(54) Title: TREATMENT OF SINUSITIS RELATED CHRONIC FACIAL PAIN AND HEADACHE WITH BOTULINUM TOXIN

(57) Abstract:
The present invention provides methods for treating sinus-evoked headaches using botulinum toxin injected or applied in multiple subcutaneous locations over divisions of the trigeminal nerve in soft tissues and dermatomes overlying the corresponding affected sinuses implicated in the etiology of the pain.
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Treatment of Sinusitis Related Chronic Facial Pain and Headache with Botulinum Toxin

This application claims benefit to U. S. Provisional Application Serial No. 60/453,037 that was filed on March 3, 2003.

TECHNICAL FIELD OF THE INVENTION

This invention relates to methods for treating headache and facial pain associated with acute recurrent or chronic sinusitis with botulinum toxin.

BACKGROUND OF INVENTION

Botulinum neurotoxin, a toxin isolated from a strain of Clostridium botulinum, a deadly toxin at higher concentrations and quantities, has been used as a valuable therapeutic for the treatment of many neuromuscular diseases (e.g., dystonia, hemifacial spasm, bruxism, spasticity, cerebral palsy, torticollis), as well as sensory disorders and cutaneous disorders (myofacial pain, migraine, tension headaches, neuropathy, hyperhydrosis). Although botulinum toxin has been used for the treatment of migraine and tension headaches, botulinum toxin has not been recognized as an effective therapy for headache and facial pain associated with acute recurrent or chronic sinusitis.

Sinus-related headaches are distinctly different from migraine headache, myofascial headaches, and headaches associated with bruxism, temporal mandibular joint syndrome (TMJ) and temporal mandibular muscle dysfunction (TMD), trigeminal neuralgia, tooth related facial pain, pain associated with elevated intraocular pressure, or internal ocular inflammation. Sinus headaches are associated with pressure, or irritating processes within the sinus cavities, sometimes associated with inflammation and impaired flow of mucus secretion. At some point in the diagnostic workup, excessive signs of inflammation within the sinus or nasal cavity, or edema within the sinus or nasal cavity is demonstrated on exam or via radiographic methods. The present inventors have discovered that botulinum toxin relieves the headache and facial pain associated with sinusitis.
SUMMARY OF THE INVENTION

The present invention provides methods of treating headache and facial pain associated with acute recurrent or chronic sinusitis in a subject in need thereof, comprising the step of administering a therapeutically effective amount of a composition comprising botulinum toxin to the nasal mucosa or to the subcutaneous structures overlying the sinuses, wherein the administration of the composition reduces the headache and facial pain associated with acute recurrent or chronic sinusitis. In a preferred embodiment, the sinuses are one or more of the sinuses selected from the group consisting of: ethmoid; maxillary; mastoid; frontal; and sphenoid. Preferably, the subcutaneous structures overlying the sinuses lie within one or more of the areas selected from the group consisting of: forehead; malar; temporal; post auricular; and lip.

Botulinum toxin may be administered to the nasal mucosa or to the subcutaneous structures overlying the sinuses by any number of methods. Preferably, the composition comprising botulinum toxin is administered by injection at one or more injection sites. More preferably, the composition comprising botulinum toxin is administered to the cutaneous projections of the trigeminal nerve innervating the sinus.

In one embodiment of the present invention, a subject is treated by administration of a composition comprising botulinum toxin, wherein the subject, prior to the onset of facial pain or headache, exhibits symptoms or history of sinus rhinorrhea (nasal hypersecretion) and purulent nasal discharge.

The methods of the present invention may be practiced with various botulinum toxin immunotypes. In one embodiment, the botulinum toxin is any one or more botulinum toxin immunotypes selected from the group consisting of: A; B; C; D; E; F; and G. Furthermore, the methods of the present invention may utilize compositions of botulinum toxin wherein the composition is administered at a dose between 0.5 and 50,000 mouse LD$_{50}$ units of botulinum toxin. In a preferred embodiment, between 15 and 200 mouse LD$_{50}$ units spread over multiple injections within a dermatome corresponding to the sinus sensory innervation.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the projection of the trigeminal nerve both to the sinuses and the cutaneous and soft tissue structures of the face.
**Figure 2** shows the major divisions and branches of the trigeminal nerve.

**DETAILED DESCRIPTION OF INVENTION**

**A. Definitions.**

As used herein, "Botulinum toxin" means a protein toxin and its complexes isolated from strains of *Clostridium botulinum*, including various immunotypes such as A, B, C1, C2, C3, D, E, F and G.

As used herein, "a therapeutically effective amount" is an amount sufficient to produce a therapeutic response. An effective amount may be determined with dose escalation studies in open-labeled clinical trials or bin studies with blinded trials.

As used herein, "subject" means a mammal.

**B. Sinusitis.**

Sinusitis is defined as any inflammatory pathology involving the ethmoid, maxillary, frontal, or sphenoid sinuses. It is generally accepted that the cause of pain occurring with acute sinusitis involves infiltration of sinus mucosa with inflammatory cells, as well as increased pressure within the sinuses. What is generally not appreciated, and is herein disclosed, is that sinusitis can cause sensitization of the trigeminal nerve in cutaneous and subcutaneous tissues overlaying the sinus structures. When sensitization of sensory nerves occurs from repeated bouts of sinusitis, the patient can experience a chronic facial pain syndrome or headache. The mechanism by which sensory nerves become up-regulated or sensitized still is not clear. Nerve sensitization is provoked by alterations in the afferent first-order-sensory nervous system, such that thresholds are lowered to the perception of pain (hyperlgesia) and central second-order or higher-neuronal alterations can occur, resulting in an exaggerated response and interpretation of sensory stimuli (central sensitization). This process has been experimentally associated with increased expression and/or responsiveness of NMDA receptors on membranes of nociceptors and possible alterations in transcription and translation of proteins within the nerve cell. The trigeminal ganglia represent a very large collection of afferent sensory neurons, which send projects not only into cutaneous regions of the head, but also internally into osseous sinus structures, and mucous membranes of the nasal and sinus cavities (see **Figure 2**). The arborization pattern of afferent sensory nerve distribution is extensive, but reactivity within any region of the afferent sensory nerve
distribution has the capability of altering the genetic and cellular-protein expression of the sensory nerve cell body within the ganglion. The process of changing cell physiology has been variously coined neuroplasticity or sensitization. Alterations can be in the form of increased expression of nerve cell receptors, such as AMPA and NMDA receptors, modulation of effectors of inflammation, alteration of cellular responses from blood-vessel neural regulation via nitric oxide, substance P, histamine, CRGP, prostaglandins, other known cellular autocrions, and not yet defined autocrions and neuropeptides. The mechanism for sensitization of human nerve cells is still not well understood, and invoking inflammatory mediators, neurogenic inflammatory autocrions, and transcriptional and phenotypic changes of nociceptors and sensory neurons as the only mechanisms for nerve sensitization is not necessary to elicit responses from therapeutic botulinum toxin for this indication. Sensitization in the periphery is thought to occur following a sufficient or prolonged exposure to inflammatory substances, causing altered physiology, possible conformational changes of certain biochemical receptors, responsiveness, and lowered thresholds for nociceptor and sensory nerve depolarization.

Sinus pain usually begins in the mid facial region over the maxillary sinus and can radiate to temporal regions, ocular regions, vertex, and over the forehead. At times, referred pain can project into the posterior cervical region or peri-auricular areas. Generalized headaches can occur. The trigeminal nucleus is somatotropically well organized, and from the brain stem area, directly extends and connects anatomically to the upper-cervical areas of the dorsal horn of the spinal cord. In addition, there are interneuronal connections between the trigeminal nucleus and other cranial nerve nuclei, the autonomic nervous system, the reticular activating system, and other descending and ascending pathways. This interconnecting system has been described as the trigeminal sensory complex. Since there are many more peripheral upper cervical and trigeminal sensory nerves synapsing on fewer central nerves, this has been described as convergence and projection. This can explain the referral patterns of head and neck pains, and the therapies employed in one area of the head and neck to affect an outcome on a another area of the head and neck with shared and referred sensory pathways.

Distinct differences in headache diagnosis have been formulated at international conventions and remain the basis for both general and research practice. For migraine headaches, the presence of episodic headaches lasting 4-48 hrs, associated with light
sensitivity (photophobia), sound sensitivity (phonophobia), nausea or vomiting, pain of a throbbing or pulsating quality, and more often unilateral than bilateral location of headache. Cluster headaches can be associated with some basal transient nasal congestion but occur over a distinct time period (cluster period) and are not associated with any persistent sinus abnormalities on MRI or computerized tomography. Myofascial and tension headaches often have a cap-like squeezing pain across and around the top of the head, often associated with a cervical musculoskeletal pain location, frequently associated with trigger points, and sometimes associated with decreased jaw motility and bruxism if the masseter and temporalis muscles are involved. Ocular-related headaches are associated with increased intra-ocular pressure or signs of intra-ocular inflammation on slit lamp microscopic exam or measured refractive error. Dental-related headaches are associated with findings on dental examination and radiographs. Trigeminal neuralgia is usually limited to one or two dermatomes and is sharp and stabbing in quality, with a rapid “on-off” episodic pattern sometimes associated with stimulation of trigger points.

Chronic-sinusitis-related headache and facial pain can linger for many months to years after an acute or subacute bout of sinus disease or bout of repeated acute sinus headaches. Often, the patient complains of continued pain when radiologic imaging studies, such as computerized tomography and magnetic resonance imaging fail to show any persisting signs of inflammation such as mucosal thickening or fluid accumulation. Often out of desperation, the surgeon performs decompressive surgery via endoscopes or direct approaches (Culdwell luc, external ethmoidectomy) with poor results with respect to the chronic pain. The above observation explains a very common clinical phenomenon associated with chronic facial pain and headache caused by sinusitis. The reason for the persisting pain despite the absence of active sinus findings is peripheral sensory nerve upregulation or sensitization. Direct treatment of sinus-related headache by botulinum toxin injected into the subcutaneous region to down-regulate sensory nerves is therapeutic.

The convention in treating sinus-related headaches involves decongestants to augment mucous clearance and drainage from sinus cavities, antibiotics to treat bacterial infection, anti-inflammatory medication (e.g. corticosteroids), and surgical decompression. Conventional analgesics such as aspirin and acetaminophen may be used. The present inventors have made the unexpected discovery that administration of botulinum toxin over the surface dermatomes containing the sensory branches corresponding to the neurons
projecting into the sinus cavity effectively treats facial and headache pain associated with sinusitis.

C. Formal Classification and Nosology of Sinus Related Head and Neck Pain.

A convention held in 1985 by the International Headache Society (I.H.S.) put forth an exhaustive classification of distinct headache syndromes. Experts in the headache therapeutic field formulated this classification, and such experts explicitly agreed on the importance of headache distinction both for practice and research. The reasons for distinctions are to promote better communication among practitioners and to provide more exacting therapy for specific headache syndromes. For instance, procedures used to treat trigeminal neuralgia, such as glycerol injections, gamma knife application, and microvascular decompression at the level of the brainstem are not effective for the treatment of recurrent sinus headache. Tryptin-related pharmaceuticals (e.g. Imitrex-TM, Zomig-TM)) would be ineffective for the treatment of sinus headache and laser iridectomy for the treatment of narrow angle glaucoma would be ineffective for the treatment of migraine. Cluster headache needs to be distinguished from migraine. Hence, one skilled in the art of treatment of pain would require specific and professionally acceptable diagnosis in order to recommend reasonable therapy or to conduct clinical trials with potentially effective new therapies. The convention held in 1985 and subsequently published in Cephalgia (1988 Vol 8 (supplement 7), 1-96) has served as a benchmark for diagnosis and classification of human headaches (nosology) for the past 15 years.

In order for the physician to function and recommend therapeutic interaction with patients suffering from pain, classification with diagnostic criteria of an affliction must be determined. Classification of disease must be operationally specified with quantitative parameters and not just descriptive. The International Headache Society (I.H.S.) formed a committee in 1995 which lead to the first adopted international headache classification, which in turn permitted uniform operational criteria for diagnosis. The I.H.S. is internationally accepted and has been incorporated into the World Health Organization (W.H.O.) classification of disease. This classification has been translated into multiple languages and competes with no other classification system (see Jes Olesen Classification of Headache in Chapter 2, The Headaches, 2nd Edition, Lippincott, Williams and Wilkins ed Olesen, Hansen, Walsh, Philadelphia, 1999). An outline of the operational classification system is presented in Table 1.
In the classification system, headaches in category 1-4 are primary headache disorders with no associated anatomic pathologic process. Groups 5-11 are headaches and cervical pain associated with some other demonstrable disease process (trauma, vascular disease, increased intracranial pressure, withdrawal from substances, systemic infection, metabolic disorder, eye, ear, nose, and throat disease, or dental disease. Group 12 relates to cranial neuralgias.

The classification is quantitative, which allows for specific diagnosis. An excellent example of operation of the classification can be noted with the diagnosis of common migraine:

**I.H.S. Classification 1.1 (Migraine without aura—common migraine)**

Diagnostic criteria for migraine without aura:

A. At least 5 attacks fulfilling B-D.

B. Headache attacks lasting 4-72 hours.

C. Headache has at least two of the following characteristics:

1. Unilateral location.

2. Pulsating quality.

3. Moderate to severe intensity (inhibits or prohibits daily activities).

4. Aggravation by walking stairs or similar routine physical activity.

D. During headache, at least one of the following:

1. Nausea and/or vomiting.

2. Photophobia and/or phonophobia.

E. At least one of the following:

1. History and/or physical and/or neurological examinations do not suggest any one of the disorders listed in groups 5-11.
2. History and/or physical and/or neurological examinations do suggest any one of the disorders listed in groups 5-11, but it is ruled out by appropriate investigations.

3. Such a disorder (groups 5-11) is present, but migraine attacks do not occur for the first time in close temporal relationship to the disorder.

The I.H.S. classification of common migraine presented above is the method most reliably used for the diagnosis of migraine headaches and has been used in large multi-centered multinational double blinded drug trials used in the investigation of triptan based drugs for treatment of migraine (The Subcutaneous Sumatriptan International Study Group. Treatment of Migraine Attacks with Sumatriptan. N Engl J Med 1991:325: 316-321). In these studies, the I.H.S. was operatively used to distinguish migraine headaches from all other types of head and neck pain syndromes.

Sinusitis related headaches and pain is distinctly different than primary headaches, such as migraine and tension headaches, because of the demonstrable evidence of sinus disease. Because of the presence of associative pathology within the sinuses, sinus related head pains are examples of secondary headache syndromes, and receive unique classification under the I.H.S. and World Health Organization diagnostic systems. Under the I.H.S., diagnostic system, sinus headache is categorized as 11.5.1 (Acute sinus headache). The diagnostic operational criteria under this system is as follows:

A. Purulent or mucous discharge in the nasal passage, either by suction or spontaneous.

B. Pathologic findings in one or more of the following tests:

1. Radiologic exam.

2. CT/MRI.

3. Transillumination.

C. Simultaneous onset of headache and sinusitis.

D. Headache location:
1. In acute frontal sinusitis, headache directly over the sinus, or to the vertex, or behind the eye.

2. In acute maxillary sinusitis, headache is located over the antral area and may radiate to the upper teeth and forehead.

3. In ethmoidal sinusitis, the headache is located between and behind the eyes and radiates to the temporal area.

4. In acute sphenoiditis, headache is located in the occipital area, the vertex, the frontal region, or behind the eye.

E. Headache disappears after the treatment of acute sinusitis.

Chronic sinusitis under the I.H.S. criteria is considered to be multiple relapses of acute sinusitis. Additionally, the World Health Organization code and diagnosis for sinus related head and neck pain is G44.845 (Headache associated with disease of the respiratory system) J01 (Acute sinusitis headache) and J32 (Chronic sinusitis).

Table 1: Outline of International Headache Society Classification of Headache Syndromes
1. Migraine
1.1 Migraine without aura
1.2 Migraine with aura
1.2.1 Migraine with typical aura
1.2.2 Migraine with prolonged aura
1.2.3 Familial hemiplegic migraine
1.2.4 Basilar migraine
1.2.5 Migraine aura without headache
1.2.6 Migraine with acute onset aura
1.3 Ophthalmoplegic migraine
1.4 Retinal migraine
1.5 Childhood periodic syndromes that may be precursors to or associated with migraine
1.5.1 Benign paroxysmal vertigo of childhood
1.5.2 Alternating hemiplegia of childhood
1.6 Complications of migraine
1.6.1 Status migrainosus
1.6.2 Migrainous infarction
1.7 Migrainous disorder not fulfilling above criteria

2. Tension-type headache
2.1 Episodic tension-type headache
2.1.1 Episodic tension-type headache associated with disorder of pericranial muscles
2.1.2 Episodic tension-type headache unassociated with disorder of pericranial muscles
2.2 Chronic tension-type headache
2.2.1 Chronic tension-type headache associated with disorder of pericranial muscles
2.2.2 Chronic tension-type headache unassociated with disorder of pericranial muscles
2.3 Headache of the tension-type not fulfilling above criteria

3. Cluster headache and chronic paroxysmal hemicrania
3.1 Cluster headache
3.1.1 Cluster headache periodicity undetermined
3.1.2 Episodic cluster headache
3.1.3 Chronic cluster headache
3.1.3.1 Unrelenting from onset
3.1.3.2 Evolved from episodic
3.2 Chronic paroxysmal hemicrania
3.3 Cluster headache-like disorder not fulfilling above criteria

4. Miscellaneous headaches unassociated with structural lesion
4.1 Idiopathic stabbing headache
4.2 External compression headache
4.3 Cold stimulus headache
4.3.1 External application of a cold stimulus
4.3.2 Ingestion of a cold stimulus
4.4 Benign cough headache
4.5 Benign exertional headache
4.6 Headache associated with sexual activity
4.6.1 Dull type
4.6.2 Explosive type
4.6.3 Postural type

5. Headache associated with head trauma
5.1 Acute posttraumatic headache
5.1.1 With significant head trauma and/or confirmatory signs
5.1.2 With minor head trauma and no confirmatory signs
5.2 Chronic posttraumatic headache
5.2.1 With significant head trauma and/or confirmatory signs
5.2.2 With minor head trauma and no confirmatory signs

6. Headache associated with vascular disorders
6.1 Acute ischemic cerebrovascular disease
6.1.1 Transient ischemic attack (TIA)
6.1.2 Thrombembolic stroke
6.2 Intracranial hematoma
6.2.1 Intracerebral hematoma
6.2.1 Subdural hematoma
6.2.3 Epidural hematoma
6.3 Subarachnoid hemorrhage
6.4 Unruptured vascular malformation
6.4.1 Arteriovenous malformation
6.4.2 Secular aneurysm
6.5 DIY
6.5.1 Giant cell arteritis
6.5.2 Ghez systemic arteritides
6.5.3 Primary intracranial arteritis
6.5.6 Carotid or vertebral artery dissection
6.5.7 Carotidynia (idiopathic)
6.5.8 Post endarterectomy headache
6.7 Venous thrombosis
6.8 Arterial hypertension
6.8.1 Acute pressor response to exogenous agent
6.8.2 Pheochromocytoma
6.8.3 Malignant (accelerated) hypertension
6.8.4 Preeclampsia and eclampsia
6.9 Headache associated with other vascular disorder

7. Headache associated with nonvascular intracranial disorder
7.1 High cerebrospinal fluid pressure
7.1.1 Benign intracranial hypertension
7.1.2 High pressure hydrocephalus
7.2 Low cerebrospinal fluid pressure
7.2.1 Postlumbar puncture headache
7.2.2 Cerebrospinal fluid leaks headache
7.3 Intracranial infection
7.4 Intracranial sarcoidosis and other non-infectious inflammatory diseases
7.5 Headache related to intrathecal injections
7.5.1 Direct effect
7.5.2 Due to chemical meningitis
7.5.3 Intracranial neoplasm
7.5.4 Headache associated with other intracranial disorders

8. Headache associated with substances or their withdrawal
8.1 Headache induced by acute substance use or exposure
8.1.1 Nitrite/nitrate induced headache
8.1.2 Monosodium glutamate induced headache
8.1.3 Carbon monoxide induced headache
8.1.4 Alcohol-induced headache
8.1.5 Other substances
8.2 Headache induced by chronic substance use or exposure
8.2.1 Ergotamine induced headache
8.2.2 Analgesics abuse headache
D. Botulinum Toxin

Treatment of headache and facial pain associated with recurrent or chronic sinusitis may be practiced by administering botulinum toxin at a biologic activity dose ranging from 0.25-50,000 mouse LD50 units. Although one of ordinary skill evaluates dosing of the botulinum toxin based on several factors, including patient-specific factors, the proper dosing, depending on the composition and botulinum toxin immunotype, may be determined by using a regional denervation bioassay. Preferably, a composition comprising a botulinum toxin is to be administered at multiple sites along any dermatoine, corresponding and sharing sensory innervations with a paranasal sinus (see Figures 1 and 2). Figure 1 shows the trigeminal dermatomes. Note that V1 corresponds to projected sensory areas of the frontal and ethmoid sinuses. V2 corresponds to the maxillary, sphenoid, and mastoid sinuses. V3 corresponds to the maxillary sinus. Figure 2 shows the projection of the trigeminal nerve both to the sinus and the cutaneous and soft tissue structures of the face. Note that the ophthalmic division of the trigeminal nerve projects the frontal and ethmoid sinuses. The
maxillary division and small portions of the mandibular division project into the maxillary sinuses. Sphenoid and mastoid sinuses also receive sensory innervation in part from the trigeminal nerve.

Administration of a composition comprising botulinum toxin by injection, according to the methods of the present invention, is accomplished without directly injecting the zygomatic minor and major muscles to avoid distortion of the lower face from the muscular effects of botulinum toxin.

The methods of the present invention may be practiced with any one or more botulinum toxin immunotypes. The present invention also contemplates the use of compositions comprising botulinum toxin and sequestration agents such as albumin which are disclosed in United States patent 7,491,403, the original specification of which reads as follows.

**Improved Pharmaceutical Botulinum Toxin Compositions**

This application claims benefit to U. S. Provisional Application Serial No. 60/435,901 that was filed on December 20, 2002.

**TECHNICAL FIELD OF THE INVENTION**

This invention relates to improved pharmaceutical compositions comprising botulinum neurotoxin and a sequestration agent. The invention further provides pharmaceutical compositions and methods for the treatment of a variety of neuromuscular diseases.

**BACKGROUND OF THE INVENTION**

Botulinum neurotoxin is a toxin isolated from a strain of *Clostridium botulinum*, that acts at the neuromuscular junction by inhibiting release of acetylcholine. Botulinum toxin is initially formed as a single-chain polypeptide that is cleaved to form a light chain that is bound to a heavy chain through a disulfide bond. The denervating effect of botulinum toxin occurs through: 1) the binding of the heavy chain to high-affinity
receptors at the presynaptic terminal; 2) internalization of botulinum toxin through endocytosis; 3) translocation of the light chain into the cytoplasm of the nerve terminal; and 4) the endo metalloprotease activity of the light chain (zinc is a cofactor) cleaves specific synaptic proteins that inhibit fusion of synaptic vesicles with the presynaptic membrane, thereby inhibiting the release of acetylcholine contained in the vesicles. Absent acetylcholine, the muscle does not receive the necessary signal for the muscle to contract.

Although a deadly toxin at higher concentrations and quantities, botulinum toxin has been used as a valuable therapeutic for the treatment of many neuromuscular diseases (e.g., dystonia, hemifacial spasm, bruxism, spasticity, cerebral palsy, torticollis), as well as sensory disorders and cutaneous disorders (myofascial pain, migraine, tension headaches, neuropathy, hyperhidrosis). Prior to this invention, the in vivo binding of albumin to botulinum toxin has never been identified as important to clinical effectiveness of botulinum-toxin-based pharmaceuticals. By enhancing regional sequestration of the neurotoxin and facilitating saturation of neurotoxin receptors on neural tissues, high-concentration-albumin formulations improve the clinical effectiveness of botulinum toxin and reduce side effects such as those resulting from diffusion of the botulinum toxin from the site of administration. There has been no prior suggestion that altering the formulation of botulinum toxin by increasing its concentration relative to the neurotoxin could enhance the effectiveness for the treatment of human disease. The existing botulinum toxin preparations currently available for clinical practice are BOTOX®, DYSPORT®, MYOBLOC®. The present invention identifies the mechanism and provides compositions of improved utility of botulinum-toxin-based pharmaceuticals by increasing the concentration of a sequestration agent and other viscous agents to enhance sequestration and improve the effectiveness where other available botulinum toxin preparations have failed.

In recent years, Borodic et al. have characterized the regional effect of botulinum toxin using muscle fiber morphometrics, cholinesterase staining, and cutaneous wrinkling from depression of facial muscle tone. (Borodic (1992) Botulinum A toxin for (expressionistic) ptosis overcorrection after frontalis sling. Ophthalmic Plastic and

Since its introduction as a therapeutic agent, the pharmaceutically measurement of the denervating or biologic activity of botulinum toxin has been the LD$_{50}$ unit using a 18-22 gram Swiss-Webster mouse, quantitated statistically by injecting cohorts of mice at different dilutions from the purified botulinum neurotoxin protein and its protein complexes. This measurement has the advantage of simplicity of a clear endpoint determination (living or dead mouse), however the LD$_{50}$ unit does not predict clinical behavior of various botulinum toxin formulations when compared in clinical studies. For instance, one preparation of type B botulinum toxin (MYOBLOC®) requires 5,000-15,000 LD$_{50}$ units to treat torticollis whereas another preparation of botulinum toxin Type A (BOTOX®) requires only 100-300 LD$_{50}$ units. Similarly, the LD$_{50}$ unit has failed to distinguish differences in therapeutic behavior of different sources of the same botulinum toxin immunotype. For instance, approximately 50-300 units of BOTOX® is required to treat blepharospasm and cervical dystonia compared to 200-1200 units of DYSPORT®, another preparation of botulinum type A toxin. Table 1 illustrates the varying doses for different diseases.

Table 1: Dosing comparisons between various pharmaceutical formulations of botulinum toxin.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Essential Blepharospasm</th>
<th>Torticollis</th>
</tr>
</thead>
<tbody>
<tr>
<td>BOTOX&quot;&quot;</td>
<td>50 U 1</td>
<td>200 U</td>
</tr>
<tr>
<td>DYSPORt¹</td>
<td>200 U</td>
<td>600-1,200 u</td>
</tr>
<tr>
<td>MYOBLOC®</td>
<td>3,000-5,000 u</td>
<td>10,000-15,000 u</td>
</tr>
</tbody>
</table>

¹Units (U) are LD$_{50}$ Units determined using 20-30 g Swiss-Webster mice, as described herein.

A. Complications Associated with Conventional Botulinum-Toxin Formulations.

Beyond effective dose requirements, substantial differences in the complication rate have been noted at therapeutic quantities of different botulinum preparations. Side effects such as those resulting from diffusion of the botulinum toxin from the site of administration appear to be dependent on the formulation of botulinum toxin. For
instance, dysphagia rates (difficulty swallowing) is a well-known complication of botulinum toxin administration when used for the treatment of cervical dystonia. (Borodic et al. (1990) Botulinum A toxin for the treatment of spasmodic torticollis. *Dysphagia and Regional Toxin Spread. Head & Neck,* 12: 392-398; incorporated herein by reference in its entirety). Differences in the rate of this complication between formulations has been well appreciated when reviewing prior art literature between 1984-1995. Furthermore differences in the rate of ptosis have been reported when comparing various immunotypes and different preparations of the same immunotype (see Table 1). It has become well accepted that this complication is the result of diffusion of botulinum toxin away from the injections sites, a property which is in conflict with the clinical goal of containing the denervating or biologic effect to a specific target region.

**Table 2:** Diffusion-related complications between various pharmaceutical formulations of botulinum toxin.

<table>
<thead>
<tr>
<th>Complication</th>
<th>BOTOX®</th>
<th>DYSPORT™</th>
<th>MYOBLOC™</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ptosis¹</td>
<td>&lt;2%</td>
<td>12-15%</td>
<td>30-40%</td>
</tr>
<tr>
<td>Dysphagia</td>
<td>&lt;2%</td>
<td>14-21%</td>
<td>10-17%</td>
</tr>
</tbody>
</table>


In 1991, Borodic *et al.* demonstrated a histologic model demonstrating a histochemical and morphologic diffusion gradient from point injections of botulinum toxin. (Borodic *et al.* (1991) Botulinum toxin: Clinical and scientific aspects. *Ophthalmology Clinics of North America* 4: 491-503; incorporated herein by reference in its entirety). The gradient was further demonstrated to be dose dependent over single muscle strips and capable of crossing fascial planes. The diffusion model was further demonstrated on the facial wrinkling pattern of the human forehead. (Borodic *et al.* (1992) Botulinum toxin for spasmodic torticollis, multiple vs single point injections per muscle. *Head and Neck* 14: 33-37). Diffusion was thereafter used to explain the
mechanism for dysphagia after surface injections of botulinum injection for the human neck and ptosis (drooping eyelid complication) after periocular injections for the treatment of essential blepharospasm. Posis results from diffusion of neuromuscular blocking activity from the lid edge to the muscular portion of the upper eyelid retractor, which lies in the upper orbital space. Dysphagia results from diffusion of neuromuscular weakening effect from the stemomastoid muscle, targeted for treatment of torticollis, to peripharyngeal musculature which generates the force for effective swallowing. From both histologic models and clinical experience, diffusion appears to be directly related to the quantity of toxin given in LD₅₀ units, that is, the greater the LD₅₀ units used, the greater the diffusion from a point injection. From literature summary from the 1980’s and early 1990’s, dysphagia is more common with use of DYSPORT® than BOTOX® at effective doses. Recently, from studies done at European centers, the differences in dysphagia rates have been confirmed (Ranoux et al. 2002) Respective potencies of DYSPORT® and BOTOX®: a double blind, randomized, crossover study in cervical dystonia.


B. Sequestration.

Albumin was initially used to formulate botulinum toxin based pharmaceuticals because of its stabilizing effect on the biologic activity of the neurotoxin at high dilutions (see Schantz, Botulinum Toxin Therapy, Marcel Dekker 1994). Dilution of the purified
botulinum toxin crystals with physiologic saline or water would cause the biologic activity and pharmaceutical properties to be lost at high dilutions. Additionally, the albumin has been reported to help keep the neurotoxin molecule from binding to glass containers. During the pre-clinical development of BOTOX® or any other botulinum toxin prepared for pharmaceutical use, there was no appreciation for the importance of albumin in the formulation other than a dilution stabilizer and excipient to keep the neurotoxin from binding to glass.

BOTOX® and DYSPORT® are derived from different strains of Clostridial species. BOTOX® is derived from the Hall strain of Clostridium botulinum originally maintained by the University of Wisconsin, whereas DYSPORT® is derived from British Microbiology Collection. Immunologic cross reactivity exists between the products as both products were derived from immunotype A strains. Despite similar immunotypes, the clinical responses between BOTOX® and DYSPORT® may be explained by the differences in the excipients used in each formulation. The difference in human serum albumin concentrations between BOTOX® and DYSPORT® are outlined in Table 3.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Albumin 1</th>
<th>LD₅₀/μg albumin</th>
</tr>
</thead>
<tbody>
<tr>
<td>BOTOX®</td>
<td>500 µg</td>
<td>0.2</td>
</tr>
<tr>
<td>DYSPORT®</td>
<td>125 µg</td>
<td>5.0</td>
</tr>
</tbody>
</table>

1Albumin is represented in mg per 100 LD₅₀ units of botulinum toxin. Other differences exist including the presence of stabilizing sugars, Lactose is used in DYSPORT® and not used in BOTOX®.

The albumin discrepancy between BOTOX® and DYSPORT® is almost identical to the difference in dose requirements observed between BOTOX® and DYSPORT® in multiple clinical studies. The correlation between the albumin ratio/clinical potency ratio is further strengthened by changes in pharmacologic properties of DYSPORT® when albumin is added to the vials using a mouse hemidiaphragm animal model. Wohlffahrt et al. noted using this model that adding albumin to one vials of DYSPORT® brought biologic activity higher using the mouse hemi-diaphragm model. (Biglalke et al (2001)
Botulinum A toxin: DYSPORT® improvement of biological availability. Exp. Neural. 168(1): 162-170. The authors suggested the increased biologic activity resulted from increased stability as measured with the mouse LD₅₀ bioassay afforded by the albumin concentration increase. (Biglalke et al. 2001) Botulinum A toxin: DYSPORT® improvement of biological availability. Exp. Neural. 168(1): 162-170). The authors explained the differences of albumin on the LD₅₀ bioassay without reference to mechanism of action in tissues or pharmacologic-pharmacokinetic importance, that is, in vivo albumin binding, enhanced sequestration, and improvement in therapeutic effects.

The same authors further observed in a rat-diaphragm preparation, that the addition of albumin to the BOTOX® preparation could not substantially increase regional denervative effects and did not advocate any changes in formulation. The findings of these researchers concluded that there was an effect of the albumin concentration on the LD₅₀ measurements however, there work did not demonstrate any increased potency of BOTOX® on regional denervation or that DYSPORT® could be enhance to give any greater denervation potency over BOTOX®. There work was limited by the in vitro nature of their experiments, that is, using a non blood perfused animal dissection of a motor nerve (phrenic nerve) and diaphragm muscle, which fails to accounts for dilutions and tissue fluid flow capable of washing injected toxin away from targeted tissue prior to binding with the nerve axon terminal receptors. The real time application requires an in vivo analysis of the effects of albumin on regional denervation as outlined in the following experiments. Their work did identify reasons for differences in LD₅₀ as measured by the mouse lethality assay. The conclusion were no improvements in potency or effectiveness could be made over existing BOTOX® preparation and is directly contrary to the conclusion derived herein. (Hanover Germany International Botulinum Toxin Meeting 2002).

Differences in potency, issues relating diffusion and containment of the biologic effect are important in the pharmacology of botulinum-based pharmaceuticals. Described herein is a method for altering compositions of botulinum based pharmaceuticals to enhance potency, increase sequestration of the botulinum toxin and limit adverse effects of botulinum-based pharmaceuticals.
SUMMARY OF THE INVENTION

The present invention provides a composition comprising botulinum toxin and a sequestration agent for use in treating various neuromuscular diseases and localized denervation. In one embodiment, the sequestration agent is present in an amount between 550 and 550,000 µg sequestration agent per 100 LD₅₀ units botulinum toxin. In another embodiment, the sequestration agent is present in an amount between 550 and 5,500 µg sequestration agent per 100 LD₅₀ units botulinum toxin. In a further embodiment, the sequestration agent is present in an amount between 5,500 and 13,000 µg sequestration agent per 100 LD₅₀ units botulinum toxin. In a preferred embodiment, the sequestration agent is present in an amount between 13,000 and 50,500 µg sequestration agent per 100 LD₅₀ units botulinum toxin. In a more preferred embodiment, the sequestration agent is present in an amount between 50,500 and 505,000 µg sequestration agent per 100 LD₅₀ units botulinum toxin. In the most preferred embodiment, the sequestration agent is formulated as encapsulated microspheres in an amount between 50,500 and 90,500 µg sequestration agent per 100 LD₅₀ units botulinum toxin.

The botulinum toxin of the present compositions may be selected from a variety of strains of Clostridium botulinum. In a preferred embodiment, the compositions of the present invention comprises a botulinum toxin selected from the group consisting of botulinum toxin types A, B, C, D, E, F and G. In a preferred embodiment, the botulinum toxin is botulinum toxin type A. In a more preferred embodiment, the botulinum toxin is botulinum toxin type A from the Hall strain of Clostridium botulinum.

In another embodiment, the compositions of the present invention comprise a botulinum toxin that consists essentially of fractionated-light-chain botulinum toxin. In yet another embodiment, the botulinum toxin consists essentially of a mixture of hybrid and chain-translocated forms of botulinum toxin. In a further embodiment, the botulinum toxin consists essentially of chimeric forms of botulinum toxin. Although the present invention may utilize any botulinum toxin, botulinum toxin fragment that retains neurotoxic activity, botulinum toxin chimeras and hybrids, chemically-modified botulinum toxin, and specific activities well known to those of ordinary skill in the art, in
one embodiment the botulinum toxin is purified to a specific activity greater than or equal
to 20 LD₅₀ units per nanogram botulinum toxin.

The present invention provides compositions of botulinum toxin and a
sequestration agent wherein the ratio of LD₅₀ units of botulinum toxin to µg sequestration
agent is less than or equal to 0.2 for botulinum toxin type A and is less than or equal to 10
for botulinum toxin type B.

Each composition of the present invention, in addition to comprising a botulinum
toxin and a sequestration agent, may further comprise a pharmaceutically acceptable
carrier and/or zinc and/or a zinc salt. In one embodiment, the botulinum toxin is
noncovalently bound to the sequestration agent. In another embodiment, the botulinum
toxin is covalently bound to the sequestration agent.

The present invention provides compositions of a botulinum toxin and a
sequestration agent, wherein the sequestration agent is selected from the group consisting
of: proteins, lipids and carbohydrates. In a preferred embodiment, the sequestration agent
is albumin, collagen, epinephrine or hyaluronate. In a more preferred embodiment, the
sequestration agent is hyaluronate. In the most preferred embodiment, the sequestration
agent is albumin.

The present invention further provides compositions comprising a botulinum
toxin and a sequestration agent, wherein the sequestration agent is an albumin, preferably
human serum albumin. Furthermore, in one embodiment, the albumin of the present
compositions is recombinantly produced. In one embodiment, the albumin is present in
an amount between 550 and 5,500 µg albumin per 100 LD₅₀ units botulinum toxin. In a
further embodiment, albumin is present in an amount between 5,500 and 13,000 µg
albumin per 100 LD₅₀ units botulinum toxin. In a preferred embodiment, albumin is
present in an amount between 13,000 and 50,500 µg albumin per 100 LD₅₀ units
botulinum toxin. In a more preferred embodiment, albumin is present in an amount
between 50,500 and 505,000 µg albumin per 100 LD₅₀ units botulinum toxin. In a most
preferred embodiment, albumin is formulated as encapsulated microspheres in an amount
between 50,500 and 90,500 µg albumin per 100 LD₅₀ units botulinum toxin.
In another embodiment, the present invention provides a composition comprising botulinum toxin and a sequestration agent, wherein the sequestration agent is present in an amount between 550 and 900,500 µg sequestration agent per 100 LD₅₀ units botulinum toxin, wherein the albumin may be formulated as a solid albumin particle.

In one embodiment of the present invention, the compositions comprise a botulinum toxin and at least one sequestration agent. In a preferred embodiment, the compositions of the present invention comprise a botulinum toxin and albumin and further comprises one or more additional sequestration agents.

The present invention also provides methods of producing localized denervation in a subject in need thereof, comprising administering an effective amount of any of the compositions of the present invention that are described herein. In one embodiment, the methods of the present invention are used to produce denervation in a subject that suffers from a neuromuscular disease associated with increased muscle tone with involuntary movement. In another embodiment, the methods of the present invention are used to produce denervation in a subject that suffers from a neuromuscular disease. Preferably, the neuromuscular disease is characterized by increased muscle tone and/or involuntary movement, including but not limited to dystonias, spinal cord injury or disease, multiple sclerosis, spasticity, cerebral palsy, stroke, and the like. Preferably, the neuromuscular disease associated with increased muscle tone and/or involuntary movement is blepharospasm or torticollis. More preferably, the neuromuscular disease associated with increased muscle tone with involuntary movement is blepharospasm.

In one embodiment, the present invention provides methods for producing denervation in a subject suffering from blepharospasm comprising administering between 10- 200 LD₅₀ units of a composition of the present invention, as described herein. In another embodiment, the present invention provides methods for producing denervation in a subject suffering from torticollis. Preferably, the effective amount of a composition of the present invention is between 10 and 3000 LD₅₀ units.

In another embodiment, the present invention provides a method of treating a condition selected from the group consisting of facial wrinkles, rhytides and cosmetic
alteration of lip and brow, in a subject in need thereof, comprising administering an effective amount of a composition of the present invention, as disclosed herein. Preferably, the effective amount is between 2.5 and 400 LD$_{50}$ units.

In yet another embodiment, the present invention provides a method of treating human headache disorders in a subject in need thereof, comprising administering an effective amount of a composition of the present invention, as disclosed herein. Preferably, the effective amount is between 5 and 1000 LD$_{50}$ units.

In a further embodiment, the present invention provides a method of treating human migraine headache disorders in a subject in need thereof, comprising administering an effective amount of a composition of the present invention, as disclosed herein. Preferably, the effective amount is between 5 and 1,000 LD$_{50}$ units.

The present invention also provides a method of treating human inflammatory conditions in a subject in need thereof, comprising administering an effective amount of a composition of the present invention, as disclosed herein. Preferably, the effective amount is between 5 and 4,000 LD$_{50}$ units.

The present invention also provides a method of treating myopathic or neuropathic pain in a subject in need thereof, comprising administering an effective amount of a composition of the present invention, as disclosed herein. Preferably, the effective amount is between 5 and 4,000 LD$_{50}$ units.

The present invention also provides a method of treating back pain or arthritic pain in a subject in need thereof, comprising administering an effective amount of a composition of the present invention, as disclosed herein. Preferably, the effective amount is between 5 and 4,000 LD$_{50}$ units.

In yet another embodiment, the present invention provides a method of treating gastrointestinal spasm and strictures in a subject in need thereof, comprising administering an effective amount of a composition of the present invention, as disclosed herein. Preferably, the effective amount is between 5 and 4,000 LD$_{50}$ units.
The present invention provides a method of treating a hyperhyrosis syndrome in a subject in need thereof, comprising administering an effective amount of a composition of the present invention, as disclosed herein. Preferably, the effective amount is between 5 and 4,000 LD₅₀ units.

The present invention also provides a method of producing the compositions described herein. In one embodiment, the method comprises mixing a sequestration agent with botulinum toxin. In another embodiment, the method comprises freeze drying or flash drying a sequestration agent with botulinum toxin. Preferably, the botulinum toxin and the sequestration agent are in a weight to weight ratio which exceeds 100 µg sequestration agent to 1 ng of botulinum toxin.

DETAILED DESCRIPTION OF THE INVENTION

The present invention describes a method and composition to enhance the clinical effectiveness of botulinum-toxin preparation for clinical use by means of increasing sequestration of botulinum neurotoxin molecules in the region of the human or mammalian body targeted for therapy through the use of a sequestration agent or "molecular anchor". Enhanced sequestration using higher concentration of macromolecules such as proteins (e.g., albumin, collagen and the like), and/or lipids and/or polysaccharides (e.g., hyaluronate, and the like) can be useful to provide a molecular anchor to neurotoxin molecules preventing diffusion away from the injection point, causing maximal saturation of botulinum neurotoxin receptors, thereby achieving greater efficacy with the amount of neurotoxin used to achieve desired clinical effects. The sequestration agent enhances containment of regional denervation, and enhances clinical outcomes. The increased sequestration allows for better delivery to nerve ending, with enhanced uptake and augmentation of denervative and other biologic effects. The invention requires a sequestration agent added to a formulation of neurotoxin which binds to the neurotoxin, prevents dissemination of the neurotoxin and demonstrates improvement in clinical response in patients who were previously treated without the carrier molecule at preferred concentrations. The sequestration agent may be an existing excipient at significantly higher concentrations than previously used (such as human
serum albumin), or a material that has not been previously used to stabilize botulinum toxin (such as sodium hyaluronate). The sequestration agent must bind to the botulinum toxin molecule and prevents its diffusion so that the neurotoxin may react with the nerve-terminal ending or any neural structure so that effectiveness of the therapy is improved.

A. Definitions.

As used herein, “Botulinum toxin” means a protein toxin and its complexes isolated from strains of Clostridium botulinum, including various immunotypes such as A, B, C₁, C₂, C₃, D, E, F and G.

As used herein, “an effective amount” is an amount sufficient to produce a therapeutic response. An effective amount may be determined with dose escalation studies in open- labeled clinical trials or bin studies with blinded trials.

As used herein “neuromuscular diseases” refer to any disease adversely affecting both nervous elements (brain, spinal cord, peripheral nerve) or muscle (striated or smooth muscle), including but not limited to involuntary movement disorders, dytonias, spinal cord injury or disease, multiple sclerosis, spasticity, cerebral palsy, and stroke.

As used herein, the term “neuromuscular diseases” refer to any disease adversely affecting both nervous elements (brain, spinal cord, peripheral nerve) or muscle (striated or smooth muscle), including but not limited to involuntary movement disorders, dytonias, spinal cord injury or disease, multiple sclerosis, spasticity, cerebral palsy, and stroke.

As used herein, the term “pharmaceutically acceptable carrier” means a chemical composition, compound, or solvent with which an active ingredient may be combined and which, following the combination, can be used to administer the active ingredient to a subject. As used herein, “pharmaceutically acceptable carrier” includes, but is not limited to, one or more of the following: excipients; surface active agents; dispersing agents; inert diluents; granulating and disintegrating agents; binding agents; lubricating agents; preservatives; physiologically degradable compositions such as gelatin; aqueous vehicles and solvents; oily vehicles and solvents; suspending agents; dispersing or
wetting agents; emulsifying agents, demulcents; buffers; salts; thickening agents; fillers; antioxidants; stabilizing agents; and pharmaceutically acceptable polymeric or hydrophobic materials and other ingredients known in the art and described, for example in Genaro, ed., 1985, Remington’s Pharmaceutical Sciences, Mack Publishing Co., Easton, Pa., which is incorporated herein by reference.

As used herein, “sequestration agent” means an agent that enhances localization and/or retention of the botulinum toxin to the site of administration.

As used herein, “subject” means a mammal.

B. Albumin.

Endogenous human serum albumin binds native circulating molecules, such as free fatty acids, bilirubin, hormones and zinc. Additionally, circulating human albumin can bind with many pharmaceutical agents which can influence potency, complication rate, clearance, and other pharmacodynamic properties of these agents. Examples include salicylates, sulfisoxazole, warfarin, phenybutazone, digitoxin, phenytoin, oxacillin, benzylpenicillin, lasix, indomethacin, diazepam, and quindine among others. Peptides and proteins also are known to bind human serum albumin. Peptide hormones such as gastrin, corticotropin, melatonin are also known to bind human serum albumin.

Several binding sites have been identified and binding has been thought to be non-covalent. Additionally, albumin can non-covalently bind cations that serve as cofactors for enzymatic reactivity of portions of the botulinum toxin polypeptide complex. Specifically, zinc is a cofactor for the endopeptidase activity of the botulinum toxin light chain which enters the target cells after heavy chain binding to the cell surface protein receptors. Higher quantities of zinc bound to albumin enhance endopeptidase activity. Zinc binding to albumin is dose dependent. Saturation of zinc binding on albumin enhances the denervating effect of botulinum toxin.

Albumin, because of larger atomic mass and other protein properties, is physiologically cleared from the injection area by lymph vessel absorption, not blood vessel absorption), a process which a much slower than removal of smaller molecular
species. The relevance to Botulinum toxin pharmaceuticals relate to the role both in maintaining biologic activity by promoting nerve contact and preventing wash out from free neurotoxin release at injection points. DYSPORT®, with its lower albumin concentration, offers less sequestration for the neurotoxin complex, and subsequently, after injected, diffusion away from the targeted anatomic area are results. The clinical effect is a greater regional diffusion of the chemodenervation, which results in increased complications (ptosis, Dysphagia see Table 2). In order to compensate for this biologic behavior, the clinicians in practice or studies have had to give four to five time as much neurotoxin to achieve the same degree of biologic activity as a higher albumin concentration. With less potent immunotypes such as botulinum toxin type B (MYOBLOC®), larger dose are needed to achieve the same regional bioeffect, hence further diffusion occurs with increased complication rates (see Table 2). Administering more botulinum toxin (higher protein load) results in higher immunity rates after repeated injections. (Borodic et al. (1996) Botulinum Toxin, Immunology and Problems with Available Materials. Neurology 46: 26-29).

MYOBLOC® is formulated at an acidic pH <6.0 which provides for increased stability and stability of the liquid formulation at room temperature. Unfortunately, the acidic pH has an adverse side effect on the structure and probably tissue carrying properties of the human serum albumin in this biologic drug’s formulation. At varying pH, the isomerization of albumin can be considerable as well as the tertiary configuration of the albumin protein and physical properties (see Peters (1996) All about Albumin. Academic Press, New York; incorporated herein by reference in its entirety). Alterations in physical properties (via changes in binding of botulinum toxin and dynamics of botulinum toxin molecular release in tissues) can be used to explain some of the considerable differences in dose requirements comparing BOTOX® and MYOBLOC® in clinical practice. With higher pH, type B formulation, similar histologic effects can be seen with equivalent LD₅₀ units (see Borodic et al. (1993) Botulinum B Toxin as an Alternative to Botulinum A Toxin, A Histologic study. Ophthalmic Plastic and Reconstructive Surgery 9(3): 182-190).

Although other proteins (e.g. gelatin, lactalbumin, lysozyme), lipids and
carbohydrates may serve as effective sequestration agents, albumin, including encapsulated albumin and solid microspheres is the preferred protein sequestration agent, in part, because of its low immunogenicity. Other proteins, polysaccharides, lipids, polymers, gels and hydrogels that are potentially suitable as sequestration agents are disclosed in U.S. Patent No.: 4,861,627, which is incorporated herein by reference in its entirety. Methods of using and making protein microspheres, including albumin microspheres, are disclosed in U.S. Patent Nos.: 6,620,617; 6,210,707; 6,100,306; and 5,069,936 which are each incorporated herein by reference in their entirety.

C. Sequestration.

The concept of sequestration has been used by the inventor to explain altered lidocaine toxicity when periocular injections are given in the absence of Wydase. (Troll et al. (1999) Diplopia after cataract surgery using 4% lidocaine in the absence of Wydase™. Clin Anesth. 11(7): 615-6). Sequestration, in the absence of Wydase, of injectable lidocaine in this circumstance causes toxicity of myofibrils of the extra-ocular muscles with contraction scarring and damage to extra-ocular movement. The lidocaine example indicates how sequestration from dynamic diffusion of an injectable drug can be important to the drug’s basic pharmacology.

There has, however, never been a suggestion or recommendation that albumin can alter regional denervation potency or enhance clinical effects or be used to treat patients not responding to BOTOX®, DYSPORT® or MYOBLOC®. The present invention provides compositions and methods that enhance the clinical effectiveness of botulinum toxin pharmaceuticals.

As pointed out in the potency section above, sequestration-the regional containment of chemodervation- is one of the most important properties of the formulations of the present invention. The property in important in enhancing potency, reducing the complication rate from diffusion, and reducing antigenicity of the botulinum toxin. Preparations which require higher dosing, that is administration of an increased protein load, are associated with higher rates of immunity (comparing 79-11 original Occulimum Batch to current BOTOX® Batch, MYOLOC® compared to BOTOX®).
Enhanced sequestration allows for lower protein load, less diffusion, and enhanced biologic effect within the region targeted for treatment. The utility of this improved composition is demonstrated by its therapeutic effectiveness when conventional formulations (e.g., BOTOX®, MYOBLOC®) currently in use have failed or given suboptimal results.

D. Dosing of High-Albumin Formulations of Botulinum Toxin.

Producing compositions of botulinum toxin that require a lower effective amount to treat a particular condition is desired, because the administration of botulinum toxin has been associated with the development of immunologic resistance. Consequently, this complication requires increased dosing (higher LD\textsubscript{50} units) to achieve a therapeutically-effective amount of the botulinum toxin.

A composition of Hall-strain-derived botulinum toxin was formulated with a specific activity of 20 LD\textsubscript{50} units/ng toxin and 900 \( \mu \)g human serum albumin to 100 LD\textsubscript{50} units of botulinum toxin(0.11 LD\textsubscript{50} unit/\( \mu \)g albumin)( US FDA IND 4891). The indication for therapy for this new formulation was aberrant regeneration of the facial nerve with involuntary synkinetic blepharospasm. The study was conducted using between 5 and 15 LD\textsubscript{50} units of botulinum type A toxin formulated with the increased amount of albumin to LD\textsubscript{50} content.

Table 5: Reduction in effective amount of botulinum toxin using high-albumin botulinum toxin compositions.

<table>
<thead>
<tr>
<th>Open-Label Trials</th>
<th>15 patients each receiving 5-15 LD\textsubscript{50} units</th>
<th>100% demonstrated decreased involuntary movement</th>
<th>No ptosis complication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Double-Blind Placebo Controlled Trials</td>
<td>30 patients (ratio 1:1 treatment/control) each receiving 15 LD\textsubscript{50} units</td>
<td>1. Degree of involuntary movements significantly better than controls.2. Subjective parameters significantly better than controls</td>
<td>No ptosis complications</td>
</tr>
</tbody>
</table>

Prior literature has indicated that existing BOTOX\textsuperscript{®} preparations require 20 LD\textsubscript{50} units to achieve favorable results for this indication. (Borodic \textit{et al.} (1993) Botulinum
Toxin for aberrant facial nerve regeneration. Dose response relationships. *Plastic and Reconstructive Surgery*, (91):6: 1042-1045. 1993). Furthermore, there has been a 20% incidence of ptosis (a diffusion complication) associated with the use of botulinum toxin for involuntary blepharospasm, based on a 100 patient study on BOTOX® for the treatment of blepharospasm and using comparable LD₅₀ doses (see new batch approval study from Allergan Pharaceuticals, 1998; incorporated herein by reference in its entirety). Comparing the incidence of this complication in the high-albumin study shown above with the BOTOX® equivalency study (19/99, compared to 0/30, P<0.01, Chi Square), it appears that the high- albumin type A botulinum toxin composition required fewer LD₅₀ units to achieve acceptable therapeutic results (reduction in effective amount of toxin) and was associated with limited diffusion into the orbit which frequently results in ptosis. The decreased incidence of this complication indicated sequestration of the effects of botulinum toxin was enhanced by the higher albumin content.

Having set out the original specification in United States patent 7,491,403, we now continue with the present specification.

**EXAMPLES**

The following Examples serve to further illustrate the present invention and are not to be construed as limiting its scope in any way.

**Example 1:** Treatment of blepharospasm.

The subject is a 52-year-old female with severe bilateral involuntary blepharospasm. Involuntary movements have prevented her from driving and maintaining gainful employment. BOTOX® was administered by injection on five separate occasions without producing any significant clinical improvement. Surgery was performed to remove a portion of the protractors of eyelid closure (orbicularis oculii). No lasting improvement was observed.

The albumin content of the BOTOX® was altered by adding 5,000 µg human serum albumin to a vial of BOTOX® (100 LD₅₀ units). The resulting composition has an
albumin concentration of 2,750 μg/glee (0.018 LD5 of μg albumin). Administration of 60 LD50 units of the high-albumin preparation produced a nearly complete resolution of symptoms. The high-albumin concentration was clinically effective even when used in subsequent administrations (4 injection cycles) for over two years.

Example 2: Treatment of hemifacial spasm.

The subject is a 62-year-old male with a history of bilateral hemifacial spasm. Botulinum-toxin therapy using BOTOX® had been ineffective. The spasms impaired his day to day ability to function. Decompression of a facial nerve was attempted surgically on two separate occasions. Both surgeries proved ineffective in attaining acceptable relief of involuntary facial spasms and produced deafness in one ear.

The albumin content of the BOTOX® was increased by adding human serum albumin sufficient to achieve a concentration of 5,250 μg/cc (0.00952 LD50/μg albumin).

Administration of 30 LD50 units of the high-albumin preparation proved highly effective and substantially relieved the clinical symptoms.

Example 3: Treatment of hemifacial spasm.

The subject is a 66-year-old man with right hemifacial spasm. Although he was successfully treated with BOTOX® for 11 years, resistance developed that rendered further injections ineffective. Immunologic-resistance testing, using a remote point injection, demonstrated an absence of circulating antibody. A trial of another botulinum toxin formulation, MYOBLOC®, was also ineffective at relieving signs and symptoms.

The albumin content of BOTOX® was increased by adding human serum albumin sufficient to achieve a concentration of 5,250 μg/glee (0.00952 LD50/μg albumin). Administration of 40 LD50 units of the high-albumin preparation proved highly effective and substantially relieved the clinical symptoms.

Example 4: Treatment of benign essential blepharospasm.

The subject is a 72-year-old university president who was diagnosed with benign
essential blepharospasm. Four prior injections of the standard BOTOX® preparation failed to achieve any significant improvement. The subject was referred for possible surgical removal of muscle and nerve to weaken muscles necessary for eyelid closure. Instead, a high-albumin preparation of botulinum toxin was administered to the usual injections sites that are specific for benign essential blepharospasm. The high-albumin preparation was produced by adding 12,250 μg/lee (0.004 LD₅₀/μg albumin). Administration of 60 LD₅₀ units of the high-albumin preparation achieved excellent results when the administration of the conventional BOTOX® formulation had failed. Three months after the initial administration of the high-albumin botulinum toxin preparation, 40 LD₅₀ units of a high-albumin preparation comprising 25,000 μg albumin per 100 LD₅₀ units (0.002 LD₅₀/μg albumin) were administered and produced greater than 80% relief of the clinical symptoms of blepharospasm.

Example 5:  Treatment of blepharospasm.

The subject is a 67-year-old female with blepharospasm that was not responsive to BOTOX® injections. Surgical removal of nerve and muscle failed to provide any relief from involuntary eyelid closures.

Albumin was added to a conventional BOTOX® preparation to produce a high-albumin preparation of botulinum toxin with a concentration of 50,250 μg albumin/cc (0.001 LD₅₀/μg albumin). Injection of 50 units the high-albumin preparation produced a greater than 50% reduction of symptoms.

Example 6:  Treatment of blepharospasm.

The subject is a 77-year-old male who noted tachyphylaxis following repeated botulinum toxin injections. Conventional formulations of botulinum toxin type B were injected without relief of blepharospasm.

Human serum albumin and 0.5 cc Heaton® (hyaluronate) were both added to a 100 LD₅₀ units of botulinum toxin type A (BOTOX®). The high-albumin preparation produced contained 25,500 μg albumin per 100 LD₅₀ units (0.005 LD₅₀/μg albumin). Administration of 60 LD₅₀ units reduced the clinically-observed involuntary-eyelid
contractions.

Example 7: Treatment of essential blepharospasm.

The subject was a 66-year-old female with essential blepharospasm. Repeated treatment with BOTOX® (type A), using a range between 40 to 300 LD₅₀ units, produced no therapeutic benefit. Botulinum toxin type B (MYOBLOC®) was administered at a dose of 10,000 LD₅₀ units within the periocular region and also failed to produce any relief. Bilateral-facial neurectomy also failed to produce any substantial relief of symptoms. Additional surgical procedures to remove muscles necessary for eyelid closure were similarly ineffective.

Human serum albumin was added to a 100 LD₅₀ units of botulinum toxin type A (BOTOX®). The high-albumin preparation produced contained 12,750 µg albumin per 100 LD₅₀ units (0.00196 LD₅₀/µg albumin). Administration of 50 LD₅₀ units produced substantial relief of symptoms for a period of three to four months, when other formulations and surgical approaches had failed.

Example 8: Treatment of severe chronic blepharospasm.

The subject is an 83-year-old male with severe chronic blepharospasm. The subject had developed ptosis, a diffusion side effect, after repeated treatments with therapeutic doses of conventional botulinum toxin formulations. The emergence of ptosis complicated the treatment of this subject by requiring lower doses of botulinum toxin. The lower dosing proved less effective.

The patient received a high-albumin formulation of botulinum toxin that was produced by mixing 25,000 µg human serum albumin 100 LD₅₀ units of BOTOX®. The high-albumin preparation contained 12,750 µg albumin per cc (0.004 LD₅₀/µg albumin). Using the high-albumin preparation, 60-70 LD₅₀ units were administered with excellent clinical results and no evidence of ptosis after the therapy. The enhanced sequestration of much higher concentrations of botulinum toxin depressed the spread of the neurotoxin into the muscles within the eye socket.
Example 9: Treatment of essential blepharospasm.

The subject is a 67-year-old woman with essential blepharospasm. The subject underwent treatment with conventional formulations of botulinum toxin without relief. In addition, these treatments produced ptosis.

A high-albumin botulinum toxin composition (20,000 μg albumin per cc; 0.0025 LD₅₀ μg albumin) was administered to the subject with a resultant clinical improvement of the blepharospasm and no diffusion-related side effects (ptosis).

Table 4: Comparison of albumin concentrations used in Examples 1-9 with other formulations.

<table>
<thead>
<tr>
<th>Example</th>
<th>Albumin Concentration (μg/cc)</th>
<th>High-Albumin Preparation (LD₅₀/μg albumin/cc)</th>
<th>BOTOX®/(LD₅₀/μg albumin/cc)</th>
<th>DYSPORT®/(LD₅₀/μg albumin/cc)</th>
<th>MYOBLOC® (LD₅₀/μg albumin/cc)</th>
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LD₅₀/mcg albumin/cc for BOTOX®, DYSPORT®, MYOBLOC® given for direct comparison.

Example 10: Preparation of a high-albumin composition of botulinum toxin.

After quantizing the biologic effect by dilution of purified botulinum toxin, a quantity of albumin is added to the lyophilized material in a quantity sufficient to exceed 500 mg per 100 LD₅₀. The increased albumin binds to botulinum toxin and enhances sequestration of the injected neurotoxin providing for better saturation of neurotoxin receptors and improved clinical effect.
Example 11: Preparation of a high-albumin composition of botulinum toxin further comprising hyaluronate.

After quantitizing the biologic effect by dilution of purified botulinum toxin, a quantity of albumin is added to the lyophilized material in a quantity sufficient to exceed 500µg per 100 LD₅₀ units. Additionally, another sequestration agent, which further enhances sequestration, is added to keep the botulinum neurotoxin from diffusing away from the injections site. Such a sequestration agent includes but is not limited to a diluted solution of sodium hyaluronate. The increased albumin non-covalently binds to botulinum toxin and an enhances the sequestration of the neurotoxin providing better saturation of neurotoxin receptors and, consequently, an improved clinical effect.

Example 12: Preparation of a high-albumin composition of botulinum toxin further comprising collagen.

After quantifying the denervating effect of a botulinum neurotoxin by dilution of a purified botulinum toxin, albumin is mixed with the lyophilized botulinum neurotoxin in a quantity sufficient to exceed 500 µg albumin per 100 LD₅₀ units. Additionally, another physical agent, which further enhances sequestration, is added to keep botulinum neurotoxin from diffusing away from the injections field. Such an agent would be a diluted mixture of animal or human collagen. The increased albumin non-covalently binds to botulinum toxin and enhances to the sequestration of the neurotoxin proving better saturation of neurotoxin receptors and improved clinical effect.


Botulinum toxin is produced as a fusion protein with albumin thereby producing an albumin molecule that is covalently linked to a botulinum toxin. The fusion protein is tested using the mouse LD₅₀ bioassay to determine the effective amount. The regional denervation rabbit ptosis bioassay and mouse hindlimb bioassay may be used to confirm the effective amount of a composition comprising the fusion protein. A clinical-dose-escalation study would be further used to confirm and refine effective amount.
Example 14: Testing PURTOX-TM and other forms of botulinum toxin.

PURTOX-TM, that is botulinum type A stabilized with recombinant serum albumin and higher concentrations of albumin will need to be formulated with attention to rSA sources and rSA concentration. Zn++concentration, albumin concentration, and the presence of complex high activity botulinum or chromatographically separated pure neurotoxin. Emphasis will be placed on measuring duration of action. changes in critical point. Each preparation will be lyophilized in a low sodium solution, with or without stabilizing sugars.

EXAMPLES

The following Example serves to further illustrate the present invention and is not to be construed as limiting its scope in any way.

Example 1

RR is a 43-year-old man who suffered from repeated bouts of sinusitis. Radiologic studies revealed sinusitis. Treatment with decongestant and corticosteroid-type anti-inflammatory medications did not produce a sustained beneficial effect. Decompressive surgery via Culdwell-Luc approach for decompression and sinus drainage failed to produce symptomatic relief. The headaches progressed to be incapacitating. The patient had no prior history of migraine or tension (muscle contraction) headaches. Pain was experienced within the mid-face radiating and involving the temporal regions. Botulinum toxin, injected over multiple points with a 30-gauge needle, produced substantial improvement and reduction in pain, allowing the patient to return to his daily activities.

Example 2

JC is a 36-year-old woman with a history of chronic headache and face pain associated with sinus surgery. Treatment with oral analgesics and decongestants failed to produce any beneficial effects. She underwent decompressive sinus surgery months before the evaluation without pain relief. Conventional oral pain medications were
ineffective.

Multiple botulinum injections to the malar region and forehead at multiple sites produced over an 80% reduction in pain that was sustained for least three months. There was no past history of migraine, muscle contraction headaches, or trigeminal neuralgia. There was a history of recurrent allergies.

Example 3

J1 is a 40-year-old with headache associated with recurrent sinusitis. MRI confirmed evidence of sinus mucosal edema and nasal exam showed excessive mucus and purulent secretions. The patient had no prior history of migraine or tension (muscle contraction) headaches. Conventional pain medications (decongestants, antibiotics, and anti-inflammatory nasal sprays) were not effective in relieving pain. Multiple injections of botulinum toxin were administered to soft tissues covering the maxillary, frontal and ethmoidal sinuses, resulting in at least a 50% reduction in pain.

Example 4

WR is a 38-year-old court clerk referred for severe frontal headaches associated with maxillary sinusitis demonstrated on radiographic evaluations. External sinus surgery was performed without relief of the headaches. Most of the pain was localized to the left maxillary sinus region, which was tender to palpation. No past history of muscle contraction headache or migraine were identified.

Multiple injections of botulinum toxin over the sinus region, and away from the surgical incisions sites, relieved 80% of the pain. She has remained responsive for at least three years, using repeated injections of type A botulinum toxin.

Example 5

Fifteen patients with severe sinus-related headaches were evaluated in this open label trial. Each patient underwent either magnetic resonance imaging or computerized tomography of the sinus cavities that showed fluid levels, mucosal thickening, or mucous accumulation. All but one patient underwent generalized anesthesia and decompression
via endoscopic osteotomies, or externally via Culdwell-Luc or frontal sinus approach. Many (> 30%) underwent multiple surgical procedures to drain and decompress the sinus cavities.

The duration of disease ranged from 2-9 years with an average of 3.9 years. Age of the patients ranged from 29-90 years. 8 patients were female and 7 male. Total dose per botulinum toxin injection cycles ranged from 25 to 90 international units, with an average of 49 IU. Injections were made over the soft tissues of the involved sinuses in multiple locations as well as the corresponding dermatome (see Figures 1 and 2). In this group, only botulinum immunotype A was used. Follow-up visits were generally made at 3 and 12 weeks.

Booster injections were given if no initial response on first injection cycle was achieved.

Response to injections was determined on week 12.

Of 15 patients treated, 12 patients benefited from the therapy (80%). A beneficial response was considered to be a positive response to the question: "Have you experienced at least a 50% reduction in the severity or frequency of the pain?" Complications were related mainly to weakness created by the botulinum toxin injections, which caused drooping of the mouth or asymmetric smile. No side effects were permanent. Duration of benefit was approximately 12 weeks for most patients, consistent with the known duration of benefit for botulinum toxin for other uses.
We claim

1. Use of a composition comprising botulinum toxin on nasal mucosa or at subcutaneous structures overlying the sinuses of a subject for treating headache and facial pain associated with acute recurrent or chronic sinusitis.

2. The use of claim 1, wherein the sinuses are one or more of the sinuses selected from the group consisting of: ethmoid; maxillary; mastoid; frontal; and sphenoid.

3. The use of claim 1, wherein the subcutaneous structures overlying the sinuses lie within one or more of the areas selected from the group consisting of: forehead; malar; temporal; post auricular; and lip.

4. The use of claim 2, wherein the subcutaneous structures overlying the sinuses lie within one or more of the areas selected from the group consisting of: forehead; malar; temporal; post auricular; and lip.

5. The use of claim 1, wherein the botulinum toxin is any one or more of immunotypes A, B, C, D, E, F, or G.

6. The use of claim 2, wherein the botulinum toxin is any one or more of immunotypes A, B, C, D, E, F, or G.

7. The use of claim 3, wherein the botulinum toxin is any one or more of immunotypes A, B, C, D, E, F, or G.

8. The use of claim 4, wherein the botulinum toxin is any one or more of immunotypes A, B, C, D, E, F, or G.

9. The use of claim 1, wherein the composition is for use by injection.

10. The use of claim 2, wherein the composition is for use by injection.

11. The use of claim 3, wherein the composition is for use by injection.
12. The use of claim 4, wherein the composition is for use by injection.

13. The use of claim 9, wherein there are at least two injection sites.

14. The use of claim 10, wherein there are at least two injection sites.

15. The use of claim 11, wherein there are at least two injection sites.

16. The use of claim 12, wherein there are at least two injection sites.

17. The use of claim 1, wherein the composition is for use on the projections of the trigeminal nerve innervating the sinus.

18. The use of claim 2, wherein the composition is for use on the projections of the trigeminal nerve innervating the sinus.

19. The use of claim 3, wherein the composition is for use on the projections of the trigeminal nerve innervating the sinus.

20. The use of claim 4, wherein the composition is for use on the projections of the trigeminal nerve innervating the sinus.

21. The use of claim 9, wherein the composition is for use on the projections of the trigeminal nerve innervating the sinus.

22. The use of claim 10, wherein the composition is for use on the projections of the trigeminal nerve innervating the sinus.

23. The use of claim 11, wherein the composition is for use on the projections of the trigeminal nerve innervating the sinus.

24. The use of claim 12, wherein the composition is for use on the projections of the trigeminal nerve innervating the sinus.
25. The use of claim 13, wherein the composition is for use on the projections of the trigeminal nerve innervating the sinus.

26. The use of claim 14, wherein the composition is for use on the projections of the trigeminal nerve innervating the sinus.

27. The use of claim 15, wherein the composition is for use on the projections of the trigeminal nerve innervating the sinus.

28. The use of claim 16, wherein the composition is for use on the projections of the trigeminal nerve innervating the sinus.

29. The use of claim 1, wherein the composition is for use on the nasal mucosa.

30. The use of claim 1, wherein the composition is for use at a dose between 0.5 and 50,000 mouse LD50 units of botulinum toxin.

31. The use of claim 1, wherein, prior to onset of headache or facial pain, the subject exhibits symptoms or history of sinus hypersecretion and purulent nasal discharge.

32. Use of botulinum toxin for the preparation of a medicament for the treatment of headache and facial pain associated with acute recurrent or chronic sinusitis.

33. The use of claim 32, wherein the botulinum toxin is any one or more of immunotypes A, B, C, D, E, F, or C.

34. The use of claim 32, wherein the botulinum toxin is in a composition comprising a sequestration agent in an amount between 550 and 550,000 µg sequestration agent per 100 LD50 units botulinum toxin.

35. The use of claim 34, wherein the sequestration agent is albumin.

36. The use of claim 5, wherein the botulinum toxin is immunotype A.
37. The use of claim 6, wherein the botulinum toxin is immunotype A.

38. The use of claim 7, wherein the botulinum toxin is immunotype A.

39. The use of claim 8, wherein the botulinum toxin is immunotype A.

40. The use of claim 1, wherein the subject no longer exhibits sinus inflammation.

41. The use of claim 30, wherein the composition is for use at a dose between 15 and 50,000 LD50 units of botulinum toxin.

42. The use of claim 41, wherein the composition is for use at a dose between 15 and 200 LD50 units of botulinum toxin.

43. The use of claim 1, wherein the composition is for use at a dose between 25-90 international units of botulinum toxin.

44. Use of a composition comprising botulinum toxin on a location on or within the head of the subject for treating headache and facial pain associated with acute recurrent or chronic sinusitis.

45. The use of claim 44, wherein the subject no longer exhibits sinus inflammation.