,取出HBS/BSA浇注液(superfusate)并储存在单独的板中。在除去浇注液后,立即将50μL刺激培养基(100nM辣椒素HBS+0.03%BSA或65nM氯化钾(KCl)HBS+0.03%BSA)加入适当的孔中。5分钟后,收集浇注液。收集后,将浇注液立即用于CGRP EIA测定,或储存在-20℃。

[1304] CGRP酶免疫(EIA)测定法

[1305] CGRP免疫测定试剂作为市售试剂盒(Bertin Pharma,法国,#A05482)的一部分购买并根据制造商的说明书制备。将板用洗涤缓冲液洗涤5次,然后加入40μL标准品和样品。然后将100μL CGRP示踪剂(制备如下:用10ml EIA缓冲液稀释的储液瓶(#A10482)(用50ml蒸馏水复溶的储备EIA缓冲液#A07000的小瓶))添加到每个孔中。然后将板用粘合带覆盖并在4℃下孵育16至20小时。孵育后,弃去板内容物,用洗涤缓冲液(制备如下:用400ml蒸馏水稀释1ml洗涤缓冲液储液(#A17000),并添加200μl Tween-20(#A12000))洗涤孔3次,然后在洗涤缓冲液中进行2分钟摇动步骤,然后再洗涤3次。除去洗涤缓冲液后,每孔添加200μL Ellman试剂(制备如下:Ellman试剂储液小瓶#A09000稀释于1ml储液洗涤缓冲液#A17000和49ml蒸馏水中)。然后将板用箔覆盖并在室温下在黑暗中孵育4小时。最后,使用Clariostar酶标仪在410nm读板。将标准品绘制在Prism V8(Graphpad)中的X-Y图上,并从标准曲线内插样品值。

[1306] 结果

[1307] 图4显示了mrBoNT/AB在抑制CGRP释放方面比rBoNT/A有效得多。rBoNT/A和mrBoNT/AB的平均pIC_{50}值分别为:6.87±0.44(对于rBoNT/A为7.34μM)和9.99±0.16(对于mrBoNT/AB为9.73nM)(平均值±SEM)。

[1308] 在图4所示结果的确认中,最大抑制的比较显示,当与1nM rBoNT/A相比时,由1nM mrBoNT/AB引起的CGRP释放抑制之间存在显著差异。具体地,mrBoNT/AB在抑制CGRP释放方面比rBoNT/A在统计学上显著更好(参见图5)。这与mrBoNT/AB特异性靶向Aδ-型纤维和C-型纤维的令人惊讶的发现一致(参见实施例1)。

[1309] 结论

[1310] 利用大鼠aDRG CGRP释放模型可以在体外疼痛模型中对不同梭菌神经毒素进行功能比较。与毒素的纤维亚型结合特异性一致,mrBoNT/AB在抑制CGRP从aDRG释放方面明显比rBoNT/A更有效。因此,具有肉毒杆菌神经毒素A(BoNT/A)轻链和转位结构域(H_N结构域)以及BoNT/B受体结合结构域(H_C结构域)的嵌合梭菌神经毒素是改进的镇痛剂。

[1311] 实施例3

[1312] 慢性偏头痛疼痛患者的治疗

[1313] Joe,43岁,被他的GP诊断为慢性偏头痛,并用包含SEQ ID NO:1(转化成双链形式)的本发明的嵌合梭菌神经毒素进行治疗。嵌合梭菌神经毒素以5,000pg的单位剂量施用,其中单个单位剂量通过肌内注射施用至每块降眉间肌、两块皱眉肌、两块咬肌、两块颞肌、两块枕肌和两块斜方肌(即总共施用11×单位剂量)。9个月后,当Joe接受下一次治疗时,他的疼痛明显减轻,不再有明显疼痛。

[1314] 实施例4

[1315] 嵌合梭菌神经毒素BoNT/AB(SEQ ID NO:1)的临床前测试

[1316] BoNT/AB嵌合体SEQ ID NO:1(转化成双链形式)在小鼠LD_{50}测定中测试,得到1.202ng/kg的结果。因此,在该测定中,1个单位的SEQ ID NO:1(转换成双链形式)对应于24.04pg。

[1317] 实施例5

[1318] 用于治疗偏头痛的嵌合梭菌神经毒素BoNT/AB(SEQ ID NO:1)的单位剂量的计算

[1319] 鉴于临床前药理学数据,已经计算出嵌合梭菌神经毒素BoNT/AB在人体中施用的合适单位剂量范围(UD)。

[1320] 计算出SEQ ID NO:1(转化成双链形式)的DAS ED_{50}为13pg/kg。ED_{50}被认为是最小的药理学活性剂量,其比在相同动物物种中4ng/kg的未观察到的副作用水平(NOAEL)低约300倍。SEQ ID NO:1(转化成双链形式)在大鼠中的ED_{50}为13pg/kg,对应于对于60kg体重的人的0.8ng剂量。

[1321] 因此,选择单位剂量的下限为1,000pg。选择单位剂量上限为5,000pg,其低于换算成60kg体重的人体剂量的来自非临床安全物种(大鼠和猴)的4ng/kg的NOAEL。

[1322] 考虑到安全性的提高,将治疗偏头痛的最大总剂量设定为175,000pg,这是根据将来自两种非临床安全物种(大鼠和猴)的4ng/kg的NOAEL换算成60kg体重的人体剂量得出的。

[1323] 有利地,在治疗偏头痛时,在达到最大剂量之前,可以将嵌合梭菌神经毒素BoNT/AB(SEQ ID NO:1[转化为双链形式])注射至更多肌肉。这是一项重大且有利的发现,可改善偏头痛的治疗,同时为临床医生提供更广泛的治疗选择。

[1324] 实施例6

[1325] 嵌合梭菌神经毒素BoNT/AB(SEQ ID NO:1)在人类中的安全性和功效

[1326] SEQ ID NO:1(转化为双链形式)通过单个单位剂量施用于人类受试者。对5个队列施用不同(增加)量的mrBoNT/AB(SEQ ID NO:1[转化成双链形式])。队列1施用2×1,000pg单位剂量的mrBoNT/AB(即2,000pg最大值),而队列5施用2×16,000pg单位剂量的mrBoNT/AB(即32,000pg最大值)。结果显示,尽管每块肌肉的剂量及其高,但所测试的所有mrBoNT/AB单位剂量(即高达16,000pg单位剂量)对肌肉麻痹均有效,可安全耐受,并且未观察到不良反应。这表明mrBoNT/AB不会从注射位点扩散开,并突出了mrBoNT/AB(SEQ ID NO:1[转化为双链形式])卓越的安全性。

[1327] 实施例7

[1328] 嵌合梭菌神经毒素BoNT/AB(SEQ ID NO:1)在人类中的安全性和功效

[1329] SEQ ID NO:1(转化为双链形式)通过单个单位剂量施用于人类受试者。对7个队列施用不同(增加)量的mrBoNT/AB(SEQ ID NO:1[转化成双链形式])至面部肌肉。队列1施用5

×20pg单位剂量的mrBoNT/AB(即100pg最大值),而队列7施用5×1,500pg单位剂量的mrBoNT/AB(即7,500pg最大值)。

[1330] 结果显示,尽管每块肌肉剂量很高,但所测试的所有mrBoNT/AB单位剂量(即高达1,500pg单位剂量)对肌肉麻痹均有效,可安全耐受,并且未观察到不良反应。这表明mrBoNT/AB不会从注射位点扩散开,并突出了mrBoNT/AB(SEQ ID NO:1[转化为双链形式])卓越的安全性。

[1331] 实施例8

[1332] 通过肌内注射治疗患有慢性偏头痛疼痛的受试者

[1333] Derek,25岁,被他的GP诊断为慢性偏头痛,并用包含SEQ ID NO:1(转化成双链形式)的本发明的嵌合梭菌神经毒素进行治疗。包含SEQ ID NO:1(转化成双链形式)的嵌合梭菌神经毒素以2,500pg的单位剂量施用,并通过如下肌内注射施用:

[1334] 2个单位剂量至Derek面部左侧的额肌肌肉,以及2个单位剂量至Derek面部右侧的额肌肌肉;

[1335] 1个单位剂量对降眉间肌;

[1336] 1个单位剂量至Derek面部左侧的皱眉肌肌肉,以及1个单位剂量至Derek面部右侧的皱眉肌肌肉;

[1337] 4个单位剂量至Derek头部左侧的颞肌肌肉,以及4个单位剂量至Derek头部右侧的颞肌肌肉;

[1338] 3个单位剂量至Derek颈部/头部左侧的枕肌肌肉,以及3个单位剂量至Derek颈部/头部右侧的枕肌肌肉;

[1339] 3个单位剂量至Derek面部左侧的斜方肌肌肉,以及3个单位剂量至Derek面部右侧的斜方肌肌肉;和

[1340] 4个单位剂量至德里克颈部左侧的颈椎旁肌群,以及4个单位剂量至德里克颈部右侧的颈椎旁肌群;

[1341] 施用总共35个单位剂量(即87,500pg)的包含SEQ ID NO:1(转化成双链形式)的嵌合梭菌神经毒素。9个月后,当Derek接受下一次治疗时,他的疼痛明显减轻,不再有明显疼痛。该治疗是安全耐受的并且没有观察到不良事件。

[1342] 实施例9

[1343] 通过皮内注射治疗患有阵发性偏头痛的受试者

[1344] Tessa,51岁,被他的GP诊断为阵发性偏头痛,并用包含SEQ ID NO:1(转化成双链形式)的本发明的嵌合梭菌神经毒素进行治疗。包含SEQ ID NO:1(转化成双链形式)的嵌合梭菌神经毒素以5,000pg的单位剂量施用,并通过如下皮内注射施用:

[1345] 1个单位剂量至Tessa面部第一侧的眶上神经区和/或1个单位剂量至面部Tessa第二侧的眶上神经区;

[1346] 1个单位剂量至Tessa面部第一侧的滑车上神经区和/或1个单位剂量至Tessa面部第二侧的滑车上神经区;

[1347] 1个单位剂量至Tessa面部第一侧的滑车内神经区和/或1个单位剂量至Tessa面部第二侧的滑车内神经区;

[1348] 1个单位剂量至Tessa面部第一侧的颧颞神经区和/或1个单位剂量至Tessa面部第

二侧的颧颞神经区;

[1349] 1个单位剂量至Tessa面部第一侧的颧面神经区和/或1个单位剂量至Tessa面部第二侧的颧面神经区;

[1350] 2个单位剂量至Tessa面部第一侧的耳颞神经区和/或2个单位剂量至Tessa面部第二侧的耳颞神经区;

[1351] 2个单位剂量至Tessa颈部第一侧枕大神经区和/或2个单位剂量至Tessa颈部第二侧枕大神经区;和/或

[1352] 1个单位剂量至Tessa颈部第一侧的枕小神经区和/或1个单位剂量至Tessa颈部第二侧的枕小神经区。

[1353] 施用总共20个单位剂量(即100,000pg)的包含SEQ ID NO:1(转化成双链形式)的嵌合梭菌神经毒素。9个月后,当Tessa接受下一次治疗时,她的疼痛明显减轻,不再有明显疼痛。该治疗是安全耐受的并且没有观察到不良事件。

[1354] 实施例10

[1355] 嵌合梭菌神经毒素BoNT/AB比BoNT/A更能有效抑制降钙素基因相关肽(CGRP)从三叉神经节神经元释放

[1356] 在由三叉神经节制备的大鼠原代神经元中评估mrBoNT/AB的作用和效力,所述三叉神经节是三叉神经的三个感觉分支发出的结构。三叉神经节是功能上参与偏头痛病理生理学的富含神经元(TGN)的关键区域。简言之,由5至8周龄的大鼠产生原代大鼠TGN培养物(参见例如,Sidders等人(2018),J Mol Biol.,14;430(18Pt A):3005-3015)。将细胞与浓度从1pM至100nM的毒素(rBoNT/A[SEQ ID NO:6转化为双链形式]或mrBoNT/AB[SEQ ID NO:1转化为双链形式])孵育后24小时后,用65mM KCl刺激培养物10分钟,以诱导CGRP的释放,CGRP是被认为是导致偏头痛发生和传播的主要神经递质。随后通过ELISA测量CGRP释放(如实施例2中所述测量;n=3,一式四份),将TGN裂解用于SNAP25的蛋白质印迹分析。

[1357] 如图7所示,mrBoNT/AB(图7B)在降低CGRP释放(图7A)和切割SNAP25(图8)方面比rBoNT/A更有效。

[1358] 这些数据进一步证明了mrBoNT/AB(当与rBoNT/A相比时)在靶向和抑制疼痛介质(例如CGRP)从偏头痛病理生理学中相关的神经元释放中的改善的功效。因此,具有肉毒杆菌神经毒素A(BoNT/A)轻链和转位结构域(H_N结构域)以及BoNT/B受体结合结构域(H_C结构域)的嵌合梭菌神经毒素的施用构成了对一般疼痛、特别是偏头痛的改进治疗是可信的。

[1359] 实施例11

[1360] 与BoNT/A相比,嵌合梭菌神经毒素BoNT/AB在人感觉神经元中显示出更高的效力

[1361] 在与疼痛相关的人体环境即源自人诱导多能干细胞(hiPSC)的感觉神经元(与细胞培养相关的方法按照制造商的说明书:https://www.anatomic.tech/,也参见例如Walsh等人(2020),Stem Cells,38,11,1400-1408)中,比较mrBoNT/AB与rBoNT/A。简言之,将源自hiPSC的感觉神经元培养14天,随后与3fM至1nM rBoNT/A(转化为双链形式的SEQ ID NO:6)或mrBoNT/AB(转化为双链形式的SEQ ID NO:1)孵育24小时,然后将它们裂解,通过蛋白免疫印迹进行SNAP25切割评估(n=3,一式三份)。图9说明与rBoNT/A相比,mrBoNT/AB在这种人细胞中切割SNAP25的效力更高。此外,这进一步支持了具有肉毒杆菌神经毒素A(BoNT/A)轻链和易位结构域(H_N结构域)和BoNT/B受体结合结构域(H_C结构域)的嵌合梭菌神经毒素的

施用通常构成了对人类疼痛、特别是人类偏头痛的改善的治疗。

[1362] 实施例12

[1363] 嵌合梭菌神经毒素BoNT/AB在与体内疼痛传递相关的神经元中切割SNAP25

[1364] 材料和方法

[1365] 将未使用过的雌性Sprague Dawley大鼠用于研究(在治疗开始时180-220g,Janvier labs,法国)。将动物置于相反的12-h光/暗循环(从18:00到06:00开灯)并在恒定温度(22±2℃)和湿度(55±5%)下维持在丰富环境中,其中可随意获取食物和水。在实验前使动物适应至少7天。该研究完全遵循ARRIVE指南、欧洲共同体委员会指令2010/63/EU和法国国家委员会法令87/848进行。

[1366] 通过肌内(IM)或皮内(ID)注射向动物施用媒介物(盐水,2只大鼠/组)或mrBoNT/AB(SEQ ID NO:1转化成双链形式)(300pg/kg,6只大鼠/组)或使用IM注射向动物施用肉毒杆菌毒素(6只大鼠/组)(用于三叉神经节分析)。通过将总剂量分配到头部和颈部的4块肌肉(右颞肌和左颞肌,右枕肌和左枕肌,各10μL注射体积)进行IM施用。通过将总剂量分配到位于4个上述肌肉上方的皮肤真皮(各10μL注射体积)进行ID施用。治疗施用后10天,对动物实施安乐死,并收获以下组织:三叉神经节、包含三叉神经核的脑干和颈脊髓。然后将组织在等渗缓冲福尔马林10%溶液(VWR,法国)中固定48小时,包埋在石蜡块中并制备组织学玻片。

[1367] 为了评价两种毒素在组织中的生物效应,对切割形式的SNAP25(c-SNAP25)进行免疫组织化学染色。在热诱导的表位修复步骤后,将内源性过氧化物酶在TBS缓冲液中的3%H_2O_2溶液中封闭10分钟。将切片与非商业性原代兔多克隆抗体(EF14007,Ipsen Innovation,法国)孵育,所述抗体仅对通过BoNT/A切割的SNAP25形式具有特异性。然后将切片与生物素化的第二抗体(抗兔IgG,Vector Laboratories,美国)孵育30分钟,然后与偶联辣根过氧化物酶的扩增系统(抗生物素蛋白-生物素)(Vector Laboratories,美国)孵育30分钟。最后,将切片与0.02%二氨基联苯胺(DAKO,USA)溶液孵育5分钟,使用苏木精(DAKO,USA)进行复染,并在光学显微镜下观察载玻片。对于三叉神经节样品,使用以下5分制量表评分系统确定c-SNAP25的量的定量:0(无染色),1(极小染色强度和密度),2(中等染色强度和密度),3(强染色强度和密度)和4(非常强的染色强度和密度)。在脊髓中,c-SNAP25阳性神经末梢的强度和密度如下分级:在5个染色最强烈的脊髓切片上分为0(无染色),1(极小),2(轻度),3(中度),4(显著),然后计算每只动物的累积评分(0-20)。对于脑干样品,使用测量c-SNAP25染色的神经纤维比例的专用图像分析方法定量SNAP25切割染色。

[1368] 结果

[1369] 如所预期的,在来自任何媒介物处理的动物的组织中没有观察到特异性c-SNAP25染色。图10显示通过IM或ID途径施用mrBoNT/AB均导致SNAP25在脑干的脊髓三叉神经感觉核处被切割,而当通过ID途径施用时,脑干的三叉神经运动核中的切割较少。运动核中较低量的SNAP25切割可导致降低的脱靶效应,例如与治疗相关的运动效应。

[1370] 图11显示通过IM或ID途径施用mrBoNT/AB导致SNAP25在颈脊髓中、特别是在背角和腹角中被切割。

[1371] 图12显示通过IM或ID途径施用mrBoNT/AB导致三叉神经节轴突中的SNAP25被切割。令人惊讶的是,肉毒杆菌毒素不切割三叉神经节轴突中的SNAP25,从而支持mrBoNT/AB

在治疗疼痛、例如治疗偏头痛中的改善效果的作用。

[1372] 实施例13

[1373] SNAP25和嵌合梭菌神经毒素BoNT/AB受体存在于与疼痛传播相关的各种人体组织中

[1374] 材料和方法

[1375] 人体组织购自ProteoGenex(美国)、Cureline(美国)和Clinisciences(法国),并评估SNAP25、SytII和SytI的存在。评估以下组织,每个组织具有n=3至5个供体:

[1376] 脑桥和延髓(包括脑干部分并包含中枢三叉神经运动和感觉核);以及

[1377] 颈脊髓(包括背角)。

[1378] 为了定量蛋白质表达,使用与实施例12中所述相同的免疫组织化学技术。用抗SNAP25(使用抗体111 008)、SytII(使用抗体105 123)和SytI(使用抗体ab126253)的抗体对组织进行染色。还用抗β-3-微管蛋白的抗体(使用抗体G7121)对组织进行染色,泛神经元标志物作为对照。

[1379] 在光学显微镜下定量染色,并使用0至4的得分对染色强度进行分级,其中得分0=无染色;1=非常弱/非常稀/不频繁染色;2=中度染色;3=强烈/频繁染色;4=非常强烈(strong)/强烈(intense)/极其频繁的染色。

[1380] 结果

[1381] 结果示于表3中。β-3-微管蛋白对照染色以及SNAP25染色在脑桥、延髓和颈脊髓中被分级为最大值(4)。同时,发现SytII和SytI在脑桥、延髓和背角层1、3和4中强烈表达。SytI在背角层2中强烈表达,而SytII在该结构中显示中度染色。

[1382] 总之,这些结果表明SNAP25、SytII和SytI在与疼痛传递相关的几种人体组织中高度表达。因此,存在合适的SytII和SytI受体用于mrBoNT/AB结合、内化、并切割这些组织中存在的神经元中的SNAP25,例如在mrBoNT/AB从施用的远端位点的神经元(例如逆向)运输之后,从而抑制疼痛传递。

[1383] 表3:与疼痛传递相关的各种人体组织中SNAP25、SytII和SytI的染色强度

[1384]

组织	结构	β-3-微管蛋白	SNAP25	SytII	SytI
脑桥	包括三叉神经运动和主要感觉核	4	4	3	3
延髓	脊髓三叉神经感觉核	4	4	3	4
颈脊髓	背角-层1、3、4	4	4	3	3
	背角-层2	4	4	2	4

[1385] 实施例14

[1386] 偏头痛患者的治疗

[1387] Timothy,33岁,被他的GP诊断为偏头痛。他通过如下施用2,500pg SEQ ID NO:1(转化为双链形式)的单位剂量(UD)进行治疗:

[1388]

肌内注射	注射部位总数	每个注射部位的剂量	每个疗程的剂量
额肌	4(每侧2块)	2.5ng	10ng
皱眉肌	2(每侧1块)	2.5ng	5ng
鼻肌	2(每侧1块)	2.5ng	5ng

眼轮匝肌	2(每侧1块)	2.5ng	5ng
颞肌	8每侧4块)	2.5ng	20ng
枕肌	6(每侧3块)	2.5ng	15ng
斜方肌	4(每侧2块)	2.5ng	10ng
总计	28		70ng

[1389] 他接受总剂量为70,000pg的SEQ ID NO:1(转化成双链形式)。治疗成功,症状得到缓解。他不需要治疗超过9个月。

[1390] 实施例15

[1391] 偏头痛患者的治疗

[1392] Joseph,31岁,被他的GP诊断为偏头痛。他通过如下施用4,000pg SEQ ID NO:1(转化为双链形式)的单位剂量(UD)进行治疗:

[1393]

肌内注射	注射部位总数	每个注射部位的剂量	每个疗程的剂量
额肌	4(每侧2块)	4ng	16ng
皱眉肌	2(每侧1块)	4ng	8ng
鼻肌	2(每侧1块)	4ng	8ng
眼轮匝肌	2(每侧1块)	4ng	8ng
颞肌	8(每侧4块)	4ng	32ng
枕肌	6(每侧3块)	4ng	24ng
斜方肌	4(每侧2块)	4ng	16ng
总计	28		112ng

[1394] 他接受总剂量为112,000pg的SEQ ID NO:1(转化成双链形式)。治疗成功,症状得到缓解。他不需要治疗超过9个月。

[1395] 上述说明书中提及的所有出版物均通过引用并入本文。在不脱离本发明的范围和精神的情况下,本发明的所述方法和系统的各种修改和变化对于本领域技术人员来说是显而易见的。尽管已经结合具体的优选实施例描述了本发明,但是应当理解,所要求保护的本发明不应当不适当地限于这些具体的实施例。实际上,对于生物化学和生物技术或相关领域的技术人员来说显而易见的用于实施本发明的所述模式的各种修改旨在落入所附权利要求的范围内。

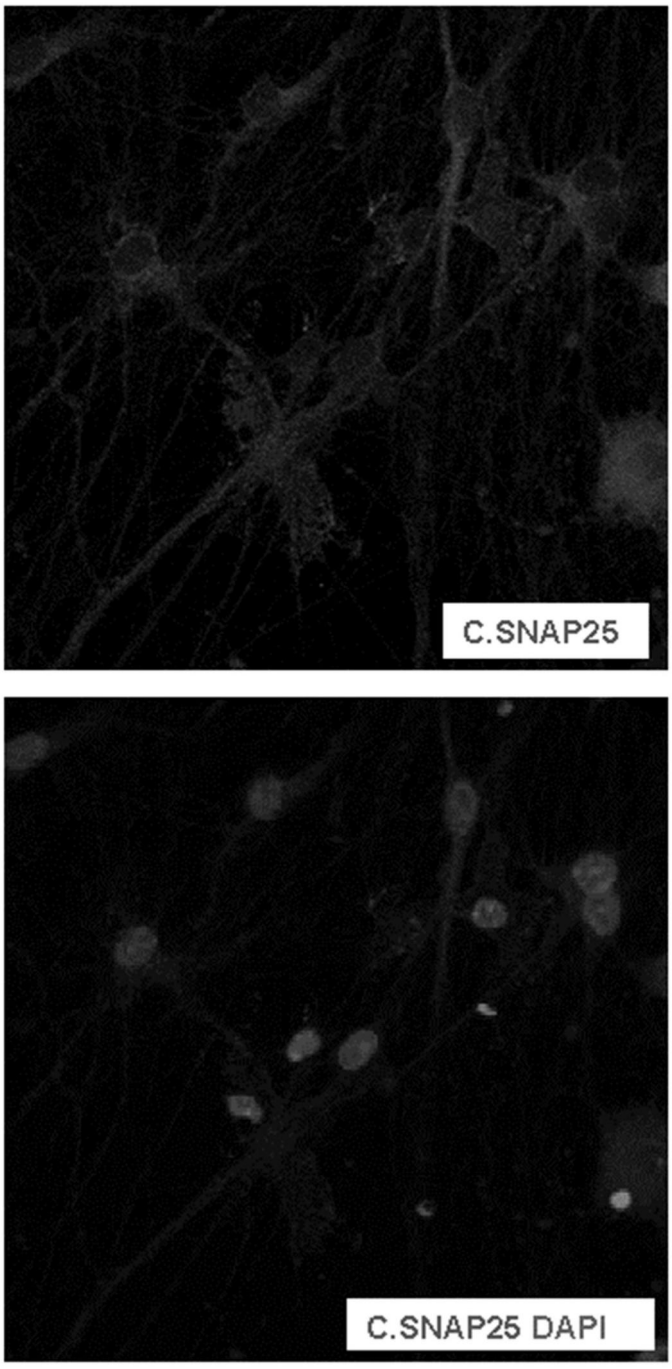


图1

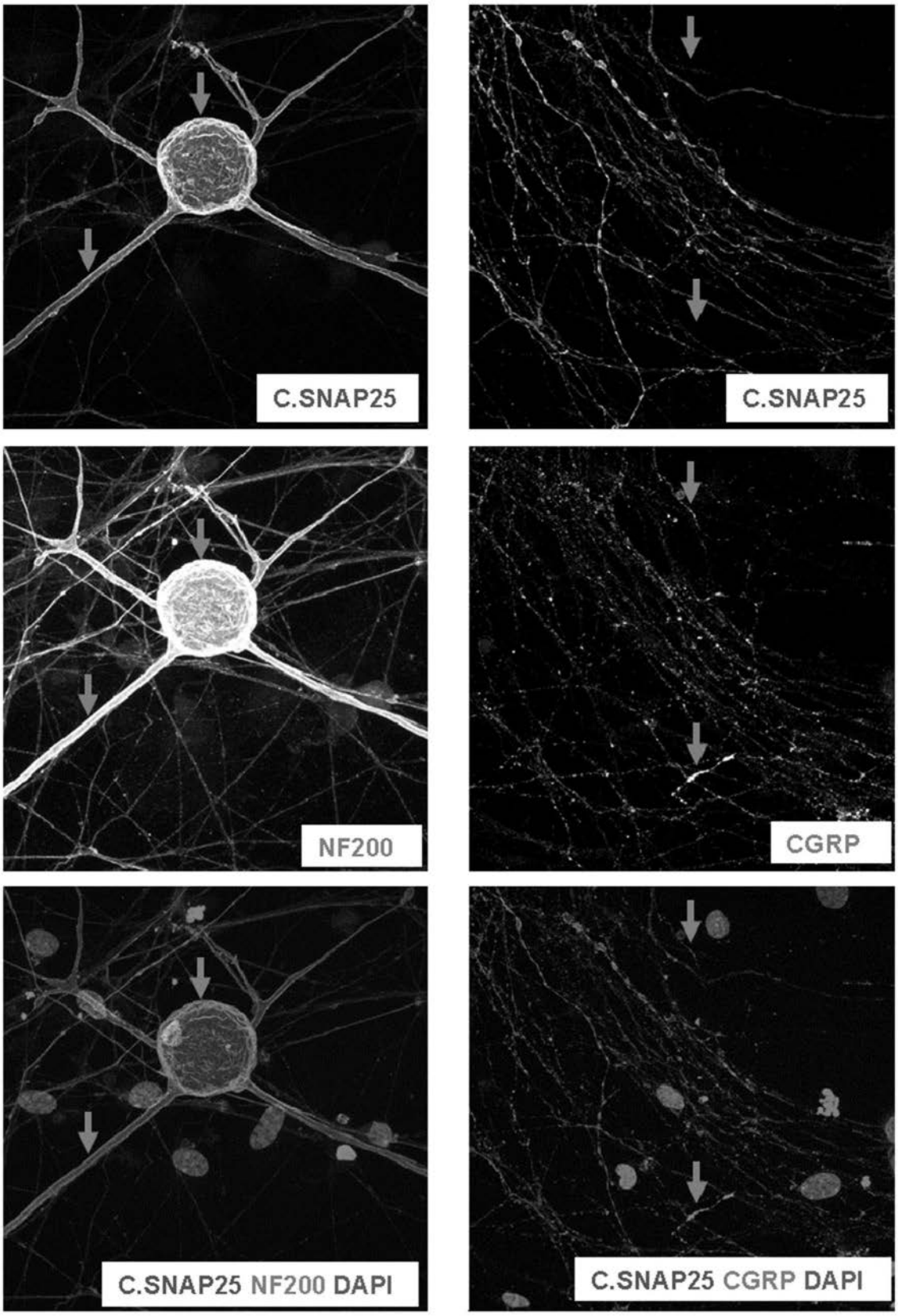

C.SNAP25
C.SNAP25
NF200
CGRP
C.SNAP25 NF200 DAPI
C.SNAP25 CGRP DAPI

图2

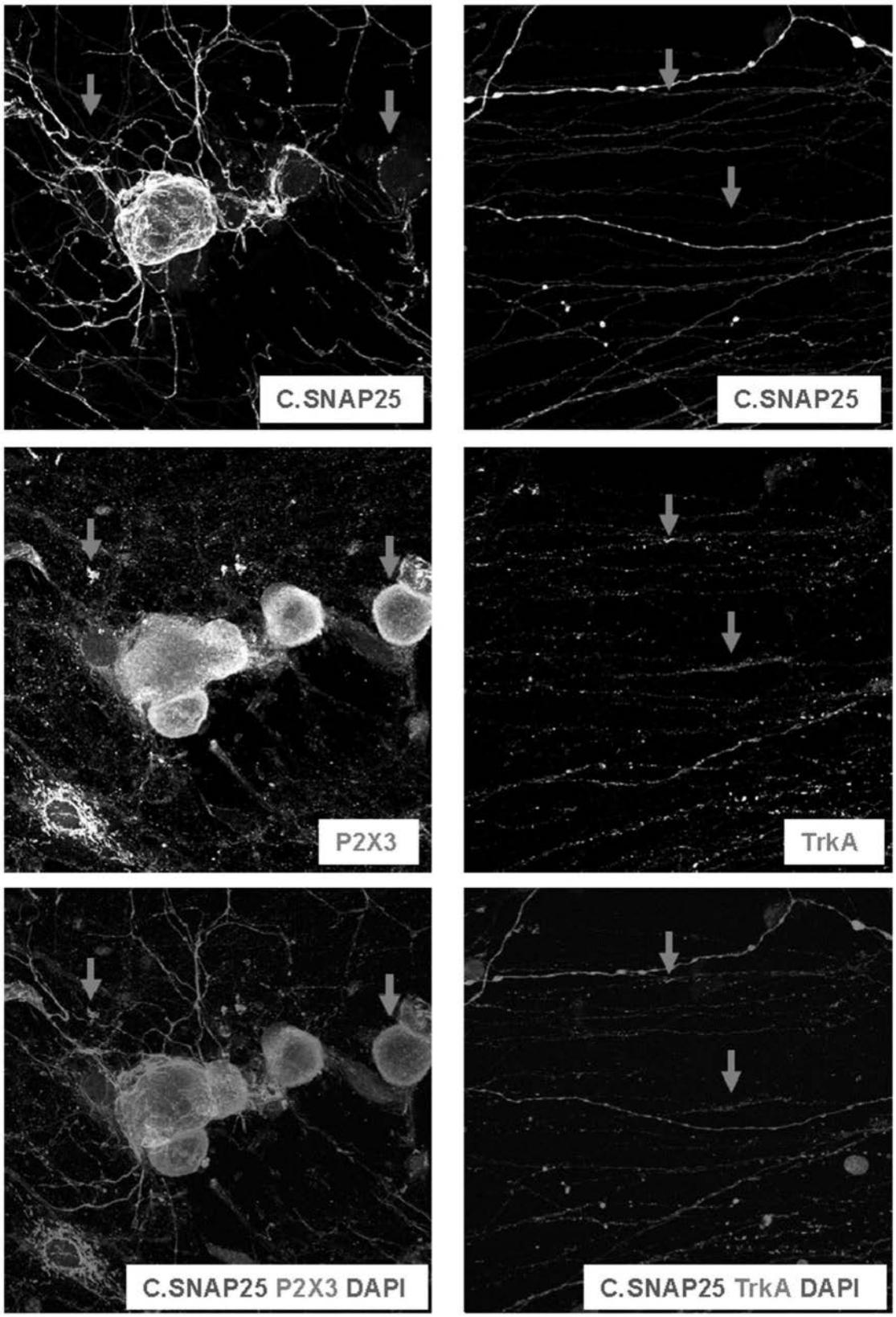

C.SNAP25
C.SNAP25
P2X3
TrkA
C.SNAP25 P2X3 DAPI
C.SNAP25 TrkA DAPI

图2续

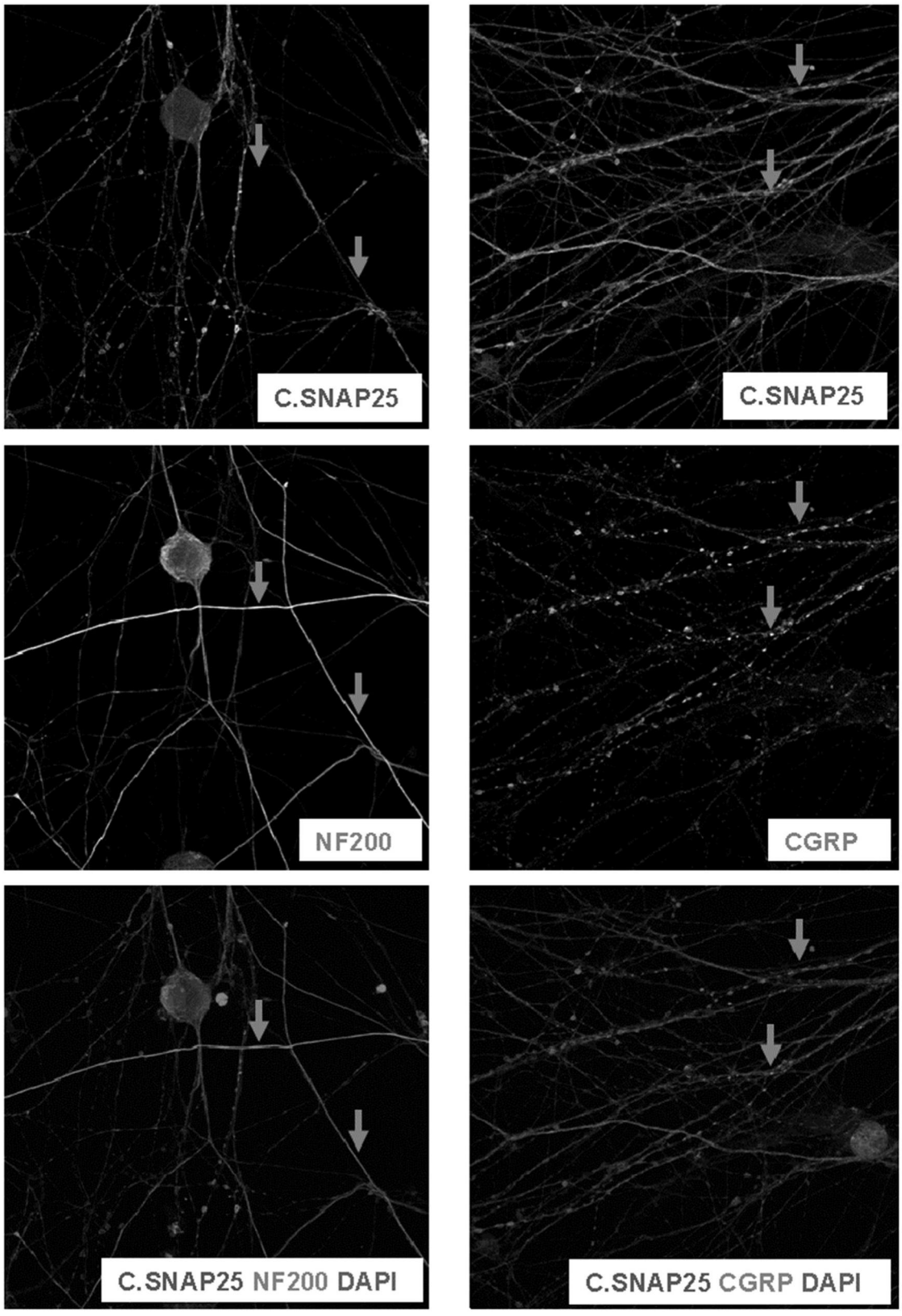

C.SNAP25
C.SNAP25
NF200
CGRP
C.SNAP25 NF200 DAPI
C.SNAP25 CGRP DAPI

图3

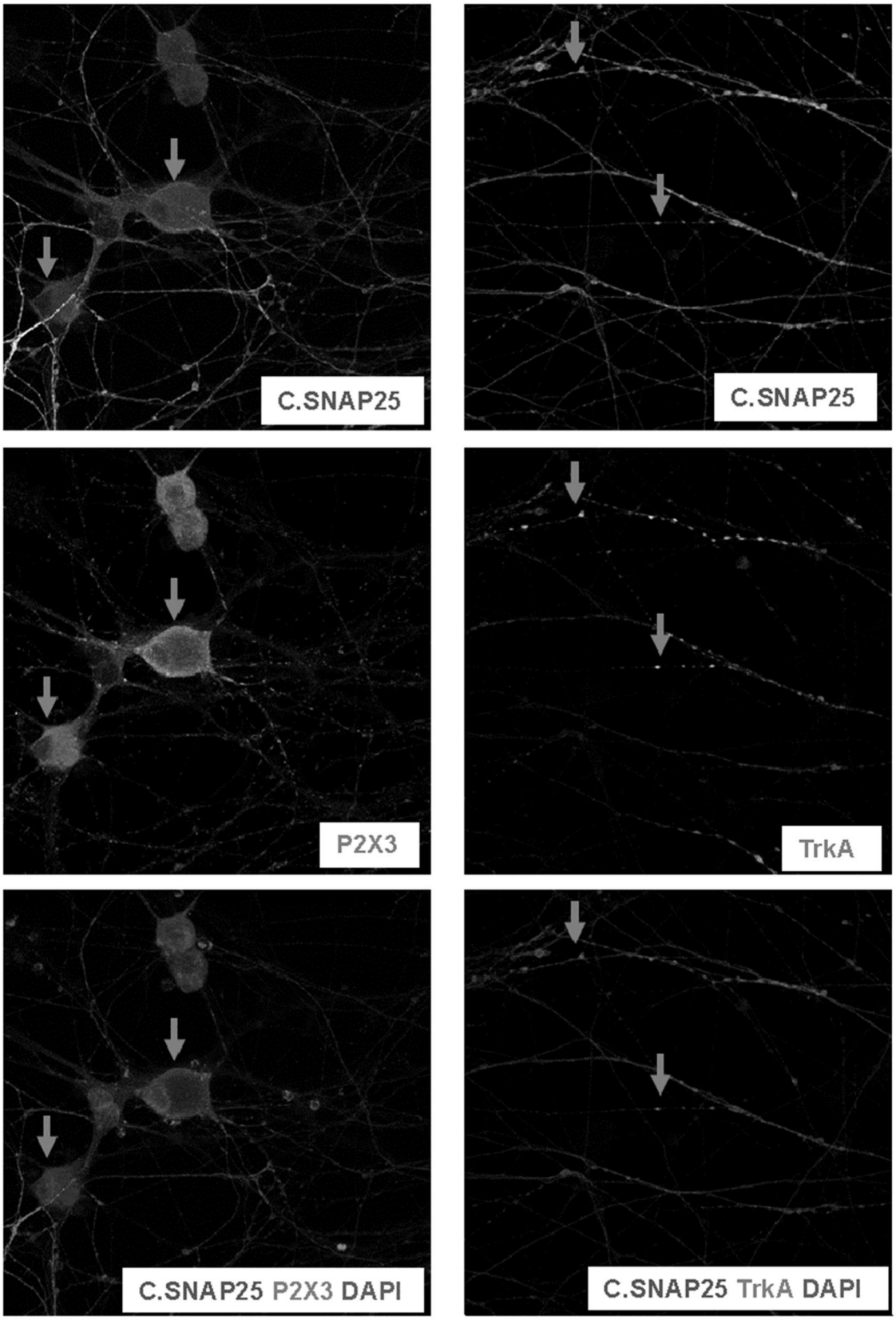

C.SNAP25
C.SNAP25
P2X3
TrkA
C.SNAP25 P2X3 DAPI
C.SNAP25 TrkA DAPI

图3续

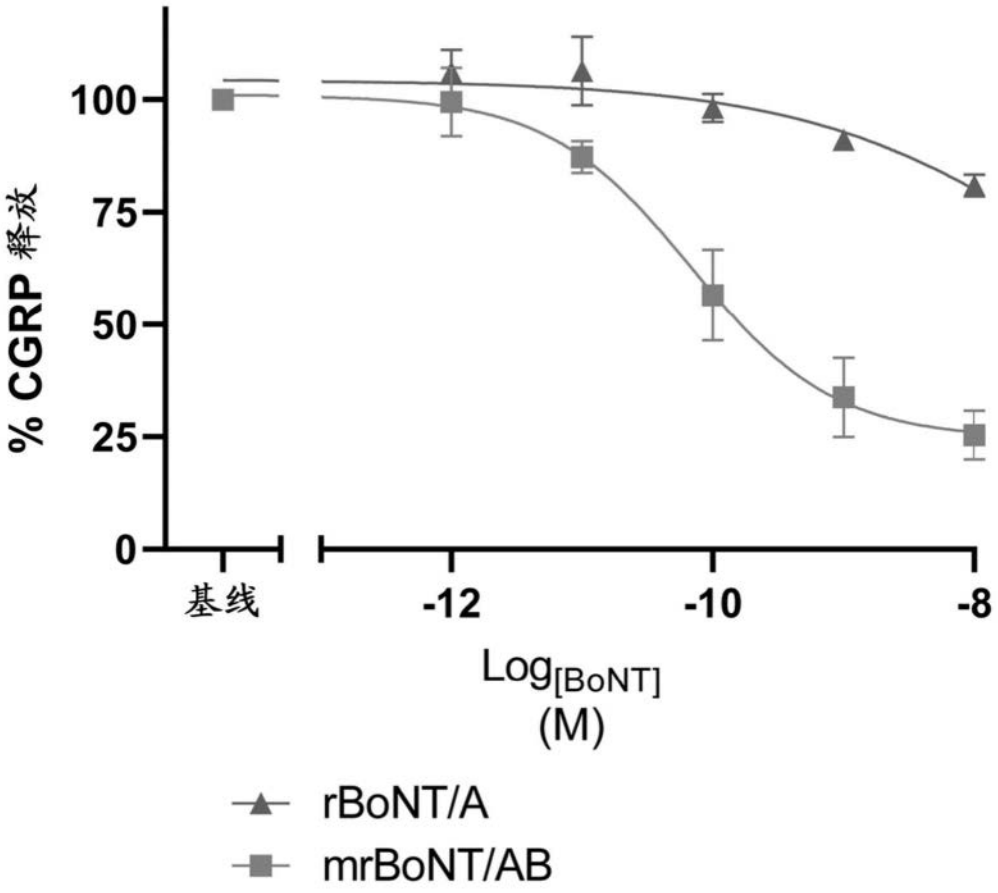


图4

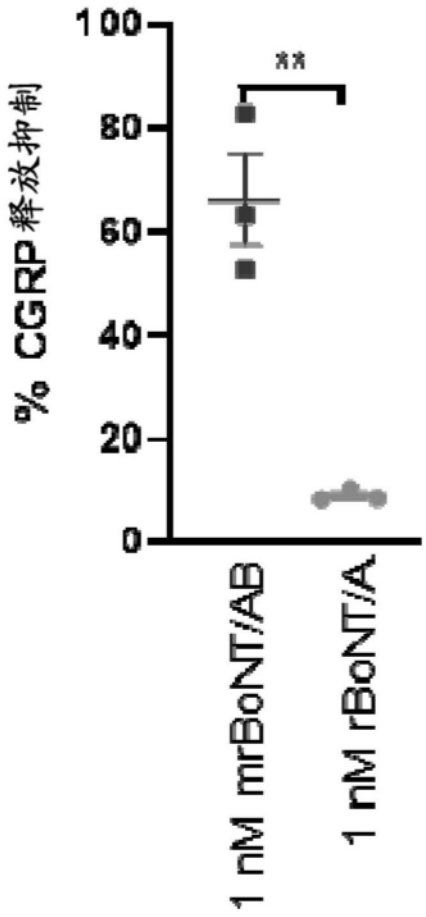
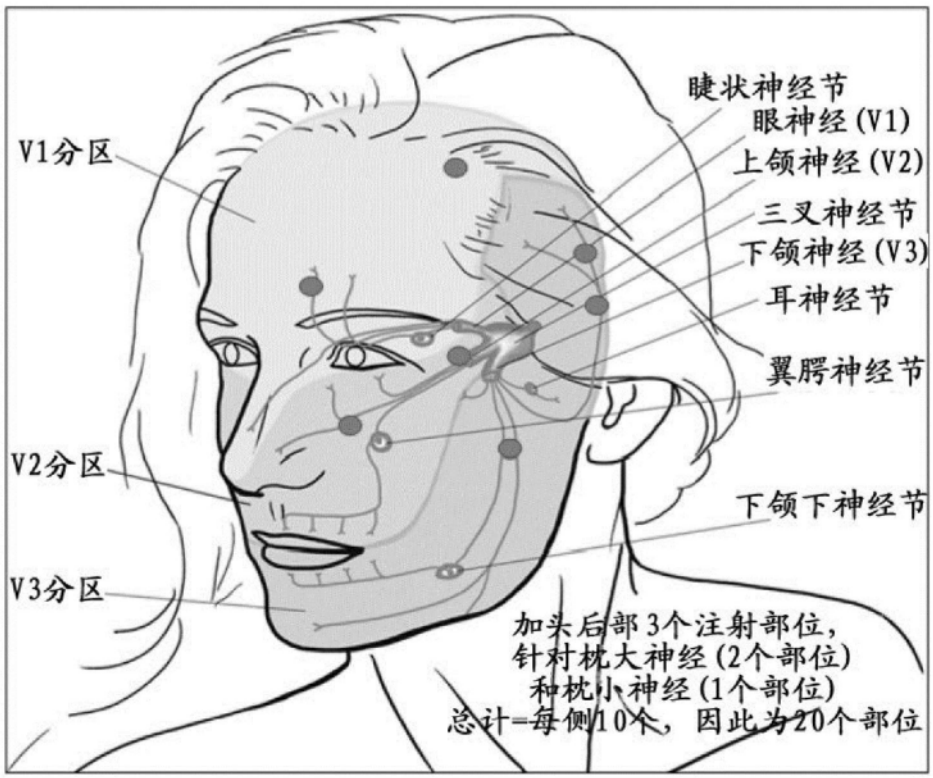


图5



区域	靶神经末端	每侧注射次数 / 总数
三叉神经，眼	眶上	1/2
	滑车上	1/2
	滑车内	1/2
三叉神经，上颌	颧颞	1/2
	颧面	1/2
三叉神经，下颌骨	耳颞	2/4
头后部	枕大	2/4
	枕小	1/2
	总计	10/20

图6

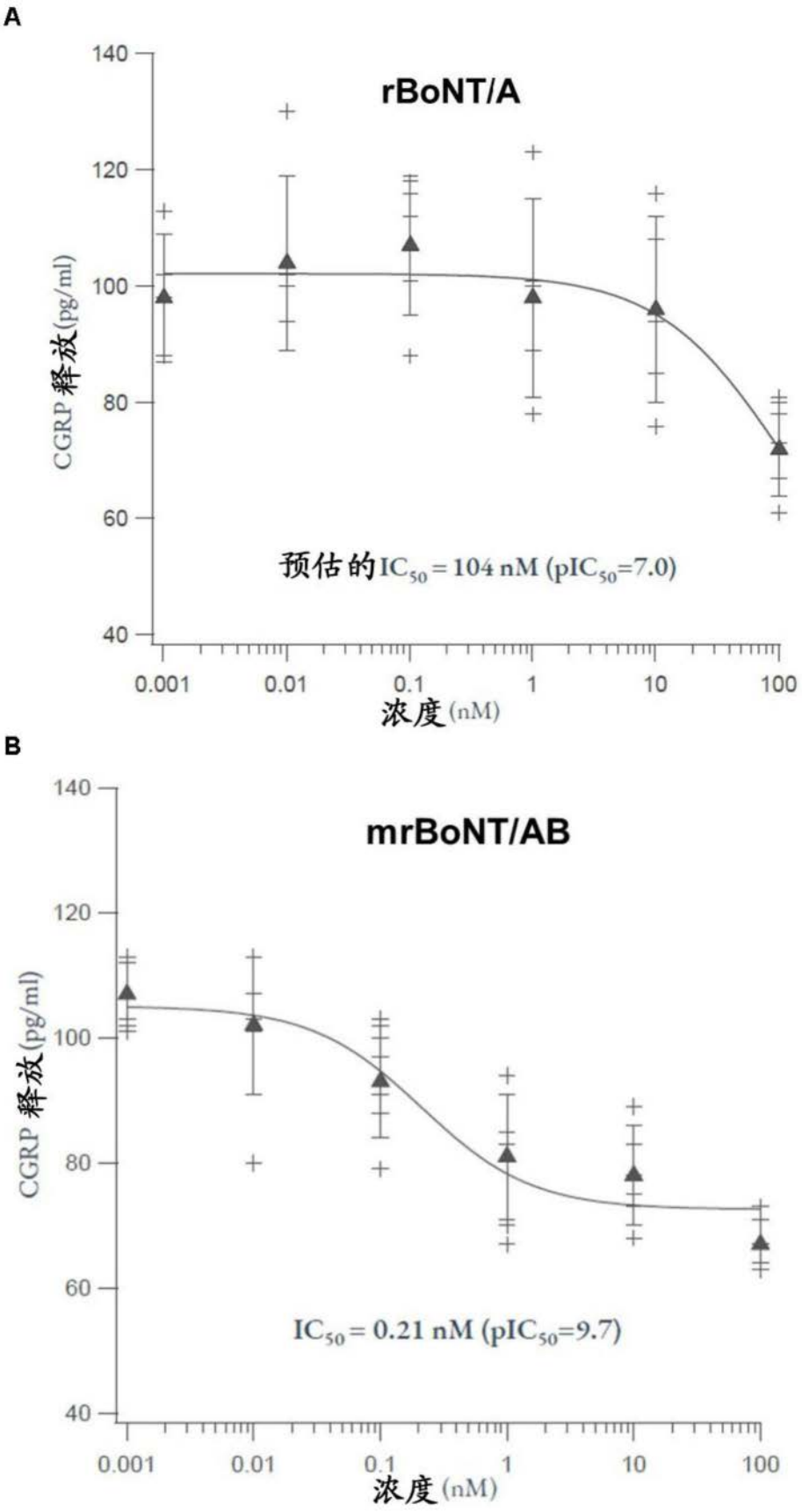

A
rBoNT/A
CGRP 释放(pg/ml)
140
120
100
80
60
40
预估的IC50 = 104 nM (pIC50=7.0)
0.001
0.01
0.1
1
10
100
浓度(nM)
B
mrBoNT/AB
CGRP 释放(pg/ml)
140
120
100
80
60
40
IC50 = 0.21 nM (pIC50=9.7)
0.001
0.01
0.1
1
10
100
浓度(nM)

图7

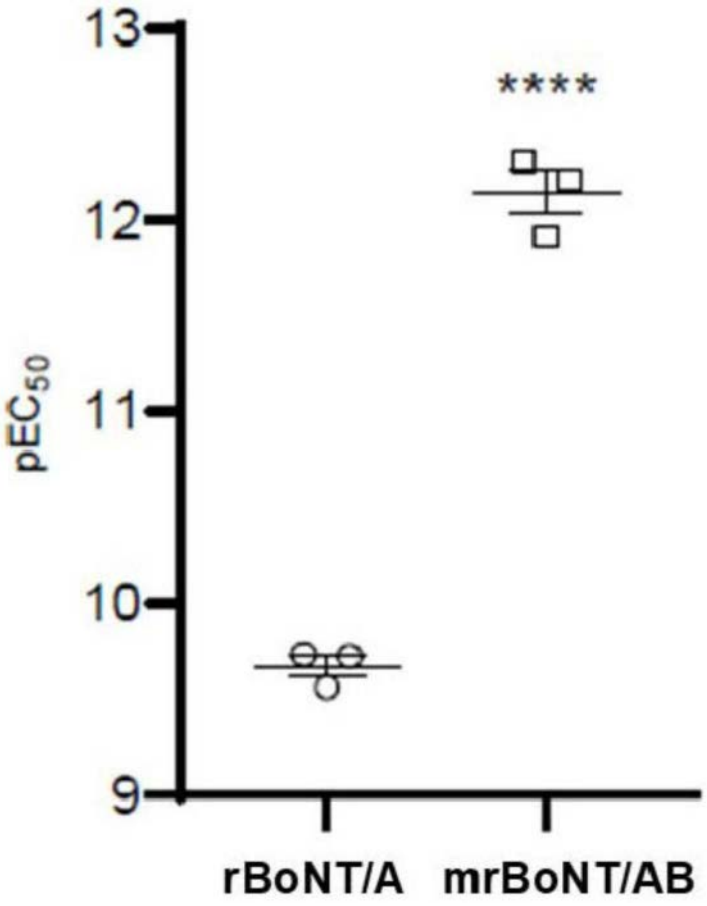


图8

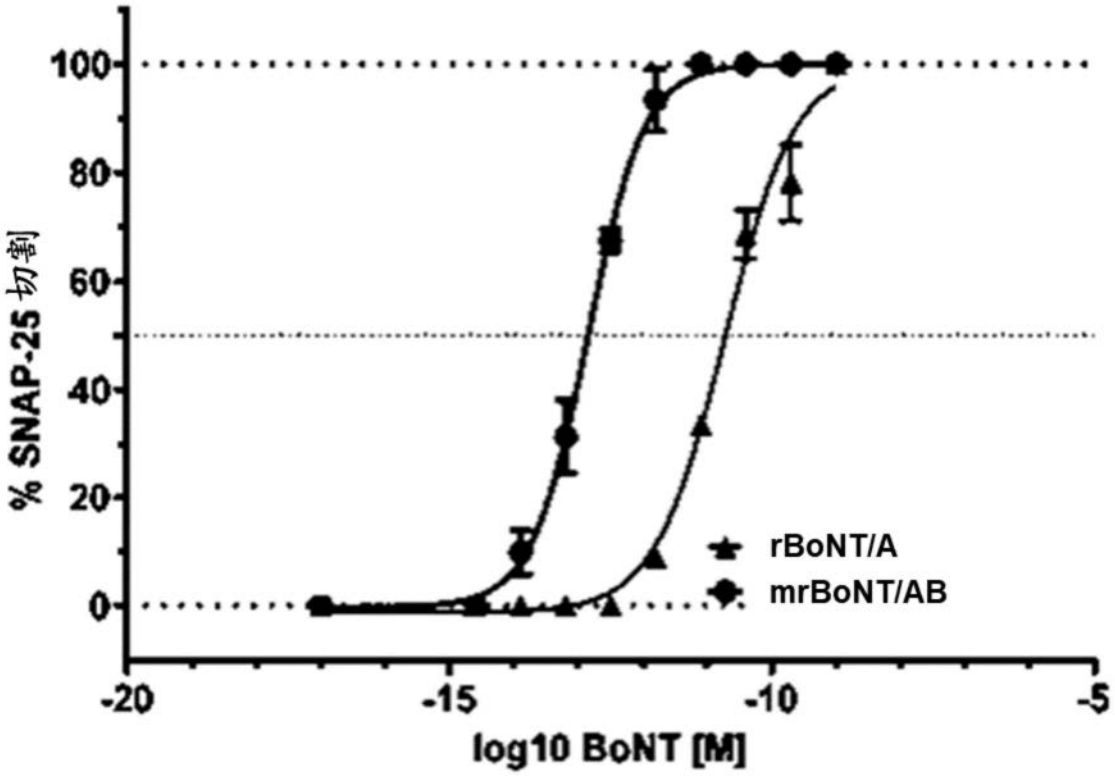

100
80
60
40
20
0
% SNAP-25 切割
-20
-15
-10
-5
log10 BoNT [M]
rBoNT/A
mrBoNT/AB

图9

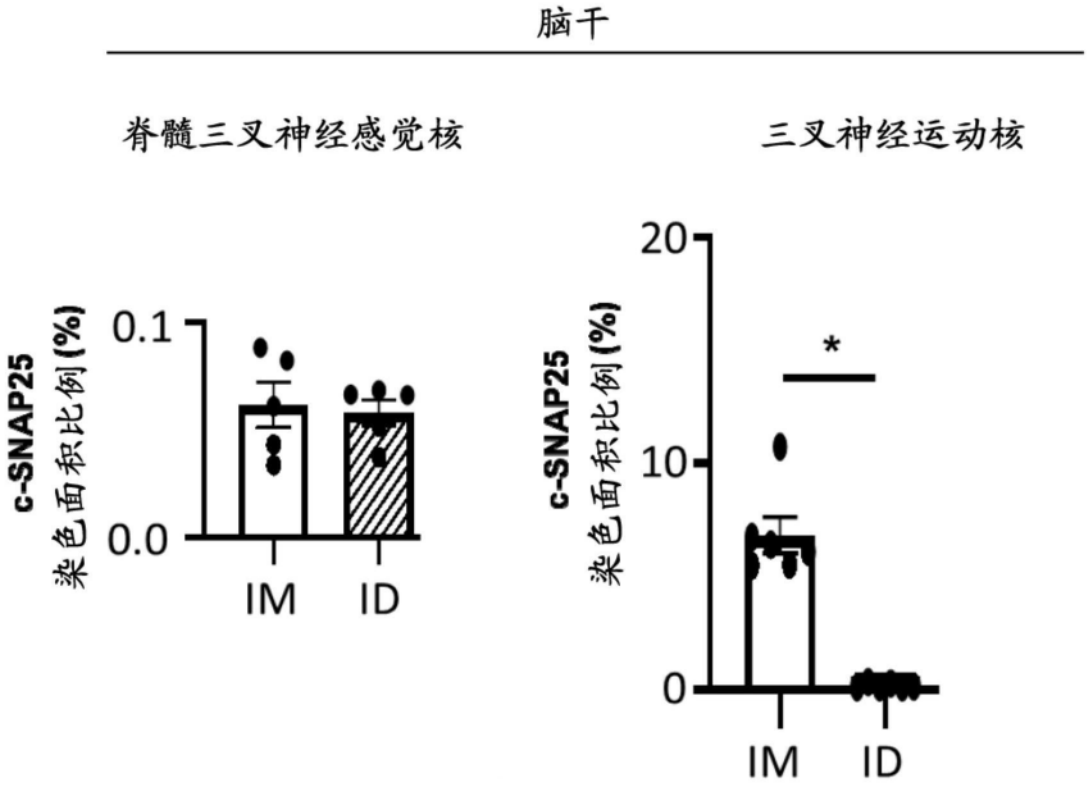

脑干
脊髓三叉神经感觉核
三叉神经运动核
c-SNAP25
染色面积比例(%)
0.1
0.0
IM
ID
20
10
0
*
IM
ID

图10

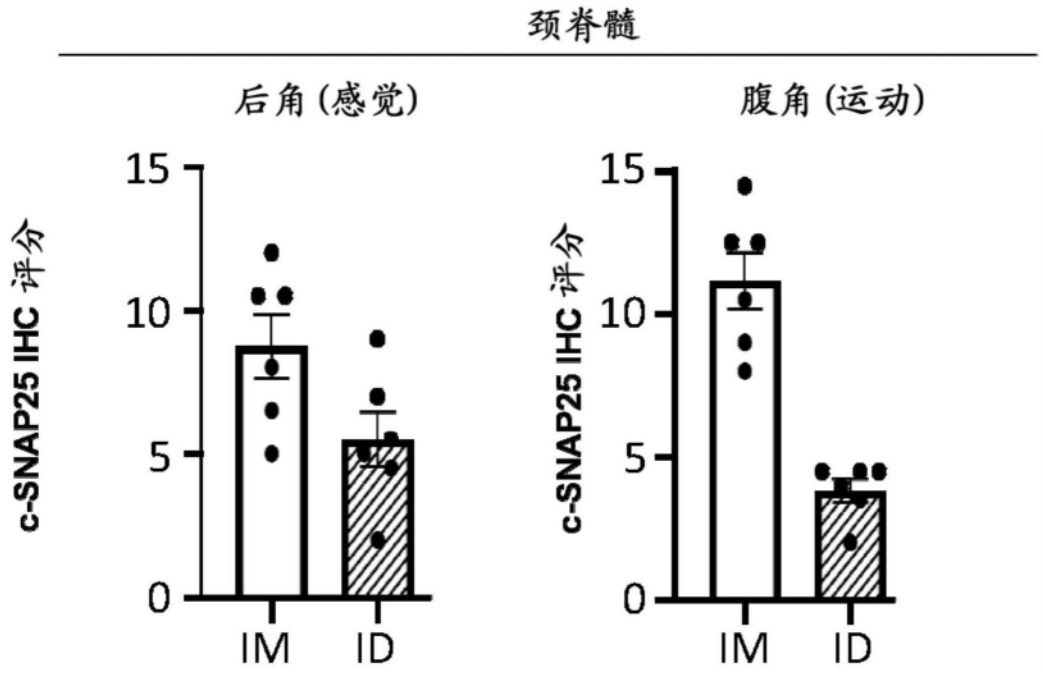

颈脊髓
后角(感觉)
腹角(运动)
c-SNAP25 IHC 评分
15
10
5
0
IM
ID

图11

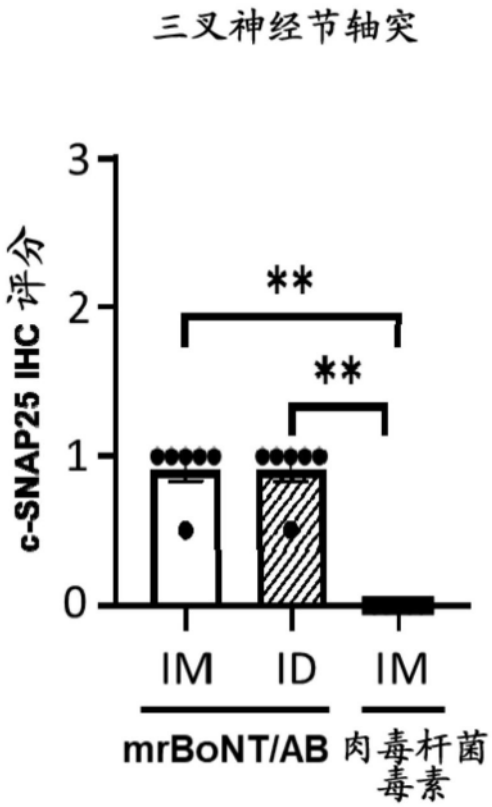
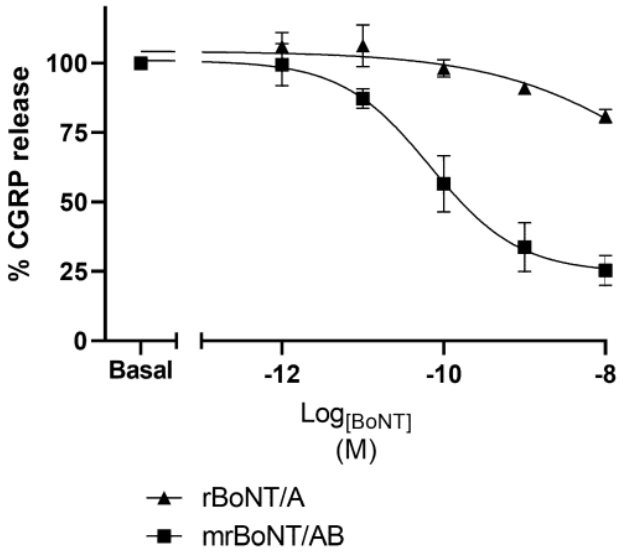

三叉神经节轴突
c-SNAP25 IHC 评分
3
2
1
0
**
**
IM
ID
IM
mrBoNT/AB
肉毒杆菌毒素

图12

Abstract

The present invention is directed inter alia to the treatment of pain. For example, there is provided a chimeric clostridial neurotoxin for use in treating pain by inhibiting release of a pain mediator from a neuron comprising an Aδ nerve fiber or a C nerve fiber, wherein the chimeric clostridial neurotoxin binds to the neuron comprising the Aδ nerve fiber or the C nerve fiber, respectively, and wherein the chimeric clostridial neurotoxin comprises a botulinum neurotoxin A (BoNT/A) light-chain and translocation domain (H_N domain), and a BoNT/B receptor binding domain (H_C domain). Also provided are methods, uses, kits, and unit dosage forms.



摘要

本发明尤其涉及疼痛的治疗。例如，提供了用于通过抑制疼痛介质从包含 Aδ 神经纤维或 C 神经纤维的神经元释放来治疗疼痛的嵌合梭菌神经毒素，其中所述嵌合梭菌神经毒素分别结合至包含 Aδ 神经纤维或 C 神经纤维的神经元，并且其中所述嵌合梭菌神经毒素包含肉毒杆菌神经毒素 A(BoNT/A)轻链和转位结构域(H_N结构域)以及 BoNT/B 受体结合结构域(H_C结构域)。还提供了方法、用途、试剂盒和单位剂型。