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(54) **Title**: PHARMACEUTICAL FORMULATIONS OF NITRITE AND USES THEREOF

(57) **Abstract**: The present invention relates to pharmaceutical compositions of nitrites such as inorganic nitrites, or any pharmaceutically acceptable salts, solvates, or prodrugs thereof, and the medical use of these compositions. The pharmaceutical compositions, which can be formulated for oral administration, can provide immediate release or extended release of the nitrite ion (NO_2^-)The pharmaceutical compositions of the invention are useful, for example, for modulating brain function, in particular improving mood and/or psychological state, in the treatment of disorders of brain development, and in the treatment and/or reduction of pain.

PHARMACEUTICAL FORMULATIONS OF NITRITE AND USES THEREOF

Cross Reference To Related Applications

This application claims benefit of priority to U.S. Provisional Application No. 61/767,017, filed February 20, 2013, which is hereby incorporated by reference.

Background of the Invention

The present invention relates to pharmaceutical compositions of nitrites and the medical use of these compositions.

Nitric oxide (NO) serves as a neurotransmitter between nerve cells and has a general role in redox signaling. Unlike most other neurotransmitters that only transmit information from a presynaptic to a postsynaptic neuron, the small, uncharged, and fat-soluble nitric oxide molecule can diffuse widely and readily enters cells. Thus, it can act on several nearby neurons, even on those not connected by a synapse. At the same time, the short half-life of NO means that such action will be restricted to a limited area, without the necessity for enzymatic breakdown or cellular reuptake. NO is also highly reactive with other free radicals, lipids, and proteins. The NO-cGMP cascade is involved in learning and memory through the maintenance of long-term potentiation (LTP). Thus, NO is an important regulator and mediator of many processes in the brain and a balance in NO levels is critical in maintaining healthy signaling and brain development, and/or maintaining a balance in psychological state.

The role of NO has also been implicated in pain, however it remains unclear as to whether inhibition of NO or production of NO is beneficial in the treatment of pain. In some studies, it has been proposed that several pain-related pathways benefit from the production of NO. In particular, the blood-flow pathway, which is normalized in the presence of NO, may help to decrease ischemic pain; the nerve transmission pathway, which decreases the irritation of the nerves in the synovium, bone, and soft tissues; the opioid receptor pathway, which might stimulate the body's normal pain reduction pathways; and the anti-inflammation pathway. Other studies proposed that the inhibition of NO is beneficial in the treatment of pain. In these studies, NO is believed to be involved in the activation of cyclooxygenase 1 (COX-1) and regulation of cyclooxygenase 2 (COX-2) expression in inflammatory responses to increase prostaglandin release thereby inducing peripheral hyperalgesia and inflammation. NO generated by activation of N-methyl-D-aspartate (NMDA) receptors has been implicated in synaptic plasticity and many of these mechanisms are involved in central sensitization, a common problem in chronic pain. Certain studies have also suggested that NO mediates the peripheral and central anti-nociceptive effects of analgesic compounds such as opioids, and nonsteroidal anti-inflammaotry drugs.

Accordingly, there is a continuing need to understand the biological functions of NO and to investigate therapeutic strategies that provide a source of NO for maintaining normal brain functions and for the treatment and/or reduction of pain.

Summary of the Invention

In general, in a first aspect, the invention features a method of treating or reducing pain, that includes administering to a subject in need thereof a pharmaceutical composition including an effective

amount of inorganic nitrite or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable excipient. In a particular embodiment, the invention features a method of treating or reducing neuropathic pain, the method including administering the pharmaceutical composition described above. In a preferred embodiment, the invention features a method of treating or reducing diabetic peripheral neuropathy, that includes administering to the subject the pharmaceutical composition as described above.

In a second aspect, the invention further includes monitoring whether the subject experiences reduced pain, wherein reduced pain is measured as a decrease in pain intensity, frequency, duration, and/or improvements in quality of life.

In some embodiments, the subject has type 1 or type 2 diabetes. In other embodiments, subject does not have a condition associated with chronic ischemia. In yet another embodiment, the subject has a predisposition to, is diagnosed with, or has chronic pain.

In any of the foregoing aspects, the chronic pain is associated with lower back pain, arthritis, headache, multiple sclerosis, fibromyalgia, shingles, nerve damage, or cancer. In some instances the pain is neuropathic pain, inflammatory pain, nociceptive pain, functional pain, musculo-skeletal pain, or central nervous system pain. In certain embodiments, the neuropathic pain is diabetic peripheral neuropathy, post-herpetic neuralgia, trigeminal neuralgia, phantom limb pain, carpal tunnel syndrome, sciatica, pudendal neuralgia, complex regional pain syndrome, sensory polyneuropathys, mono-neuropathies, or central pain syndrome. In a preferred embodiment, the pain is diabetic peripheral neuropathy.

In a third aspect, the invention features a method of treating a mood disorder or a disorder of brain development, the method including administering to a subject in need thereof a pharmaceutical composition including an effective amount of inorganic nitrite or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable excipient.

In some embodiments, the mood disorder is selected from the group consisting of: major depressive disorders, depressive disorders, bipolar disorders, substance induced mood disorders, alcohol induced mood disorders, and benzodiazepine induced mood disorders. In other embodiments, the disorder of brain development is selected from the group consisting of: impairment of learning and memory, autistic disorder, Rett syndrome, childhood disintegrative disorder, pervasive developmental disorder-not otherwise specified (PDD-NOS), and Asperger syndrome.

In any of the foregoing aspects, the pharmaceutical composition includes from about 10 mg to about 100 mg or from about 20 mg to about 200 mg of inorganic nitrite, wherein the inorganic nitrite is $NaNO_2$, or KNO_2. In preferred embodiments, the inorganic nitrite is $NaNO_2$. In some aspects, the pharmaceutical composition is administered with a second agent, wherein the second agent is selected from the group consisting of: a non-steroidal anti-inflammatory drug (NTHE), a corticosteroid, acetaminophen, an opioid, a muscle relaxant, an anti-anxiety drug, an anti-depressant, an anti-convulsant drug, an antipsychotic, an antiepileptic drug, a selective serotonin reuptake inhibitor (SSRI), a norepinephrin inhibitor, and a mood stabilizer.

In one embodiment, the pharmaceutical composition is administered one or more times a day. In a second embodiment, the pharmaceutical composition is administered for at least two to twenty days. In a third embodiment, the administration occurs for at least two days, at least three days, at least four days, at least five days, at least six days, at least seven days, at least ten days, or at least fifteen days. In a

fourth embodiment, the dose is about 0.5 to about 2000 µg/kg; about 0.5 to about 1000 µg/kg; about 0.5 µg/kg to about 500 µg/kg; about 0.5 µg/kg to about 250 µg/kg; about 0.5 µg/kg to about 100 µg/kg; or about 0.5 µg/kg to about 50 µg/kg. In a preferred embodiment, the dose is about 165 µg/kg; about 16.5 µg/kg; or about 8.25 µg/kg.

In certain embodiments, the pharmaceutical composition is formulated for topical, enteral, or parenteral administration. In other embodiments, the pharmaceutical composition is formulated as a solid dosage form for oral administration. In preferred embodiments, the pharmaceutical composition is a tablet or capsule.

In any of the foregoing embodiments, the pharmaceutical composition includes a pharmaceutically acceptable excipient for delayed release of the inorganic nitrite, or pharmaceutically acceptable salt thereof, such that, when orally administered to a subject, the inorganic nitrite or pharmaceutically acceptable salt thereof is not substantially released in the stomach of the subject.

By "chronic pain" is meant pain that lasts longer than three to six months or pain that extend beyond the expected period of healing. Chronic pain may originate with an initial trauma/injury or infection, or may be an ongoing cause of pain associated with neuropathic pain (e.g., diabetic peripheral neuropathy, post-herpetic neuralgia, trigeminal neuralgia, phantom limb pain, carpal tunnel syndrome, sciatica, pudendal neuralgia, complex regional pain syndrome, sensory polyneuropathies, mono-neuropathies, or central pain syndrome), headaches, joint pain, backaches, sinus pain, muscle pain, nerve pain, and pain affecting specific parts of the body, such as shoulders, pelvis, and neck. Chronic pain may also be associated with lower back pain, arthritis, headache, multiple sclerosis, fibromyalgia, shingles, nerve damage, or cancer.

As used herein, the term "delayed release" refers to a pharmaceutical preparation, e.g., an orally administered formulation, which passes through the stomach substantially intact and dissolves in the small and/or large intestine (e.g., the colon). In some embodiments, delayed release of the active agent (e.g., nitrite as described herein) results from the use of an enteric coating of an oral medication (e.g., an oral dosage form).

The term an "effective amount" of an agent, as used herein, is that amount sufficient to effect beneficial or desired results, such as clinical results, and, as such, an "effective amount" depends upon the context in which it is being applied.

The terms "extended release" or "sustained release" interchangeably refer to a drug formulation that provides for gradual release of a drug over an extended period of time, e.g., 6-12 hours or more, compared to an immediate release formulation of the same drug. Preferably, although not necessarily, results in substantially constant blood levels of a drug over an extended time period that are within therapeutic levels and fall within a peak plasma concentration range that is between, for example, 0.05-10 µM, 0.1-10 µM, 0.1-5.0 µM, or 0.1-1 µM.

As used herein, the terms "formulated for enteric release" and "enteric formulation" refer to pharmaceutical compositions, e.g., oral dosage forms, for oral administration able to provide protection from dissolution in the high acid (low pH) environment of the stomach. Enteric formulations can be obtained by, for example, incorporating into the pharmaceutical composition a polymer resistant to dissolution in gastric juices. In some embodiments, the polymers have an optimum pH for dissolution in the range of approximately 5.0 to 7.0 ("pH sensitive polymers"). Exemplary polymers include methacrylate acid copolymers that are known by the trade name Eudragit® (e.g., Eudragit® L100,

Eudragit® S100, Eudragit® L-30D, Eudragit® FS 30D, and Eudragit® L100-55), cellulose acetate phthalate, cellulose acetate trimellitiate, polyvinyl acetate phthalate (e.g., Coateric®), hydroxyethylcellulose phthalate, hydroxypropyl methylcellulose phthalate, or shellac, or an aqueous dispersion thereof. Aqueous dispersions of these polymers include dispersions of cellulose acetate phthalate (Aquateric®) or shellac (e.g., MarCoat 125 and 125N). An enteric formulation reduces the percentage of the administered dose released into the stomach by at least 50%, 60%, 70%, 80%, 90%, 95%, or even 98% in comparison to an immediate release formulation. Where such a polymer coats a tablet or capsule, this coat is also referred to as an "enteric coating."

The term "pharmaceutical composition," as used herein, represents a composition containing a compound described herein (e.g., inorganic nitrite, or any pharmaceutically acceptable salt, solvate, or prodrug thereof), formulated with a pharmaceutically acceptable excipient, and typically manufactured or sold with the approval of a governmental regulatory agency as part of a therapeutic regimen for the treatment of disease in a mammal. Pharmaceutical compositions can be formulated, for example, for oral administration in unit dosage form (e.g., a tablet, capsule, caplet, gelcap, or syrup); for topical administration (e.g., as a cream, gel, lotion, or ointment); for intravenous administration (e.g., as a sterile solution free of particulate emboli and in a solvent system suitable for intravenous use); or in any other formulation described herein.

A "pharmaceutically acceptable excipient," as used herein, refers any ingredient other than the compounds described herein (for example, a vehicle capable of suspending or dissolving the active compound) and having the properties of being nontoxic and non-inflammatory in a patient. Excipients may include, for example: antiadherents, antioxidants, binders, coatings, compression aids, disintegrants, dyes (colors), emollients, emulsifiers, fillers (diluents), film formers or coatings, flavors, fragrances, glidants (flow enhancers), lubricants, preservatives, printing inks, sorbents, suspensing or dispersing agents, sweeteners, or waters of hydration. Exemplary excipients include, but are not limited to: butylated hydroxytoluene (BHT), calcium carbonate, calcium phosphate (dibasic), calcium stearate, croscarmellose, cross-linked polyvinyl pyrrolidone, citric acid, crospovidone, cysteine, ethylcellulose, gelatin, hydroxypropyl cellulose, hydroxypropyl methylcellulose, lactose, magnesium stearate, maltitol, maltose, mannitol, methionine, methylcellulose, methyl paraben, microcrystalline cellulose, polyethylene glycol, polyvinyl pyrrolidone, povidone, pregelatinized starch, propyl paraben, retinyl palmitate, shellac, silicon dioxide, sodium carboxymethyl cellulose, sodium citrate, sodium starch glycolate, sorbitol, starch (corn), stearic acid, stearic acid, sucrose, talc, titanium dioxide, vitamin A, vitamin E, vitamin C, and xylitol.

The term "pharmaceutically acceptable prodrugs" as used herein, represents those prodrugs of the compounds of the present invention which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of humans and animals with undue toxicity, irritation, allergic response, and the like, commensurate with a reasonable benefit/risk ratio, and effective for their intended use, as well as the zwitterionic forms, where possible, of the compounds of the invention.

The term "pharmaceutically acceptable salt," as use herein, represents those salts which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of humans and animals without undue toxicity, irritation, allergic response and the like and are commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable salts are well known in the art. For example, pharmaceutically acceptable salts are described in: Berge et al., *J. Pharmaceutical Sciences* 66:1-19,

1977 and in *Pharmaceutical Salts: Properties, Selection, and Use*, (Eds. P.H. Stahl and C.G. Wermuth), Wiley-VCH, 2008. The salts can be prepared *in situ* during the final isolation and purification of the compounds of the invention or separately by reacting the free base group with a suitable organic or inorganic acid. Representative acid addition salts include acetate, adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, fumarate, glucoheptonate, glycerophosphate, hemisulfate, heptonate, hexanoate, hydrobromide, hydrochloride, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, toluenesulfonate, undecanoate, valerate salts, and the like. Representative alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, magnesium, and the like, as well as nontoxic ammonium, quaternary ammonium, and amine cations, including, but not limited to ammonium, tetramethylammonium, tetraethylammonium, methylamine, dimethylamine, trimethylamine, triethylamine, ethylamine, and the like.

The terms “pharmaceutically acceptable solvate” or “solvate,” as used herein, means a compound of the invention wherein molecules of a suitable solvent are incorporated in the crystal lattice. A suitable solvent is physiologically tolerable at the administered dose. For example, solvates may be prepared by crystallization, recrystallization, or precipitation from a solution that includes organic solvents, water, or a mixture thereof. Examples of suitable solvents are ethanol, water (for example, mono-, di-, and tri-hydrates), *N*-methylpyrrolidinone (NMP), dimethyl sulfoxide (DMSO), *N*,*N*'-dimethylformamide (DMF), *N*,*N*'-dimethylacetamide (DMAC), 1,3-dimethyl-2-imidazolidinone (DMEU), 1,3-dimethyl-3,4,5,6-tetrahydro-2-(1H)-pyrimidinone (DMPU), acetonitrile (ACN), propylene glycol, ethyl acetate, benzyl alcohol, 2-pyrrolidone, benzyl benzoate, and the like. When water is the solvent, the solvate is referred to as a “hydrate.”

The term “reducing,” as used herein, refers to treatment that alleviates one or more symptoms or conditions of a disease, disorder, or conditions described herein (e.g., pain). Treatment can be initiated, for example, following (“post-exposure prophylaxis”) an event that precedes the onset of the disease, disorder, or conditions in a subject that has been predisposed or previously diagnosed with the disease and/or condition. Treatment that includes administration of a compound of the invention, or a pharmaceutical composition thereof, can be acute, short-term, or chronic. The doses administered may be varied during the course of the treatment.

By “improvement in mood or psychological state” is meant a positive change in a subject’s emotional state.

By “modulates brain function” is meant to regulate or adjust the levels of NO in the brain such that there is homeostatic signaling occurring in the brain.

By “predisposition or is diagnosed” is meant a population of subjects (e.g. mammals, including humans and non-humans) that has been pre-selected as having a condition associated with pain, a mood disorder and/or an imbalance in psychological state, or a disorder of brain development. The conditions associated with pain include but is not limited to: musculo-skeletal pain (after trauma, infections), neuropathic pain caused by diabetes, infections, metabolic disorders, exposure to toxins, traumatic injury, spinal cord injury, tumors, compression, inflammation, dental pain, episiotomy pain, deep and visceral

pain (e.g., heart pain, bladder pain, or pelvic organ pain), muscle pain, eye pain, orofacial pain (e.g., odontalgia, trigeminal neuralgia, glossopharyngeal neuralgia), abdominal pain, gynecological pain (e.g., dysmenorrhea and labor pain), pain associated with nerve and root damage due to trauma, compression, inflammation, toxic chemicals, metabolic disorders, hereditary conditions, infections, vasculitis and autoimmune diseases, central nervous system pain, such as pain due to spinal cord or brain stem damage, cerebrovascular accidents, tumors, infections, demyelinating diseases including multiple sclerosis, low back pain, sciatica, and post-operative pain. Mood disorders include but are not limited to: major depressive disorders, depressive disorders, bipolar disorders, substance induced mood disorders, alcohol induced mood disorders, and benzodiazepine induced mood disorders. Disorders of brain development include but are not limited to: impairment in learning and memory, autistic disorder, Rett syndrome, childhood disintegrative disorder, pervasive developmental disorder-not otherwise specified (PDD-NOS), and Asperger syndrome.

The term "prodrug," as used herein, represents compounds which are rapidly transformed *in vivo* to the parent compound of the above formula. Prodrugs also encompass bioequivalent compounds that, when administered to a human, lead to the *in vivo* formation of nitrite ion (NO_2^-) or nitrous oxide (NO). A thorough discussion is provided in T. Higuchi and V. Stella, Pro-drugs as Novel Delivery Systems, Vol. 14 of the A.C.S. Symposium Series, and Edward B. Roche, ed., Bioreversible Carriers in Drug Design, American Pharmaceutical Association and Pergamon Press, 1987, each of which is incorporated herein by reference. Preferably, prodrugs of the compounds of the present invention are pharmaceutically acceptable such as those described in EP 1336602A1, which is herein incorporated by reference.

As used herein, and as well understood in the art, "treatment" is an approach for obtaining beneficial or desired results, such as clinical results. Beneficial or desired results can include, but are not limited to, alleviation or amelioration of one or more symptoms or conditions; diminishment of extent of disease, disorder, or condition; stabilized (i.e. not worsening) state of disease, disorder, or condition; preventing spread of disease, disorder, or condition; delay or slowing the progress of the disease, disorder, or condition; amelioration or palliation of the disease, disorder, or condition; and remission (whether partial or total), whether detectable or undetectable. "Treatment" can also mean prolonging survival as compared to expected survival if not receiving treatment. As used herein, the terms "treating" and "treatment" can also refer to delaying the onset of, retarding or reversing the progress of, or alleviating either the disease or condition to which the term applies, or one or more symptoms of such disease or condition.

The term "unit dosage forms" refers to physically discrete units suitable as unitary dosages for human subjects and other mammals, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect, in association with any suitable pharmaceutical excipient or excipients.

As used herein, the term "plasma concentration" refers to the amount of nitrite ion present in the plasma of a treated subject (e.g., as measured in a rabbit using an assay described below or in a human).

By "about" is meant ±20% of the recited value.

Other features and advantages of the invention will be apparent from the following Detailed Description, the drawings, and the claims.

Brief Description of the Drawings

Figures 1A-1B show the results from the RAND 36 Questionnaire. Figure 1A shows results from the physical quality of life assessment in the placebo, 40 mg, and 80 mg group. Figure 1B shows results from the psychological quality of life assessment in the placebo, 40 mg, and 80 mg group.

Figures 2A-2B show the results from the WIQ. Figure 2A shows results from the WIQ in the FAS population. Figure 2B shows results from the WIQ in the diabetic population.

Detailed Description

The invention features methods to treat and/or alleviate pain, particularly neuropathic pain (e.g., diabetic peripheral neuropathy, post-herpetic neuralgia, trigeminal neuralgia, phantom limb pain, carpal tunnel syndrome, sciatica, pudendal neuralgia, and central pain syndrome), modulate brain function, to improve mood and/or psychological state, and treat disorders of brain development, such as autism.

Nitrite

Inorganic Nitrite

The pharmaceutically acceptable compositions of the invention include inorganic nitrite, e.g., a salt or ester of nitrous acid (HNO_2), or a pharmaceutically acceptable salt thereof. Nitrite salts can include, without limitation, salts of alkali metals, e.g., sodium, potassium; salts of alkaline earth metals, e.g., calcium, magnesium, and barium; and salts of organic bases, e.g., amine bases and inorganic bases. Compounds of the invention also include all isotopes of atoms occurring in the intermediate or final compounds. Isotopes include those atoms having the same atomic number but different mass numbers. For example, isotopes of hydrogen include tritium and deuterium. The term "compound," as used herein with respect to any inorganic nitrite or pharmaceutically acceptable salt, solvate, or prodrug thereof. All compounds, and pharmaceutical acceptable salts thereof, are also meant to include solvated (e.g., hydrated) forms. Nitrite has the chemical formula NO_2^- and may exist as an ion in water. Sodium nitrite has the chemical formula $NaNO_2$ and typically dissolves in water to form the sodium ion Na^+ and the nitrite ion NO_2^-. It will further be understood that the present invention encompasses all such solvated forms (e.g., hydrates) of the nitrite compounds. Exemplary nitrite compounds are described in WO 2008/105730, which is hereby incorporated by reference.

In addition to sodium nitrite, representative inorganic nitrite compounds include: ammonium nitrite (NH_4NO_2), barium nitrite ($Ba(NO_2)_2$; e.g., anhydrous barium nitrite or barium nitrite monohydrate), calcium nitrite ($Ca(NO_2)_2$; e.g., anhydrous calcium nitrite or calcium nitrite monohydrate), cesium nitrite ($CsNO_2$), cobalt(II) nitrite ($Co(NO_2)_2$), cobalt(III) potassium nitrite ($CoK_3(NO_2)_6$; e.g., cobalt(III) potassium nitrite sesquihydrate), lithium nitrite ($LiNO_2$; e.g., anhydrous lithium nitrite or lithium nitrite monohydrate), magnesium nitrite ($MgNO_2$; e.g., magnesium nitrite trihydrate), postassium nitrite (KNO_2), rubidium nitrite ($RbNO_2$), silver(I) nitrite ($AgNO_2$), strontium nitrite ($Sr(NO_2)_2$), and zinc nitrite ($Zn(NO_2)_2$).

The compounds of the present invention can be prepared in a variety of ways known to one of ordinary skill in the art of chemical synthesis. Methods for preparing nitrite salts are well known in the art and a wide range of precursors and nitrite salts are readily available commercially. Nitrites of the alkali and alkaline earth metals can be synthesized by reacting a mixture of nitrogen monoxide (NO) and nitrogen dioxide (NO_2) with a corresponding metal hydroxide solution, as well as through the thermal

decomposition of the corresponding nitrate. Other nitrites are available through the reduction of the corresponding nitrates.

The present compounds can be prepared from readily available starting materials using the methods and procedures known in the art. It will be appreciated that where typical or preferred process conditions (i.e., reaction temperatures, times, mole ratios of reactants, solvents, pressures, etc.) are given, other process conditions can also be used unless otherwise stated. Optimum reaction conditions may vary with the particular reactants or solvent used, but such conditions can be determined by one of ordinary skill in the art by routine optimization procedures.

Suitable pharmaceutically acceptable salts include, for example, sodium nitrite, potassium nitrite, or calcium nitrite. Still other exemplary salts are found in Remington's Pharmaceutical Sciences, 17th ed., Mack Publishing Company, Easton, Pa., 1985, p. 1418, Berge et al., *J. Pharmaceutical Sciences* 66:1-19, 1977 and *Pharmaceutical Salts: Properties, Selection, and Use*, (Eds. P.H. Stahl and C.G. Wermuth), Wiley-VCH, 2008, each of which is incorporated herein by reference in its entirety.

Pharmaceutical Compositions

When employed as pharmaceuticals, inorganic nitrite, e.g., a salt of nitrous acid (HNO_2) such as $NaNO_2$, or a pharmaceutically acceptable salt, solvate, or prodrug thereof can be administered in the form of pharmaceutical compositions. These compositions can be prepared in a manner well known in the pharmaceutical art, and can be administered by a variety of routes, depending upon whether local or systemic treatment is desired and upon the area to be treated. Administration may be topical, parenteral, intravenous, intra-arterial, subcutaneous, intramuscular, intracranial, intraorbital, ophthalmic, intraventricular, intracapsular, intraspinal, intracisternal, intraperitoneal, intranasal, aerosol, by suppositories, or oral administration. In one embodiment, the inorganic nitrite is administered in a pharmaceutical composition taught in U.S. Patent Application No. 12/904,791, hereby incorporated by reference.

The pharmaceutical composition can contain one or more pharmaceutically acceptable carriers. In making a pharmaceutical composition for use in a method of the invention, the inorganic nitrite, pharmaceutically acceptable salt, solvate, or prodrug thereof is typically mixed with an excipient, diluted by an excipient or enclosed within such a carrier in the form of, for example, a capsule, sachet, paper, or other container. When the excipient serves as a diluent, it can be a solid, semisolid, or liquid material (e.g., normal saline), which acts as a vehicle, carrier or medium for the active ingredient. Thus, the compositions can be in the form of tablets, powders, lozenges, sachets, cachets, elixirs, suspensions, emulsions, solutions, syrups, and soft and hard gelatin capsules. As is known in the art, the type of diluent can vary depending upon the intended route of administration. The resulting compositions can include additional agents, such as preservatives.

The therapeutic agents of the invention can be administered alone, or in a mixture, in the presence of a pharmaceutically acceptable excipient or carrier. The excipient or carrier is selected on the basis of the mode and route of administration. Suitable pharmaceutical carriers, as well as pharmaceutical necessities for use in pharmaceutical formulations, are described in *Remington: The Science and Practice of Pharmacy*, 21st Ed., Gennaro, Ed., Lippencott Williams & Wilkins (2005), a well-known reference text in this field, and in the USP/NF (United States Pharmacopeia and the National Formulary). Examples of suitable excipients are lactose, dextrose, sucrose, sorbitol, mannitol, starches,

gum acacia, calcium phosphate, alginates, tragacanth, gelatin, calcium silicate, microcrystalline cellulose, polyvinylpyrrolidone, cellulose, water, syrup, and methyl cellulose. The formulations can additionally include: lubricating agents such as talc, magnesium stearate, and mineral oil; wetting agents; emulsifying and suspending agents; preserving agents such as methyl- and propylhydroxy-benzoates; sweetening agents; and flavoring agents. Other exemplary excipients are described in *Handbook of Pharmaceutical Excipients*, 6th Edition, Rowe et al., Eds., Pharmaceutical Press (2009).

The pharmaceutical composition can include nitrate salts, or prodrugs thereof, or other therapeutic agents. Exemplary nitrate salts are described in WO 2008/105730. Exemplary therapeutic agents that may be included in the compositions described herein are provided herein.

The pharmaceutical compositions can be formulated so as to provide immediate, extended, or delayed release of the active ingredient after administration to the patient by employing procedures known in the art.

The compositions can be formulated in a unit dosage form, each dosage containing, e.g., 0.1-500 mg of the active ingredient. For example, the dosages can contain from about 0.1 mg to about 50 mg, from about 0.1 mg to about 40 mg, from about 0.1 mg to about 20 mg, from about 0.1 mg to about 10 mg, from about 0.2 mg to about 20 mg, from about 0.3 mg to about 15 mg, from about 0.4 mg to about 10 mg, from about 0.5 mg to about 1 mg; from about 0.5 mg to about 100 mg, from about 0.5 mg to about 50 mg, from about 0.5 mg to about 30 mg,, from about 0.5 mg to about 20 mg, from about 0.5 mg to about 10 mg, from about 0.5 mg to about 5 mg; from about 1 mg from to about 50 mg, from about 1 mg to about 30 mg,, from about 1 mg to about 20 mg, from about 1 mg to about 10 mg, from about 1 mg to about 5 mg; from about 5 mg to about 50 mg, from about 5 mg to about 20 mg, from about 5 mg to about 10 mg; from about 10 mg to about 100 mg, from about 20 mg to about 200 mg, from about 30 mg to about 150 mg, from about 40 mg to about 100 mg, from about 40 mg to about 80 mg of the active ingredient, or from about 50 mg to about 80 mg of the active ingredient. For preparing solid compositions such as tablets, the principal active ingredient is mixed with one or more pharmaceutical excipients to form a solid bulk formulation composition containing a homogeneous mixture of a compound of the present invention. When referring to these bulk formulation compositions as homogeneous, the active ingredient is typically dispersed evenly throughout the composition so that the composition can be readily subdivided into equally effective unit dosage forms such as tablets and capsules. This solid bulk formulation is then subdivided into unit dosage forms of the type described above.

Compositions for Oral Administration

The pharmaceutical compositions contemplated by the invention include those formulated for oral administration ("oral dosage forms"). Oral dosage forms can be, for example, in the form of tablets, capsules, a liquid solution or suspension, a powder, or liquid or solid crystals, which contain the active ingredient(s) in a mixture with non-toxic pharmaceutically acceptable excipients. These excipients may be, for example, inert diluents or fillers (e.g., sucrose, sorbitol, sugar, mannitol, microcrystalline cellulose, starches including potato starch, calcium carbonate, sodium chloride, lactose, calcium phosphate, calcium sulfate, or sodium phosphate); granulating and disintegrating agents (e.g., cellulose derivatives including microcrystalline cellulose, starches including potato starch, croscarmellose sodium, alginates, or alginic acid); binding agents (e.g., sucrose, glucose, sorbitol, acacia, alginic acid, sodium alginate, gelatin, starch, pregelatinized starch, microcrystalline cellulose, magnesium aluminum silicate,

carboxymethylcellulose sodium, methylcellulose, hydroxypropyl methylcellulose, ethylcellulose, polyvinylpyrrolidone, or polyethylene glycol); and lubricating agents, glidants, and antiadhesives (e.g., magnesium stearate, zinc stearate, stearic acid, silicas, hydrogenated vegetable oils, or talc). Other pharmaceutically acceptable excipients can be colorants, flavoring agents, plasticizers, humectants, buffering agents, and the like.

Formulations for oral administration may also be presented as chewable tablets, as hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent (e.g., potato starch, lactose, microcrystalline cellulose, calcium carbonate, calcium phosphate or kaolin), or as soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium, for example, peanut oil, liquid paraffin, or olive oil. Powders, granulates, and pellets may be prepared using the ingredients mentioned above under tablets and capsules in a conventional manner using, e.g., a mixer, a fluid bed apparatus or a spray drying equipment.

Controlled release compositions for oral use may be constructed to release the active drug by controlling the dissolution and/or the diffusion of the active drug substance. Any of a number of strategies can be pursued in order to obtain controlled release and the targeted plasma concentration vs time profile. In one example, controlled release is obtained by appropriate selection of various formulation parameters and ingredients, including, e.g., various types of controlled release compositions and coatings. Thus, the drug is formulated with appropriate excipients into a pharmaceutical composition that, upon administration, releases the drug in a controlled manner. Examples include single or multiple unit tablet or capsule compositions, oil solutions, suspensions, emulsions, microcapsules, microspheres, nanoparticles, patches, and liposomes. In certain embodiments, compositions include biodegradable, pH, and/or temperature-sensitive polymer coatings.

Dissolution or diffusion controlled release can be achieved by appropriate coating of a tablet, capsule, pellet, or granulate formulation of compounds, or by incorporating the compound into an appropriate matrix. A controlled release coating may include one or more of the coating substances mentioned above and/or, e.g., shellac, beeswax, glycowax, castor wax, carnauba wax, stearyl alcohol, glyceryl monostearate, glyceryl distearate, glycerol palmitostearate, ethylcellulose, acrylic resins, dl-polylactic acid, cellulose acetate butyrate, polyvinyl chloride, polyvinyl acetate, vinyl pyrrolidone, polyethylene, polymethacrylate, methylmethacrylate, 2-hydroxymethacrylate, methacrylate hydrogels, 1,3 butylene glycol, ethylene glycol methacrylate, and/or polyethylene glycols. In a controlled release matrix formulation, the matrix material may also include, e.g., hydrated methylcellulose, carnauba wax and stearyl alcohol, carbopol 934, silicone, glyceryl tristearate, methyl acrylate-methyl methacrylate, polyvinyl chloride, polyethylene, and/or halogenated fluorocarbon.

The liquid forms in which the compounds and compositions of the present invention can be incorporated for administration orally include aqueous solutions, suitably flavored syrups, aqueous or oil suspensions, and flavored emulsions with edible oils such as cottonseed oil, sesame oil, coconut oil, or peanut oil, as well as elixirs and similar pharmaceutical vehicles.

Coatings

The pharmaceutical compositions formulated for oral delivery, such as tablets or capsules of the present invention can be coated or otherwise compounded to provide a dosage form affording the advantage of delayed or extended release. The coating may be adapted to release the active drug

substance in a predetermined pattern (e.g., in order to achieve a controlled release formulation) or it may be adapted not to release the active drug substance until after passage of the stomach, e.g., by use of an enteric coating (e.g., polymers that are pH-sensitive ("pH controlled release"), polymers with a slow or pH-dependent rate of swelling, dissolution or erosion ("time-controlled release"), polymers that are degraded by enzymes ("enzyme-controlled release" or "biodegradable release") and polymers that form firm layers that are destroyed by an increase in pressure ("pressure-controlled release")). Exemplary enteric coatings that can be used in the pharmaceutical compositions described herein include sugar coatings, film coatings (e.g., based on hydroxypropyl methylcellulose, methylcellulose, methyl hydroxyethylcellulose, hydroxypropylcellulose, carboxymethylcellulose, acrylate copolymers, polyethylene glycols and/or polyvinylpyrrolidone), or coatings based on methacrylic acid copolymer, cellulose acetate phthalate, hydroxypropyl methylcellulose phthalate, hydroxypropyl methylcellulose acetate succinate, polyvinyl acetate phthalate, shellac, and/or ethylcellulose. Furthermore, a time delay material such as, for example, glyceryl monostearate or glyceryl distearate, may be employed.

For example, the tablet or capsule can comprise an inner dosage and an outer dosage component, the latter being in the form of an envelope over the former. The two components can be separated by an enteric layer which serves to resist disintegration in the stomach and permit the inner component to pass intact into the duodenum or to be delayed in release.

When an enteric coating is used, desirably, a substantial amount of the drug is released in the lower gastrointestinal tract.

In addition to coatings that effect delayed or extended release, the solid tablet compositions may include a coating adapted to protect the composition from unwanted chemical changes (e.g., chemical degradation prior to the release of the active drug substance). The coating may be applied on the solid dosage form in a similar manner as that described in *Encyclopedia of Pharmaceutical Technology,* vols. 5 and 6, Eds. Swarbrick and Boyland, 2000.

Formulations for Colonic Drug Release

In some embodiments, colon-targeted drug delivery systems can be used. Exemplary approaches include, but are not limited to:

(a) covalent linkage of the drug with the carrier to form a prodrug that is stable in the stomach and small intestine and releases the drug in the large intestine upon enzymatic transformation by the intestinal microflora; examples of these prodrugs include azo-conjugates, cyclodextrin-conjugates, glycoside-conjugates, glucuronate conjugates, dextran-conjugates, polypeptide and polymeric conjugates;

(b) approaches to deliver intact molecule to the colon, such as coating with pH-sensitive polymers to release the drug at neutral to alkaline pH, or coating with biodegradable polymers which release the drug upon degradation by the bacteria in the colon;

(c) embedding the drug in biodegradable matrices and hydrogels which release the drug in response to the pH or biodegradation;

(d) time released systems where once the multicoated formulation passes the stomach, the drug is released after a lag time of 3-5 hrs which is equivalent to the transit time of the small intestine;

(e) using redox-sensitive polymers where a combination of azo and disulfide polymers, provide drug release in response to the redox potential of the colon;

(f) using bioadhesive polymers which selectively adhere to the colonic mucosa slowly releasing the drug; and

(g) osmotic controlled drug delivery where the drug is released through semi-permeable membrane due to osmotic pressure.

Routes of Administration

The compositions described herein may be administered to a patient in a variety of forms depending on the selected route of administration, as will be understood by those skilled in the art and as relating to the particular disease or condition to be treated. The compositions used in the methods described herein may be administered, for example, by topical, enteral, or parenteral applications. Topical applications include but are not limited to epicutaneous, inhalation, enema, eye drops, ear drops, and applications through mucous membranes in the body. Enteral applications include oral administration, rectal administration, vaginal administration, and gastric feeding tubes. Parenteral administration includes intravenous, intraarterial, intracapsular, intraorbital, intracardiac, intradermal, transtracheal, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural, intrastemal, intraperitoneal, subcutaneous, intramuscular, transepithelial, nasal, intrapulmonary, intrathecal, rectal, and topical modes of administration. Parenteral administration may be by continuous infusion over a selected period of time.

For intravenous or intrathecal delivery or direct injection, the composition must be sterile and fluid to the extent that the composition is deliverable by syringe. In addition to water, the carrier can be an isotonic buffered saline solution, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyetheylene glycol, and the like), and suitable mixtures thereof. Proper fluidity can be maintained, for example, by use of coating such as lecithin, by maintenance of required particle size in the case of dispersion and by use of surfactants. In many cases, it is preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol or sorbitol, and sodium chloride in the composition. Long-term absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate or gelatin.

The choice of the route of administration will depend on whether a local or systemic effect is to be achieved. For example, for local effects, the composition can be formulated for topical administration and applied directly where its action is desired. For systemic, long term effects, the composition can be formulated for enteral administration and given via the digestive tract. For system, immediate and/or short term effects, the composition can be formulated for parenteral administration and given by routes other than through the digestive tract.

Parenteral Administration

Within the scope of the present invention are also parenteral depot systems from biodegradable polymers. These systems are injected or implanted into the muscle or subcutaneous tissue and release the incorporated drug over extended periods of time, ranging from several days to several months. Both the characteristics of the polymer and the structure of the device can control the release kinetics which can be either continuous or pulsatile. Polymer-based parenteral depot systems can be classified as

implants or microparticles. The former are cylindrical devices injected into the subcutaneous tissue whereas the latter are defined as spherical particles in the range of 10 – 100 µm. Extrusion, compression or injection molding are used to manufacture implants whereas for microparticles, the phase separation method, the spray-drying technique and the water-in-oil-in-water emulsion techniques are frequently employed. The most commonly used biodegradable polymers to form microparticles are polyesters from lactic and/or glycolic acid, e.g. poly(glycolic acid) and poly(L-lactic acid) (PLG/PLA microspheres). Of particular interest are in situ forming depot systems, such as thermoplastic pastes and gelling systems formed by solidification, by cooling, or due to the sol-gel transition, cross-linking systems and organogels formed by amphiphilic lipids. Examples of thermosensitive polymers used in the aforementioned systems include, N-isopropylacrylamide, poloxamers (ethylene oxide and propylene oxide block copolymers, such as poloxamer 188 and 407), poly(N-vinyl caprolactam), poly(siloethylene glycol), polyphosphazenes derivatives and PLGA-PEG-PLGA.

Dosing Regimes

The present methods for modulating brain function, in particular improving mood and/or psychological state, in the treatment of disorders of brain development, and in the treatment and/or reduction of pain are carried out by administering an inorganic nitrite for a time and in an amount sufficient to result in the improvement of mood and/or psychological state, in the treatment and/or reduction of pain, and in the treatment of disorders of brain development.

The amount and frequency of administration of the compositions can vary depending on, for example, what is being administered, the state of the patient, and the manner of administration. In therapeutic applications, compositions can be administered to a patient suffering from pain (e.g., neuropathic pain, neuropathy, diabetic peripheral neuropathy) in an amount sufficient to relieve or least partially relieve the symptoms of pain (e.g., discomfort, soreness, tightness, or stiffness) and its complications (e.g., fatigue, sleeplessness, weakened immune system, depression, anxiety, stress, irritability, or disability). The dosage is likely to depend on such variables as the type and extent of progression of the pain (e.g., as determined by the "Pain Ladder" guideline from the World Health Organization), the severity of the pain (e.g., acute, subacute, or chronic), the age, weight and general condition of the particular patient, the relative biological efficacy of the composition selected, formulation of the excipient, the route of administration, and the judgment of the attending clinician. Effective doses can be extrapolated from dose- response curves derived from in vitro or animal model test system. An effective dose is a dose that produces a desirable clinical outcome by, for example, improving a sign or symptom of pain or slowing its progression.

The amount of inorganic nitrite per dose can vary. For example, a subject can receive from about 0.1 µg/kg to about 10,000 µg/kg. Generally, the nitrite is administered in an amount such that the peak plasma concentration ranges from 150 nM-250 µM. Exemplary dosage amounts can fall between about 0.1 to about 2000 µg/kg; about 0.5 to about 1000 µg/kg; about 0.5 to about 2000 µg/kg; about 100 to about 1500 µg/kg; about 0.5 µg/kg to about 500 µg/kg; about 0.5 µg/kg to about 250 µg/kg; about 0.5 µg/kg to about 100 µg/kg; about 0.5 µg/kg to about 50 µg/kg; about 100 to about 350 µg/kg; about 340 to about 750 µg/kg; or about 750 to about 1000 µg/kg. Exemplary dosages can be about 8.25 µg/kg, about 10 µg/kg, about 16.5 µg/kg, about 20 µg/kg, about 30 µg/kg, about 50 µg/kg, about 100 µg/kg, about 165

μg/kg, about 200 μg/kg, about 500 μg/kg, about 750 μg/kg, about 1000 μg/kg, about 1250 μg/kg, about 1500 μg/kg, about 1750 μg/kg, or about 2000 μg/kg. Exemplary peak plasma concentrations can range from 0.05-10 μM, 0.1-10 μM, 0.1-5.0 μM, or 0.1-1 μM. The peak plasma concentrations may be maintained for 2-14 hours, 4-14 hours, 6-14 hours, 6-12 hours, or 6-10 hours.

The frequency of treatment may also vary. The subject can be treated one or more times per day (e.g., once, twice, three, four or more times) or every so-many hours (e.g., about every 2, 4, 6, 8, 12, or 24 hours). Preferably, the pharmaceutical composition is administered 1 or 2 times per 24 hours. The time course of treatment may be of varying duration, e.g., for two, three, four, five, six, seven, eight, nine, ten, or more days, two weeks, 1 month, 2 months, 4 months, 6 months, 8 months, 10 months, or more than one year. For example, the treatment can be twice a day for three days, twice a day for seven days, twice a day for ten days. Treatment cycles can be repeated at intervals, for example weekly, bimonthly or monthly, which are separated by periods in which no treatment is given. The treatment can be a single treatment or can last as long as the life span of the subject (e.g., many years).

Kits

Any of the pharmaceutical compositions described herein can be used together with a set of instructions, i.e., to form a kit. The kit may include instructions for use of the pharmaceutical compositions as a therapy as described herein. For example, the instructions may provide dosing and therapeutic regimes for use of the compounds of the invention for modulating brain function, in particular improving mood and/or psychological state, in the treatment of disorders of brain development, and in the treatment and/or reduction of pain.

Methods of Treatment

The present invention provides nutritional and pharmaceutical compositions of nitrite, e.g., inorganic nitrite, or a pharmaceutically acceptable prodrug thereof, for both prophylactic and therapeutic nutritional supplementation, specifically in maintaining a balance in psychological state and alleviating pain (e.g., chronic pain). Specifically, the present invention relates to novel compositions of nitrite, e.g., inorganic nitrite, or a pharmaceutically acceptable prodrug thereof, that can be used to treat patients with acute pain, subacute pain, or chronic pain (e.g., pain that lasts longer than three to six months or pain that extend beyond the expected period of healing, and/or pain that originates from an initial trauma/injury or infection, or pain that may be an ongoing cause associated with neuropathic pain (e.g., diabetic peripheral neuropathy, post-herpetic neuralgia, trigeminal neuralgia, phantom limb pain, carpal tunnel syndrome, sciatica, pudendal neuralgia, complex regional pain syndrome, sensory polyneuropathies, mono-neuropathies, or central pain syndrome, headaches, joint pain, backaches, sinus pain, muscle pain, nerve pain, and pain affecting specific parts of the body, such as shoulders, pelvis, and neck, and/or pain that is associated with lower back pain, arthritis, headache, multiple sclerosis, fibromyalgia, shingles, nerve damage, or cancer. The present invention also relates to novel compositions of nitrite, e.g., inorganic nitrite, or a pharmaceutically acceptable prodrug thereof, that can be used to treat patients with mood disorders and/or an imbalance in psychological state, and disorders of brain development.

Pain

Following the clinical trials described in the Examples below, it was observed that patients who were taking the compositions of the invention also reported a reduction in pain. Therefore, it was postulated that the compositions of the invention may be useful in the treatment or reduction of pain in general and neuropathic pain in preferred embodiments.

Neuropathic pain

Neuropathic pain can take a variety of forms depending on its origin and can be characterized as acute, subacute, or chronic depending on the duration. Acute pain can last anywhere from a couple hours to less than 30 days. Subacute pain can last from one to six months and chronic pain is characterized as pain that lasts longer than three to six months or pain that extend beyond the expected period of healing. In neuropathic pain, the pain may be described as being peripheral neuropathic if the initiating injury occurs as a result of a complete or partial transection of a nerve or trauma to a nerve plexus. Peripheral neuropathy can result from traumatic injuries, infections, metabolic disorders, diabetes, and/or exposure to toxins. Alternatively, neuropathic pain is described as being central neuropathic following a lesion to the central nervous system, such as a spinal cord injury or a cerebrovascular accident. The methods of the invention include administration of the compositions described herein to treat neuropathic pain. Types of neuropathic pain include but are not limited to: diabetic peripheral neuropathy, post-herpetic neuralgia, trigeminal neuralgia, phantom limb pain, carpal tunnel syndrome, sciatica, pudendal neuralgia, complex regional pain syndrome, sensory polyneuropathies, mono-neuropathies, and central pain syndrome.

Neuropathy

The compositions described herein are useful for the treatment of neuropathy, in particular, diabetic peripheral neuropathy. Neuropathy can have many causes such as sustained injury or exposure to toxins or chronic diseases (e.g., Parkinson's multiple sclerosis, autoimmune diseases, and diabetes), with diabetes as the biggest risk factor. In diabetic patients, it typically takes many years for neuropathy to develop as nerve damages result over time due to prolonged exposure to the damaging effects of high blood glucose levels. The longer a subject has diabetes, the higher the risk of developing neuropathy. The compositions described herein can be administered prophylactically to a subject having diabetes to prevent or reduce the risk of developing diabetic peripheral neuropathy or therapeutically to treat diabetic peripheral neuropathy.

Without wishing to be bound by theory, the compositions of nitrite described herein may be especially beneficial to a diabetic subject through its indirect effect on the sorbitol-aldose reductase pathway, which is implicated in diabetic complications. In diabetic subjects with a high hyperglycemic state, the affinity for aldose reductase for glucose increases which leads to higher levels of sorbitol and lower NADPH. NADPH specifically acts to promote nitric oxide (NO) and glutathione production, which results in vasodilation. The oxidation of NADPH to NADP+ is also necessary to prevent reactive oxygen species from forming. The lower NADPH levels in diabetic subjects inhibit NO production and may alone lead to neuronal cell death and pain. Thus, the ability to replenish the NO supply would in some instances ameliorate symptoms of neuropathic pain in subjects with diabetes.

Inflammatory pain

Inflammatory pain is a form of pain that is caused by tissue injury or inflammation (e.g., in postoperative pain or rheumatoid arthritis). Following a peripheral nerve injury, symptoms are typically experienced in a chronic fashion, distal to the site of injury and are characterized by hyperesthesia (enhanced sensitivity to a natural stimulus), hyperalgesia (abnormal sensitivity to a noxious stimulus), allodynia (widespread tenderness associated with hypersensitivity to normally innocuous tactile stimuli), and/or spontaneous burning or shooting lancinating pain. In inflammatory pain, symptoms are apparent, at least initially, at the site of injury or inflamed tissues and typically accompany arthritis-associated pain, musculo-skeletal pain, and postoperative pain. The different types of pain may coexist or pain may be transformed from inflammatory to neuropathic during the natural course of the disease, as in post-herpetic neuralgia.

Nociceptive pain

Nociceptive pain is the pain experienced in response to a noxious stimulus, such as a needle prick or during trauma or surgery. Nociceptive pain may be divided into superficial and deep, and deep pain into deep somatic and visceral. Superficial pain is initiated by activation of nociceptors in the skin or superficial tissues. Deep somatic pain is initiated by stimulation of nociceptors in ligaments, tendons, bones, blood vessels, fasciae and muscles, and is dull, aching, poorly-localized pain. Visceral pain originates in the viscera (organs). Visceral pain may be well-localized, but often it is extremely difficult to locate, and several visceral regions produce referred pain when damaged or inflamed, where the sensation is located in an area distant from the site of pathology or injury.

Other types of pain

Functional pain refers to conditions in which there is no obvious peripheral pathology or lesion to the nervous system. This particular form of pain is generated by abnormal function of the nervous system and conditions characterized by such pain include fibromyalgia, tension-type headache, and irritable bowel syndrome.

Common conditions associated with chronic pain include but are not limited to back injuries (e.g., slipped or bulging discs, spinal stenosis, compression fractures, soft tissue damage, traumatic fractures, and structural deformities), headaches (e.g., muscle tension headaches, eye strain headaches, migraines, cluster headaches), joint pain (e.g., osteoarthritis, rheumatoid arthritis, and repetitive strain injury), fibromyalgia, and pain associated with cancer (e.g., leukemia, brain cancer, bladder cancer, breast cancer, cervical cancer, colorectal cancer, endometrial cancer, esophageal cancer, head and neck cancer, liver cancer, lung cancer, lymphoma, ovarian cancer, pancreatic cancer, prostate cancer, renal cancer, skin cancer, stomach cancer, testis cancer, thyroid cancer, and urothelial cancer).

The pharmaceutical compositions and methods described herein may be useful for the treatment, reduction, or prevention of various forms of pain, namely inflammatory pain, nociceptive pain, functional pain, and neuropathic pain, whether acute or chronic. Exemplary conditions that may be associated with pain include, for example, soft tissue, joint, bone inflammation and/or damage (e.g., acute trauma, osteoarthritis, or rheumatoid arthritis), myofascial pain syndromes (fibromylagia), headaches (including cluster headache, migraine, and tension type headache), myocardial infarction, angina, ischemic cardiovascular disease, post-stroke pain, sickle cell anemia, peripheral vascular occlusive disease,

cancer, inflammatory conditions of the skin or joints, diabetic neuropathy, and acute tissue damage from surgery or traumatic injury (e.g., burns, lacerations, or fractures).

The present invention may also be useful for the treatment or reduction of musculo-skeletal pain (after trauma, infections, and exercise), neuropathic pain caused by spinal cord injury, tumors, compression, inflammation, dental pain, episiotomy pain, deep and visceral pain (e.g., heart pain, bladder pain, or pelvic organ pain), muscle pain, eye pain, orofacial pain (e.g., odontalgia, trigeminal neuralgia, glossopharyngeal neuralgia), abdominal pain, gynecological pain (e.g., dysmenorrhea and labor pain), pain associated with nerve and root damage due to trauma, compression, inflammation, toxic chemicals, metabolic disorders, hereditary conditions, infections, vasculitis and autoimmune diseases, central nervous system pain, such as pain due to spinal cord or brain stem damage, cerebrovascular accidents, tumors, infections, demyelinating diseases including multiple sclerosis, low back pain, sciatica, and post-operative pain.

Assessment of Efficacy

The compositions described herein can be tested for efficacy in any standard animal model of pain. Various models test the sensitivity of normal animals to intense or noxious stimuli (physiological or nociceptive pain). These tests include responses to thermal, mechanical, or chemical stimuli. Thermal stimuli usually involve the application of hot stimuli (typically varying between 42 -55 ºC) including, for example: radiant heat to the tail (the tail flick test), radiant heat to the plantar surface of the hindpaw (the Hargreaves test), the hotplate test, and immersion of the hindpaw or tail into hot water. Immersion in cold water, acetone evaporation, or cold plate tests may also be used to test cold pain responsiveness. Tests involving mechanical stimuli typically measure the threshold for eliciting a withdrawal reflex of the hindpaw to graded strength monofilament von Frey hairs or to a sustained pressure stimulus to a paw (e.g., the Ugo Basile analgesiometer). The duration of a response to a standard pinprick may also be measured. When using a chemical stimulus, the response to the application or injection of a chemical irritant (e.g., capsaicin, mustard oil, bradykinin, ATP, formalin, acetic acid) to the skin, muscle joints or internal organs (e.g., bladder or peritoneum) is measured. In particular, the assessment of pain upon administration of the compositions of the invention in diabetic peripheral neuropathy can be studied in animal models such as the streptozotocin-induced diabetic rats and mice, which serves as a model of peripheral neuropathy of type 1 diabetes (see, e.g., Tesch et al., *Nephrology*. **12**(3):261-266, 2007), the leptin deficient (ob/ob) mouse model, which serves as a model of peripheral neuropathy of type 2 diabetes (see, e.g., Drel et al., *Diabetes*. **55**(12):3335-3343, 2006), the nonobese diabetic (NOD) mouse model, spontaneously induced Ins2 Akita mouse model, Db/Db leptin receptor deficient mouse model, WBN/Kob spontaneous diabetic rat model, SDT fatty rat model, high fat diet C5BL/6J mouse model, and Rhesus monkey PDN model (see, e.g., Islam, *J Diabetes Res*. 2013: 149452, 2013).

In addition, various tests assess pain sensitization by measuring changes in the excitability of the peripheral or central components of the pain neural pathway. In this regard, peripheral sensitization (i.e., changes in the threshold and responsiveness of high threshold nociceptors) can be induced by repeated heat stimuli as well as the application or injection of sensitizing chemicals (e.g., prostaglandins, bradykinin, histamine, serotonin, capsaicin, or mustard oil). Central sensitization (i.e., changes in the excitability of neurons in the central nervous system induced by activity in peripheral pain fibers) can be

induced by noxious stimuli (e.g., heat), chemical stimuli (e.g., injection or application of chemical irritants), or electrical activation of sensory fibers.

Various pain tests developed to measure the effect of peripheral inflammation on pain sensitivity can also be used to study the efficacy of the compositions described herein (Stein et al., *Pharmacol. Biochem. Behav.* (1988) 31: 445-451; Woolf et al., *Neurosci.* (1994) 62: 327-331). Additionally, various tests assess peripheral neuropathic pain using lesions of the peripheral nervous system. One such example is the "axotomy pain model" (Watson, *J. Physiol.* (1973) 231:41). Other similar tests include the SNL test which involves the ligation of a spinal segmental nerve (Kim and Chung *Pain* (1992) 50: 355), the Seltzer model involving partial nerve injury (Seltzer, *Pain* (1990) 43: 205-18), the spared nerve injury (SNI) model (Decosterd and Woolf, *Pain* (2000) 87:149), chronic constriction injury (CCI) model (Bennett (1993) *Muscle Nerve* 16: 1040), tests involving toxic neuropathies such as diabetes (streptozocin model), pyridoxine neuropathy, taxol, vincristine, and other antineoplastic agent-induced neuropathies, tests involving ischaemia to a nerve, peripheral neuritis models (e.g., CFA applied peri-neurally), models of post-herpetic neuralgia using HSV infection, and compression models.

Chronic pain has been characterized as a disease affecting brain structure and function. Magnetic resonance imaging studies have shown abnormal anatomical and functional connectivity, even during rest involving areas related to the processing of pain. Persistent pain has also been shown to cause grey matter loss, reversible once the pain has resolved. Thus, measures of neuroplasticity can be used to assess the efficacy of the compositions described herein. Brain electroencephalogram (EEG) can be used to measure the changes in relative beta activity, alpha activity, and theta activity in subjects taking with the composition compared to subjects not taking the composition.

Neuropathy affects the motor fibers or the large sensory fibers and traditional tools such as electromyography and nerve conduction studies are useful for determining the efficacy of administration of the compositions described herein to a subject. Quantitative sensory testing can also be used to determine efficacy of treatment. Quantitative sensory testing involves the application of controlled mechanical, thermal, or chemical stimuli. Subjects report their perception of the stimulus and indicate the point at which it becomes painful, which allows an evaluation of the subject's sensory threshold for various types of stimuli. Other *in vivo* assays to measure effectiveness of the compositions include monofilament testing and nerve conduction velocity testing. Skin biopsies maybe also be useful for monitoring response to therapy or disease progression. Skin biopsies require a small sample of the epidermis be taken, using local anesthetic, from anywhere on the body. The biopsy sample is immunolabeled with an antibody against PGP9.5, a panaxonal marker so the small sensory nerve endings in the skin can be seen and counted using a light microscope. Skin biopsies allow quantitative measurement of the sensory nerve endings in the epidermis because they exist as individual nerve endings that can be counted. Normative data are available to show the normal density of cutaneous nerve endings and provide a point of comparison for the test subject. Other *ex vivo* efficacy testing include histopathology or biopsy of tissues to look for changes in axonal atrophy, axonal dystrophy in myelination (i.e., demyelination), and reduced numbers of large myelinated fibers.

In all of the above tests, outcome measures may be assessed, for example, according to behavior, electrophysiology, neurochemistry, or imaging techniques to detect changes in neural activity. In all of the above tests, an improvement in pain reduction can also be assessed by determining the pharmacological and non-pharmacological characteristics of pain such as pain intensity (as measure on a

standardized pain scale), pattern (e.g., constant, intermittent), location, radiation, frequency, timing, and duration, impact on quality of life (sleep, function, appetite, and mood).

Mood Disorders and Imbalance of Psychological State

NO signaling in the brain can modulate a range of processes such as various forms of plasticity (long term potentiation and depression, LTP and LTD), regulating rhythmic activity, including gut motility, respiratory rhythm, circadian rhythms, locomotor, and thalamocortical oscilliation. The compositions described herein are effective NO-donor compounds delivering NO to specific sites, therefore these compositions may also be useful in modulating brain function, in particular improving mood and/or psychological state, and in the treatment of mood disorders, by maintaining a balance in the levels of NO.

The term mood disorder refers to the underlying or longitudinal emotional state observed in a subject. Two groups of mood disorders are broadly recognized; the division is based on whether a manic or hypomanic episode has ever been present. Thus, there are depressive disorders, of which the best-known and most researched is major depressive disorder (MDD) and bipolar disorder (BD), characterized by intermittent episodes of mania or hypomania, usually interlaced with depressive episodes. There are also forms of depression of MDD and BD that are less severe and are known as dysthymic disorder (in relation to MDD) and cyclothymic disorder (in relation to BD).

Other types of depressive disorders include but are not limited to: atypical depression, melancholic depression, psychotic major depression, catatonic depression, postpartum depression, seasonal affective disorder, and depressive disorder not otherwise specified (DD-NOS). Bipolar disorders include: bipolar I, bipolar II, cyclothymia, and bipolar disorder not otherwise specified (BD-NOS). Mood disorders can also be classified as substance induced. These substance-induced mood disorders include: alcohol-induced mood disorders, bezodiazepine-induced mood disorders, and stimulant-induced mood disorders (e.g., amphetamine, methamphetamine, and cocaine)

Disorders of Brain Development

NO is also involved in learning and memory mechanism through mediation of specific forms of LTP. As such, the compositions described herein may also be useful in treating disorders of brain development such as: impairment in learning and memory, autistic disorder, Rett syndrome, childhood disintegrative disorder, pervasive developmental disorder-not otherwise specified (PDD-NOS), and Asperger syndrome. Autism is a disorder of neural development characterized by impaired social interaction and communication, and by restricted and repetitive behavior.

Autism affects information processing in the brain by altering how nerve cells and their synapses connect and organize. Austism is among one of the three recognized disorders in the autism spectrum (ASDs), the other two being Asperger syndrome, which lacks delays in cognitive development and language, and pervasive developmental disorder. As autism is caused by neurological dysfunctions, the compositions of the invention may also be useful in the treatment of autism and related neural development disorders.

Rett syndrome, originally termed as cerebroatrophic hyperammonemia, is a neurodevelopmental disorder of the grey matter of the brain that almost exclusively affects females but has also been found in male patients. The clinical features include small hands and feet and a deceleration of the rate of head growth (including microcephaly in some). Repetitive stereotyped hand movements, such as wringing

and/or repeatedly putting hands into the mouth, are also common clinical features. Subjects with Rett syndrome are prone to gastrointestinal disorders and up to 80% have seizures and typically have no verbal skills. About 50% of individuals affected are not ambulatory. Scoliosis, growth failure, and constipation are very common features of Rett syndrome and can be problematic. Rett syndrome is listed under the broad category of pervasive developmental disorders.

Childhood disintegrative disorder (CDD), also known as Heller's syndrome and disintegrative psychosis, is a rare condition characterized by late onset (>3 years of age) of developmental delays in language, social function, and motor skills. CDD has some similarity to autism, and is sometimes considered a low-functioning form of it, but an apparent period of fairly normal development is often noted before a regression in skills or a series of regressions in skills. Many children are already somewhat delayed when the disorder becomes apparent, but these delays are not always obvious in young children. The age at which this regression can occur varies, and can be from age 2-10 with the definition of this onset depending largely on opinion. Some children describe or appear to be reacting to hallucinations, but the most obvious symptom is that skills apparently attained are lost.

Combination Therapy/Treatment

The compositions and methods of the invention can also be used in conjunction with other remedies known in the art that are used to treat pain, mood disorders and/or imbalances in psychological state, or disorders of brain development including non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids, acetaminophen, opioids, muscle relaxants, anti-anxiety drugs, anti-depressants, anti-convulsant drugs, antipsychotics, mood stabilizers, lithium, and serotonin reuptake inhibitors (SSRIs). The compositions and methods of the invention can also be used in conjunction with other forms of treatment including but not limited to: cognitive-behavioral therapies, music therapies, art therapies, group therapies, psychotherapies, physical exercise, pet therapies, communication therapies, educational therapies, and family therapies. The choice of specific treatment may vary and will depend upon the severity of the pain, mood disorder, or disorder of brain development, the subject's general health, and the judgment of the attending clinician.

For the treatment of neuropathic pain, the compositions of the invention can be used prior to, concurrently with, or subsequent to administration of tricyclic antidepressants (TCAs), such as amitriptyline , selective serotonin reuptake inhibitors (SSRIs) or norepinephrine inhibitors, such as duloxetine, milnacipran, and venlafaxine, antiepileptic drugs (AEDs), such as gabapentin, pregabalin, topiramate, and levetiracetam, and other neuropathic pain agents, such as nortriptyline, bupropion, desipramine, nonsteroidal anti-inflammatories, opioids (e.g., codeine, lydrocodone, hydromorphone, methadone, morphine, oxycodone), lidocaine, gallium maltolate, and cannabinoids.

The present compositions can also be formulated in combination with one or more additional active ingredients, which can include a pharmaceutical agent such NSAIDs (e.g., aspirin, ibuprofen, ketoprofen, ketorolac tromethamine, and naproxen), corticosteroids (e.g., prednisolone, methylprednisolone, hydrocortisone, amcinonide, fluocinonide, flunisolide, prednicarbate, betamethasone, and triamcinolone acetonide), acetaminophen, opioids (e.g., morphine, fentanyl, oxcodone, codeine), muscle relaxants (e.g., carisoprodol, cyclobenzaprine, and diazepam), anti-anxiety drugs (e.g., duloxetine, fluoxetine, alprazolam, escitalopram, and lorazepam), anti-depressants (e.g., desipramine, amitriptyline, agomelatine, etoperidone, and phenelzine), anti-convulsant drugs (e.g., lithium carbonate,

lithium citrate, topiramate, oxcarbazepine, and valproic acid), antipsychotics (e.g., aripiprazole, clozapine, risperidone, asenaphine, and olanzapine), and SSRIs (e.g., citalopram, paroxetine, fluvoxamine, and sertraline).

In one embodiment, any of the foregoing compounds may be formulation with an inorganic nitrite (e.g., sodium nitrite) or administered along with an inorganic nitrite (e.g., sodium nitrite) to a patient suffering from pain (e.g., diabetic neuropathy or another neuropathic pain). When co-administered, the two compounds are desirably administered within 24 hours of each other (e.g., within 12 hours, 8 hours, 4, hours, 2 hours, 1 hour, 30 minutes, 15 minutes, or substantially simultaneously).

In some embodiments, the composition also includes an inorganic nitrate; in other embodiments, the composition excludes inorganic nitrates. For example, the present composition can include inorganic nitrite and nitrates in a ratio that is between 1-5 to 1-100 nitrite:nitrate, e.g., 1-5, 1-10, 1-30, 1-50, 1-70, or 1-100 nitrite:nitrate.

EXAMPLES

The following list of abbreviations and definitions of terms are used in the examples described hereafter.

Abbreviations	**Term**
ABI	Ankle Brachial Index
ACS	Acute Coronary Syndrome
AUC	Area Under Curve
AE	Adverse Event
BID	Twice Daily
CBC	Complete Blood Count
CFR	Code of Federal Regulations
CHF	Congestive Heart Failure
CNS	Central Nervous System
C_{max}	Maximum Plasma Drug Concentration
C_{tau}	Average Drug Concentration over Dosing Interval
DBP	Diastolic Blood Pressure
DLT	Dose-Limiting Toxicity
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
FDA	Food and Drug Administration
FMD	Flow-Mediated Vasodilation
G6PD	Glucose-6 Phosphate Dehydrogenase
HbA1c	Hemoglobin A1c
ICF	Informed Consent Form
IL-6	Interleukin-6
IP	Investigational Product
LOCF	Last Observation Carried Forward
MDRD	Modification of Diet in Renal Disease Study
MetHb	Methemoglobin
NO	Nitric Oxide
NYHA	New York Heart Association
PAD	Peripheral Artery Disease
PD	Pharmacodynamic
PI	Principal Investigator
PK	Pharmacokinetic
QoL	Quality of Life
RAND 36	RAND 36-Item Short Form Health Survey

SAE	Serious Adverse Event
SBP	Systolic Blood Pressure
SD	Standard Deviation
SICAM	Soluble Intercellular Adhesion Molecule
SOC	System Organ Class
TIA	Transient Ischemic Attack
VCAM	Vascular cell adhesion protein
WIQ	Walking Impairment Questionnaire

Example 1: Phase 2a Clinical Studies

Study Rationale and Details

Sodium nitrite was investigated as a new therapy for improving function in subjects with PAD. The overall goal of this dose-ranging study was to evaluate the safety, pharmacokinetics, tolerability, and potential biological activity of multiple doses of oral sodium nitrite in subjects with PAD. As described in detail above, the primary pathophysiology of PAD is related to the limitation in blood flow of the lower extremities, resulting in limited exercise tolerance and decreased quality of life. A common feature of PAD is endothelial dysfunction, decreased NO bioavailability, and depletion of NO stores, a finding that may be compounded when PAD and metabolic diseases, such as diabetes, coexist. Sodium nitrite is an inorganic salt that is found and metabolized in vivo. At physiological concentrations, sodium nitrite is known to cause vasodilation.

The primary objective of this early stage clinical study was to evaluate the safety and tolerability of multiple doses of twice daily 40 mg and 80 mg sodium nitrite compared with placebo over a 10 week treatment period. The secondary objective of this study was to evaluate the pharmacokinetics of sodium nitrite and to demonstrate the pharmacodynamic effect of sodium nitrite on measures of biologic activity and functional measures of walking distance and claudication symptoms. Finally, the relationship between doses, plasma concentration of sodium nitrite, and pharmacodynamic effects were characterized and evaluated. In this study, multiple assessments of biological activity and ambulatory function were made during standardized tests of arterial reactivity and claudication-limited exercise. The pharmacodynamic assessments included: brachial artery flow-mediated vasodilation (FMD), six-minute walk test, selected biomarkers of interest, quality of life questionnaires (WIQ & RAND 36).

The primary endpoints included: clinical safety and tolerability data including spontaneous AE reporting, ECGs, vital signs, nursing/physician observation, and clinical laboratory values. The secondary endpoints included flow-mediated vasodilation responses, maximal distance covered during a six-minute walk test, plasma pharmacokinetics (including but not limited to AUC, C_{max}, C_{tau}) of sodium nitrite and the relationship to the pharmacodynamic assessments performed in this study, and quality of life (WIQ & RAND 36). Furthermore, exploratory pharmacodynamic/biomarker endpoints included changes in markers of inflammation, oxidative stress, metabolic function, angiogenesis, or other markers of atherosclerotic disease, as data permitted (e.g. sodium nitrite, nitrite, nitrate, soluble intercellular adhesion molecule (SICAM), Vascular cell adhesion protein (VCAM), F2-isoprostanes and Interleukin-6 (IL-6)).

The trial type was a randomized, double-blind, placebo-controlled, dose ranging, parallel design multiple dosing study targeted on subjects with PAD. Subjects were at least 35 years of age, but not greater than 85 years of age. If the subject experienced claudication, the subjects also had a 1 month

history of stable PAD symptoms. Subjects were assigned to either the placebo or sodium nitrite treatment group in accordance with the randomization schedule generated prior to the start of the study. Subjects were randomized into the study by means of an interactive web response system (IWRS) through electronic data capture (EDC) to receive one of the treatment regimens of either placebo, 40 mg BID or 80 mg BID. As this was a double-blind study, subjects, investigators, and site staff were blinded. TheraVasc and CPC were also blinded. In the case of a medical emergency or in the event of a serious medical condition, when knowledge of the investigational product was essential for the clinical management or welfare of the subject, an investigator or other physician managing the subject could unblind that subject's treatment code. The investigator made every effort to contact the CPC Medical Monitor before unblinding to discuss options. If the blind was broken for any reason and the investigator was unable to contact CPC prior to unblinding, the investigator must notify CPC as soon as possible following the unblinding incident without revealing the subject's study treatment assignment, unless the information was important to the safety of subjects remaining in the study. In addition, the investigator would record the date and reason for revealing the blinded treatment assignment for that subject in the appropriate data collection tool. If an expedited regulatory report to one or more regulatory agencies was required, the report identified the subject's treatment assignment. When applicable, a copy of the regulatory report was sent to investigators in accordance with relevant regulations, CPC policy, or both.

The Investigational Product (IP)

Capsules of sodium nitrite at dose strength of 40 mg and 80 mg per capsule which were to be stored at controlled room temperature (20 - 25 °C, 68-77 °F). Matching placebo capsules were also supplied and stored at controlled room temperature. TV1001 was supplied in 50 count bottles dispensed in accordance with the visit schedule described in Table 1. IP was stored under secure conditions. Bilcare, Global Clinical Supplies labeled, stored and distributed the sodium nitrite and matching placebo. IP was assigned and administered as described below. Table 2 describes details of the study drug.

Table 1. Schedule of Assesments

Visit Name	Screening	Visit 1 Randomization	Visit 2	Visit 3	Safety Visit[1]	Visit 4	Visit 5	Phone Call 1	Phone Call 2	Visit 6	Visit 7	Safety Visit[1]	Termination Visit 8	Follow-up Phone call	Early Term
Timing (days)	-21 to -14 days	Day 0	Day 1	Day 4	Day 7	Day 14	Day 28	Day 42	Day 56	Day 70	Day 71	Day 71 + 1	6 Days after V7	7 Days after V8 or ET	n/a
Allowable Variance			± 4 hours	± 1 day	± 1 day	± 2 days	± 2 days	± 2 days	± 2 days	± 2 days	± 1 day	± 1 day	± 1 days	± 1 days	n/a
Informed Consent	X														
Demographics	X														
Medical and Medication History	X	X													
Physical Examination	X												X		X
Vital Signs[2]	X	X	X	X	X	X	X			X	X	X	X		X
12-Lead ECG		X					X						X		X
Clinical Safety Labs	X		X	X		X	X			X	X		X		X
Met-Hb Labs[3]			X	X	X	X	X			X	X	X	X		X
PK Sample			X	X		X	X			X	X		X		X
PK and Met-Hb over 7 time points[3]		X													
Urine Pregnancy Test	X	X				X							X		X
PD Biomarkers		X								X					X
Ankle Brachial Index (ABI)	X														
FMD[4]		X								X					X
QoL (WIQ, RAND 36)		X								X					X
Six-Minute Walk Test		X								X					X
Study Medication Dispensed		X				X	X				X				
Adverse Events		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant Medications		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Evaluate Inclusion/Exclusion Criteria	X	X													
Evaluate Study Stopping Criteria		X	X	X	X	X	X			X	X	X			

1This visit was only required if Met-Hb is 8% or greater.
2Vital signs are supine prior to first dose of IP and postural after first dose.
3Repeated blood draws occured at baseline (pre-dose) and post-dose at 15 minutes ± 5 minutes, 30 minutes ± 5 minutes, 1 hour ± 10 minutes, 2 hours ± 10 minutes, 4 hours ± 10 minutes, and 6 hours ± 10 minutes.
4FMD may be done 7 days prior to the rest of Visit 1 and 5 days prior to the rest of Visit 6.

Table 2. Study Drug

Study Drug	TV1001	Placebo
Form	Capsule	Capsule
Available Unit does strength(s)	40 and 80 mg	40 and 80 mg matched
Route/Administration	Administered orally	Administered orally
Supplier	TheraVasc Inc.	TheraVasc Inc.
Manufacturer	UPM Pharmaceuticals 6200 Seaforth Street, Baltimore, MD 21224	UPM Pharmaceuticals 6200 Seaforth Street, Baltimore, MD 21224

Subjects were instructed to return unused study medication and empty packaging at each study visit; all returned capsules were counted and recorded on the appropriate form. Compliance was calculated as the number of capsules taken divided by the number of capsules expected. If a subject was taking fewer capsules than expected, the site staff would counsel the subject on the importance of IP compliance. Investigators were responsible for receipt and proper storage of study medication, as well as for maintaining records of product delivery to site, inventory at site, dispensing of product to each subject, and return of product to TheraVasc, or designee, at the end of the study. All used, unused and partially used medication packages were returned according to TheraVasc, or designee, instructions.

The study was stopped if there were significant changes in safety parameters or significant AEs considered to be related to treatment with study medication (i.e., an imbalance in the safety profile in subjects receiving active drug vs. placebo). An individual subject was withdrawn at the discretion of the responsible investigator and the site study team for the reasons listed below as well as for other safety reasons that may not be listed. In the event one or more subjects were withdrawn, additional subjects were enrolled to ensure an adequate number of subjects complete the cohort. Specific reasons for an individual subject to withdraw included but was not limited to:

- Subjects with a pattern of severe adverse events in any SOC, or cardiac monitoring findings as determined by the investigator and/or the sponsor.
- Subjects with methemoglobin value ≥ 15% on any one occasion during study participation.
- Subjects with normal baseline blood pressure who experienced any of the following: an increase in blood pressure to 160 mm Hg systolic and/or 90 mmHg diastolic that persists over 24 hours, an increase from baseline blood pressure of 30 mm Hg systolic and/or 15 mm Hg diastolic that persists over 24 hours, any symptomatic increase in blood pressure.
- Subjects with stable elevated blood pressure at baseline who experienced any of the following: an increase in blood pressure to 180 mm Hg systolic and/or 100 mmHg diastolic that persists over 24 hours, an increase from baseline blood pressure of 20 mm Hg systolic and/or 10 mm Hg diastolic that persists over 24 hours, any symptomatic increase in blood pressure.
- Subjects who experienced a decrease from baseline blood pressure of ≥ 20 mm Hg systolic with or without an increase of 10 beats per minute (BPM) pulse and the presence of symptoms.

Any subject who developed hypertension or hypotension requiring intervention were followed to resolution, preferably until any intervention therapy was withdrawn.

There were no Data Monitoring Committee (DMC) in place for this study and safety was monitored by the designated Study Medical Expert. A Steering Committee was formed comprising the Sponsor's CEO, two clinicians with experience in clinical trials, a medical regulatory expert and a

researcher with expertise in sodium nitrite and its biological effects. CPC provided monthly status reports to the Committee on subject recruitment at each site, monitored reports of the site activities, and other non-safety information regarding the trial. Similar reports were provided in a blinded manner by the distributor of the bottle kits relative to the number of kits distributed to each site, returned bottles, and any issues that arose in randomization or distribution of the IP, assuring that no information was provided to the Committee as to the actual randomization. The Committee would discuss the reports and if any protocol deviations or non-compliance to the investigator's agreement or general investigational plan were noted, action was promptly taken to correct such deviations and secure compliance or discontinue shipments of the investigational drug to the investigator, end the investigator's participation in the investigation, require that all investigational drug be returned to the sponsor, and notified to the FDA. The Committee monitored subject accrual at each site and when necessary discontinue sites that were failing to enroll subjects and add additional sites. The Committee met within two calendar days upon receiving any information that could affect subject safety. The Committee discussed all safety information with CPC, and reported to the FDA and all active clinical investigators any information relevant to the safety of the drug as required under 21 CFR 312.32. The committee made annual reports on the progress of the investigations in accordance with 21 CFR 312.33. No interim analysis was planned for this study.

Study Visits

The study visits included the following components:

Screening

This visit was conducted within 14 to 21 days of Visit 1 – Randomization. A signed informed consent form (ICF) was obtained before any study-specific assessments were performed. The following screening assessments were performed: (1) Informed Consent, (2) Demographics, (3) Medical and Medication History, (4) Physical Exam, (5) Supine Vital Signs, (6) Clinical Safety Labs, (7) Urine Pregnancy Test, (8) Ankle Brachial Index, and (9) Evaluate Inclusion/Exclusion Criteria.

Visit 1-Randomization

This was considered Day 0 of the study. Subjects were randomized at this visit and given first dose of study medication. The following assessments were performed: (1) Update Medical and Medication History, (2) 12-Lead ECG, (3) Urine Pregnancy Test, (4) FMD (may be performed within 7 days prior to Visit 1), (5) Quality of Life Questionnaires (WIQ and RAND 36), (6) Six-Minute Walk Test, (7) Evaluate Inclusion/Exclusion Criteria, (8) Study Medication Dispensed (the dose of study medication occurred in clinic. Subjects remained on clinic site for safety follow-up until the last PK sampling was complete), (8) PK Sampling (pre-dose and post-dose: 15, 30 minutes ± 5 minutes, and 1, 2, 4, 6 hours ± 10 minutes), (9) MetHb Sampling (pre-dose and post-dose: 15, 30 minutes ± 5 minutes, and 1, 2, 4, 6 hours ± 10 minutes), (10) PD Biomarkers, (11) Postural Vital Signs, (12) Adverse Event/Concomitant Medication Assessment (adverse events were captured following administration of the first dose), and (13) Evaluate Study Stopping Criteria.

Visit 2 (Day 1)

This visit was conducted 1 day (24 hours) +/- 4 hours following the time of first dose administration at Visit 1. The subject must have taken the morning dose of the study medication in clinic 30 minutes (+/- 10 min) before PK sampling. The following assessments were performed: (1) Administration of morning dose of study medication, (2) Clinical Safety Labs, (3) PK Sampling, (4) MetHb Sampling, (4) Postural Vital Signs, (5) Adverse Event/Concomitant Medication Assessment, and (6) Evaluate Study Stopping Criteria

Visit 3 (Day 4)

This visit was conducted 4 +/- 1 days following Visit 1. The subject must have taken the morning dose of the study medication in clinic 30 minutes (+/- 10 min) before PK sampling. The following assessments were performed: (1) Administration of morning dose of study medication, (2) Clinical Safety Labs, (3) PK Sampling, (4) MetHb Sampling, (5) Postural Vital Signs, (6) Adverse Event/Concomitant Medication Assessment, (7) Evaluate Study Stopping Criteria (if the subject does not meet stopping criteria but does experience an increase in MetHb to 8% or higher, and optional safety visit at Day 7 was scheduled as described below).

Optional Safety Visit (Day 7)

This visit was conducted only if the subject had a MetHb at Visit 3 of 8% or higher. It should be conducted 7 days following Visit 1 +/- 1 day. The subject must have taken the morning dose of the study medication in clinic 30 minutes (+/- 10 min) before MetHb sampling. The following assessments were performed: (1) Administration of morning dose of study medication, (2) MetHb Sampling, (3) Postural Vital Signs, (4) Adverse Event/Concomitant Medication Assessment, and (5) Evaluate Study Stopping Criteria

Visit 4 (Day 14)

This visit was conducted 14 +/- 2 days following Visit 1. The subject must have taken the morning dose of the study medication in clinic 30 minutes (+/- 10 min) before PK sampling. The following assessments were performed: (1) Administration of morning dose of study medication, (2) Clinical Safety Labs, (3) PK Sampling, (4) MetHb Sampling, (5) Urine Pregnancy Test, (6) Postural Vital Signs, (7) Adverse Event/Concomitant Medication Assessment, (8) Evaluate Study Stopping Criteria, (9) Study Medication Compliance, and (10) Study Medication Dispensed

Visit 5 (Day 28)

This visit was conducted 28 +/- 2 days following Visit 1. The subject must have taken the morning dose of the study medication in clinic 30 minutes (+/- 10 min) before PK sampling. The following assessments were performed: (1) Administration of morning dose of study medication, (2) Clinical Safety Labs, (3) PK Sampling, (4) MetHb Sampling, (5) Postural Vital Signs, (6) 12-Lead ECG, (7) Adverse Event/Concomitant Medication Assessment, (8) Evaluate Study Stopping Criteria, (9) Study Medication Compliance, and (10) Study Medication Dispensed

Phone Call 1

A phone call was placed to the subject 42 +/- 2 days following Visit 1. The subject was questioned regarding any adverse events and changes to concomitant medications.

Phone Call 2

A phone call was placed to the subject 56 +/- 2 days following Visit 1. The subject was questioned regarding any adverse events and changes to concomitant medications.

Visit 6 (Day 70)

This visit was conducted 70 +/- 2 days following Visit 1. The subject must have taken the morning dose of the study medication in clinic 30 minutes (+/- 10 min) before PK sampling. The following assessments were performed: (1) Administration of morning dose of study medication (2) Clinical Safety Labs, (3) PK Sampling, (4) MetHb Sampling, (5) PD Biomarkers, (5) Postural Vital Signs, (6) FMD (may be performed within 5days prior to Visit 6), (7) Quality of Life Questionnaires (WIQ and RAND 36), (8) Six-Minute Walk Test, (9) Adverse Event/Concomitant Medication Assessment, (10) Evaluate Study Stopping Criteria, and (11) Study Medication Compliance .

Visit 7 (Day 71)

This visit was conducted 1 day + 1 day following Visit 6. The subject must have taken the morning dose of the study medication (dose escalation) in clinic 30 minutes (+/- 10 min) before PK sampling. The following assessments were performed: (1) Study Medication Dispensed, (2) Administration of morning dose of study medication (upon dispensing and administering study medication, subjects were instructed to increase from 1 capsule BID to 2 capsules BID as described. Subject remained in clinic for a 1 ½ hours post dose observation), (3) Clinical Safety Labs, (4) PK Sampling, (5) MetHb Sampling (subject remained in clinic until results were available), (6) Postural Vital Signs, (7) Adverse Event/Concomitant Medication Assessment, (8) Evaluate Study Stopping Criteria (if the subject did not meet stopping criteria but experienced an increase in MetHb to 8% or higher, a safety visit at Day 70+2 was scheduled as described below in Optional Safety Visit (Visit 7 + 1), (9) Safety Assessment (immediately prior to subject departure), (10) Evaluation of MetHb results, and (11) Seated Vitals – Pulse Rate and BP.

Optional Safety Visit (Visit 7 + 1)

This visit was conducted only if the subject has a MetHb at Visit 7 of 8% or higher. It was conducted 1 +1 day following Visit 7. The subject must have taken the morning dose of the study medication in clinic 30 minutes (+/- 10 min) before MetHb sampling. The following assessments were performed: (1) Administration of morning dose of study medication, (2) MetHb Sampling, (3) Postural Vital Signs, (4) Adverse Event/Concomitant Medication Assessment, and (5) Evaluate Study Stopping Criteria.

Visit 8- Termination (Vist 7 + 6)

This visit was conducted 6 +/- 1 days following Visit 7. The subject must have taken the morning dose of the study medication in clinic 30 minutes (+/- 10 min) before PK sampling. This would be the final dose and study visit. The following assessments were performed: (1) Physical Exam, (2) Clinical Safety Labs, (3) PK Sampling, (4) MetHb Sampling, (5) Urine Pregnancy Test, (6) Postural Vital Signs, (7) 12-

Lead ECG, (8) Adverse Event/Concomitant Medication Assessment, and (9) Study Medication Compliance

Follow-up Phone Call

A phone call was placed to the subject 7 +/- 1 days following Visit 8. If this subject early terminates from the study, a phone call was placed to the subject 7 days following the ET visit +/- 1 day. The subject was questioned regarding any adverse events and changes to concomitant medications.

Early Termination Visit (ET)

In the case that a subject must withdraw early from study participation for any reason prior to Visit 6, every effort was made to complete an early termination visit. The subject must have taken the morning dose of the study medication in clinic 30 minutes (+/- 10 min) before PK sampling unless the subject was withdrawn for safety and should stop taking IP immediately. The following assessments were performed: (1) Administration of morning dose of study medication, if applicable, (2) Physical Exam, (3) Clinical Safety Labs, (4) PK Sampling, (5) MetHb Sampling, (6) PD Biomarkers, (7) Postural Vital Signs, (8) 12-Lead ECG, (9) FMD (may be performed within 5 days prior to ET Visit), (10) Quality of Life Questionnaires (WIQ and RAND 36), (11) Six-Minute Walk Test, (12) Urine Pregnancy Test, (13) Adverse Event/Concomitant Medication Assessment, and (14) Study Medication Compliance. Moreover, if early termination occurred after Visit 6 but before the appropriate visit window for Visit 8, all procedures required at Visit 8 were completed.

Selection and Withdrawal of Subjects

The inclusion criteria included subjects between the ages of 35 and 85 years. Subjects must be either male or females post-menopausal, sterilized or using suitable birth control. Suitable birth control must be total abstinence, male partner sterilization or double barrier method paired with using oral contraception, injectable progestogen, implants of levonorgestrel, estrogenic vaginal ring, percutaneous contraceptive patches, or intrauterine device (IUD). A history of peripheral artery disease (PAD) was confirmed by medical chart or an ankle brachial index at rest of ≤ 0.90. If subjects received a medical standard treatment for cardiac risk factors, subject must have been on a stable treatment for at least 1 month prior to Screening. If included in this regimen, treatments such as cilostazol, pentoxifylline, statins, or angiotensin converting enzymes (ACE)-inhibitors; supervised exercise rehabilitation training; participation in a formal smoking cessation program or prescription of medications for smoking cessation were not changed significantly in the last month and were not expected to change over the duration of the study. If subjects experienced claudication symptoms, subjects must have stable lower extremity symptoms for at least 1 month (e.g. no change in claudication symptoms) prior to Screening. Subjects were required to provide written informed consent and willingness as documented by a signed informed consent form.

The exclusion criteria included subjects with non-atherosclerotic PAD (e.g. Buerger's vasculitis), lower extremity surgical or percutaneous revascularization, evidence of graft failure or other peripheral vascular surgical procedure within the last 6 months prior to Screening, anticipated lower extremity revascularization within the treatment period, myocardial infarction, unstable angina, cerebrovascular accident or transient ischemic attack (TIA) within 3 months prior to Screening, poorly controlled diabetes (HgA1c > 10.0), poorly controlled hypertension (systolic blood pressure (SBP) ≥ 160 mmHg or diastolic

blood pressure (DBP) ≥ 100 mmHg) despite therapy, systolic blood pressure ≤100 mmHg on current medical regimen, hypersensitivity to sodium nitrite or related compounds, and renal insufficiency documented as eGFR < 30 mL/minute/1.73 m^2 (Modification of Diet in Renal Disease Study MDRD). Exclusion criteria also included subjects who were pregnant or nursing women, who had a life expectancy of < 6 months, a chronic illness that may increase the risks associated with this study in the opinion of the investigator, an active malignancy requiring active anti-neoplastic therapy that, in the opinion of the investigator, interfered with study treatment or participation (although stable basal cell skin cancer was allowed and cancer being treated solely with hormonal therapy was allowed), an active infection (i.e. systemic or osteomyelitis), a NYHA CHF Class III or IV, has had recent hospitalization (< 30 days) for acute coronary syndrome (ACS), myocardial infarction (MI), congestive heart failure (CHF) or stroke, recent (< 30 days) coronary revascularization had previously been treated with angiogenic factors or stem cell therapy within 1 year prior to Screening, was involved in another PAD clinical trial within past 1 month prior to Screening, had exposed tendon, muscle or bone or a diagnosis of critical leg ischemia (CLI), was previously amputated within 3 months prior to Screening, or had a planned amputation that would limit walking (although small toe is allowed). Exclusion criteria also included subjects whose ability to perform the 6 minute walk test was limited by symptoms other than claudication, who was diagnosed with alcohol or other substance abuse, had a history of methemoglobinema, (metHb ≥ 15%), who had an inability to speak English (due to need for administering standardized English-language questionnaire), who had evidence of anemia or a history of chronic hemolytic condition, including sickle cell disease, who had chronic use of anti-migraine medication such as Imitrex or sumatriptan, and a positive screen for glucose-6-phosphate dehydrogenase (G6PD) deficiency at screening. Subjects who chronically took the following medications: Allopurinol, PDE-5 inhibitors, sedative tricyclic antidepressants, sedative antihistamines, meperidine and related narcotic central nervous system (CNS) depressants, and nitrates were also excluded.

The withdrawal criteria allowed a subject to withdraw from the study at any time at his/her own request. The subject may also have been withdrawn at the Investigator's request if it was the Investigator's opinion that it was not in the subject's best interest to continue in the study. The subject was withdrawn if he or she met stopping criteria described above. In the event a subject was withdrawn from the study for any reason, the subject was followed-up with reasonable effort made to determine the reason for their withdrawal from the study and an ET visit as described above. Telephone calls, certified letters and offers of transportation assistance were considered reasonable effort. A summary of subject withdrawals is provided in Table 3.

Table 3. Subject Withdrawals

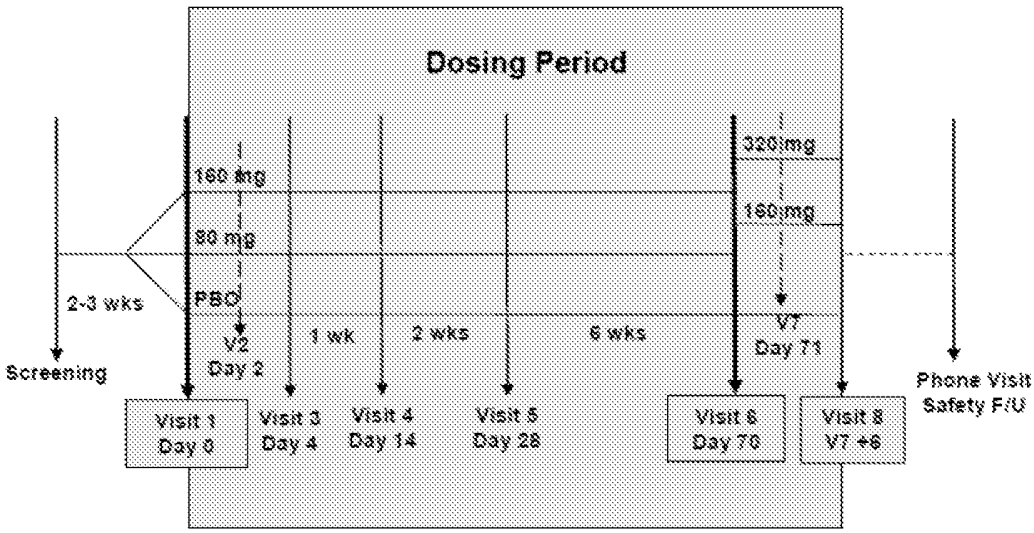
SUMMARY OF SUBJECT DISPOSITION

	Placebo n=18	40 mg n=19	80 mg n=18
Subjects who completed the study	15(83.3%)	17(89.5%)	15(83.3%)
Subjects who withdrew prior to completion	3(16.7%)	2(10.5%)	3(16.7%)
Reasons for withdrawal:			
Adverse Event	0(0.0%)	1(5.3%)	2(11.1%)
Met withdrawal-new hypotension	1(5.6%)	0(0.0%)	1(5.6%)
Subject Request-lack of energy	0(0.0%)	1(5.3%)	0(0.0%)
Subject request- refused to continue	1(5.6%)	0(0.0%)	0(0.0%)
Subject request-no benefit	1(5.6%)	0(0.0%)	0(0.0%)

Treatment of Subjects

The three dosing arms were placebo, 40 mg BID and 80 mg BID sodium nitrite as illustrated in Table 4 below. All doses were given as a twice-daily oral dose for 10 weeks. On the day following the 10 week dosing period and completion of efficacy assessments, subjects in each treatment arm entered a 6 day dose-escalation period (dose-doubling). Subjects in the 40 mg sodium nitrite BID group were dose-escalated to 80 mg sodium nitrite BID for 6 days, and subjects in the 80 mg sodium nitrite BID were dose-escalated to 160 mg sodium nitrite BID for 6-days. Placebo subjects doubled the number of placebo capsules taken BID. All study medication was stopped at the end of the 6-day dose-escalation period.

Table 4. Dosing Arms



Subjects chronically taking Imitrex (sumatriptan), allopurinol, PDE-5 inhibitors, sedative tricyclic antidepressants, sedative antihistamines, meperidine and related narcotic CNS depressants, and nitrates were prohibited from participating in this study.

Subjects were instructed to return unused study medication at each study visit; all returned capsules were counted and recorded on the appropriate form. Compliance was calculated as the number of capsules taken divided by the number of capsules expected. The number of capsules taken was calculated by subtracting the number of capsules left from 50, the number of capsules in the bottle. If a

subject took fewer capsules than expected, the site staff counseled the subject on the importance of IP compliance. Investigators were responsible for receipt and proper storage of study medication, as well as for maintaining records of product delivery to site, inventory at site, dispensing of product to each subject, and return of product to TheraVasc, or designee, at the end of the study. All used, unused, and partially used medication packages were returned according to TheraVasc, or designee, instructions.

Assessment of Efficacy

The efficacy parameters included: (1) flow-mediated vasodilation (FMD), six-minute walk test, pharmacokinetics (PK), biomarkers/pharmacodynamic (PD) markers, and quality of life (QoL) questionnaires.

Of significance to the present invention are the responses relating to quality of life. Quality of life was measured by two questionnaires: WIQ and RAND 36. The two questionnaires were administered in the same sequence: WIQ first, followed by the RAND 36. The WIQ was a disease-specific instrument that measures community-based walking. The questionnaire consisted of four subscales (pain severity, distance, speed, and stairs). The WIQ was verbally administered to the subject by the Investigator, or designee. The RAND 36 was an instrument that measured general health issues. Study staff directed subjects to complete the RAND 36 on their own. Staff did not try to interpret the questions for the subject. If the subject did not understand a particular question, the study staff instructed the subject to interpret the meaning of the question to the best of his or her ability and provide an answer that seems most accurate to the subject. No family members or other individuals were allowed to answer questions or complete the questionnaire for the subject. All questionnaires were completed directly on the written source document pages. The study coordinator reviewed all questionnaires to ensure that there was only one response to each question, each question has been answered and any necessary corrections have been initialed and dated by the Investigator (or designee) or subject, accordingly. The results from the RAND 36 physical and psychological assessments are detailed in Figures 1A-B. RAND 36 showed a trend toward improvement in quality of life assessment and significant improvement in pain assessment in the 40 mg group. Results from the WIQ assessments are detailed in Figures 2A-B. WIQ showed no change in assessment in walking distance and a trend toward improvement in walking speed and stair climbing.

Assessment of Safety

The following safety parameters were assessed: medical and medication history, concomitant medication usage, physical examination, vital signs, 12-lead ECG, clinical chemistries, CBC, urinalysis, and adverse events. Urine pregnancy testing was completed for women of child-bearing potential who have not been surgically sterilized. Assessment of acute adverse events (i.e., drop in blood pressure, dizziness) was performed after administration of 1st dose for each dose level of sodium nitrite. Dose-limiting toxicity (DLT) was defined as Grade 3 and clinically significant hematological events, particularly MetHb.

Overall, no severe adverse effects were observed in treated groups. Dose dependent hypotensive affects were observed demonstrating the hemodynamic affects of the treatment. Moreover, Methemoglobin levels were of no concern, even at the 160 mg dose escalation.

Demographic information (Table 5) and a complete medical history (Table 6) were obtained at the Screening Visit. Medical history for any ongoing ailments and for 5 years prior to screening and

medication history for the past 1 month were documented. Medical and medication history was reviewed with the subject prior to randomization to ensure all data were accurate and complete to date.

Table 5. Demographic Data

		Placebo n=18	40-mg n=19	80-mg n=18
Age at informed consent (years)		64.9 +/- 8.98	65.3 +/- 8.86	67.9 +/- 9.99
Gender	Male	13(72.2%)	15(78.9%)	13(72.2%)
	Female	5(27.8%)	4(21.1%)	5(27.7%)
Race/Ethnicity				
	Black or African American	5(27.8%)	6(31.6%)	8(44.4%)
	White	12(66.7%)	12(63.2%)	10(55.6%)
	Other	1(5.6%)	1(5.3%)	0(0.0%)
Weight(kg)		88.07 +/- 27.24	79.32 +/- 13.53	88.99 +/- 16.70
Height(cm)		173.18 +/- 13.29	172.01 +/- 9.87	172.18 +/- 9.95
Screening BMI (kg/m2)		29.32 +/- 8.31	26.71 +/- 2.99	30.01 +/- 5.03
ABI in index limb at screening		0.56 +/- 0.15	0.62 +/- 0.20	0.69 +/- 0.17
Diabetes Diagnosis		10(55.6%)	14(73.7%)	14(77.8%)
Hb A1c (% Hb) at screening		6.97 +/- 1.48	6.99 +/- 1.27	6.71 +/- 0.94

Table 6. Medical History Background

	Placebo N=18	40-mg N=19	80-mg N=18
PAD in last 5 years	18(100%)	19(100%)	18(100%)
Peripheral revascularization in last 5 years	8(44.4%)	2(10.5%)	8(44.4%)
Coronary artery disease in last 5 years	6(33.3%)	5(26.3%)	7(38.9%)
Angina	2(11.1%)	0	4(22.2%)
Myocardial infarction	0	2(10.5%)	2(11.1%)
Coronary revascularization in last 5 years	1(5.6%)	0	4(22.2%)
Congestive Heart Failure	1(5.6%)	0	1(5.6%)
Cerebrovascular disease in last 5 years	2(11.1%)	3(15.8%)	5(27.8%)
Ischemic stroke	0	1(5.3%)	1(5.6%)
TIA.mini-stroke	1(5.6%)	0	1(5.6%)
Hypertension	16(88.9%)	18(94.7%)	16(88.9%)
Dyslipidemia	15(83.3%)	18(94.7%)	16(88.9%)
Diabetes type 1	0	1(5.3%)	0
Diabetes type 2	10(55.6%)	12(63.2%)	12(66.7%)
Deep vein thrombosis/Pulmonary Embolism	0	0	2(11.1%)
Stent/Balloon/Bypass	5(27.8%)	0	1(5.6%)

ABI assessments were measured at the screening visit in order to assess if the subject was appropriate according to inclusion criteria. ABI assessments were done only after the subject had been resting in a supine position for at least 10 minutes. The ABI was defined as the ratio between the higher of the two pedal systolic blood pressures (dorsalis pedis and posterior tibialis) and the higher of the two systolic brachial pressures. A continuous wave Doppler, between 5 and 10 MHz, was used to measure the systolic pressures in both the dorsalis pedis and posterior tibial arteries in each leg, as well as the brachial arteries in each arm. The higher of the 2 arm pressures and the higher of the 2 ankle pressures

for each leg were used for the calculation. The ABI was calculated for both legs. The ABI must be less than 0.90 in at least one extremity to qualify for the study.

Site staff recorded any medication taken by a subject after randomization into the study, including prescribed, nutritional supplements and over-the-counter medications, and the reason for its use as a concomitant medication. If a subject required treatment by any medications listed as a prohibited concomitant therapy, he or she would be withdrawn from study participation and completed an ET visit.

A complete physical examination was performed at Screening and included height, weight and assessments of the following systems: general appearance; eyes; ears, nose, and throat; head and neck; chest and lungs; cardiovascular; abdomen; musculoskeletal; lymphatic; dermatological; neurological; and extremities. At Visit 8 or Early Termination a follow-up physical exam assessed weight and any changes to the above mentioned systems. Any significant changes noted at Visit 8 was documented as an adverse event unless otherwise noted by the PI or designee.

Supine vital signs were measured at the Screening Visit. The subject rested in a supine position for a minimum of 3 minutes prior to obtaining vital sign measurements. Vital signs included BP and pulse rate. Postural vital signs, including both supine and standing measurements of blood pressure and pulse rate, were recorded at all study visits following the first dose of IP administration. Measurements were performed as follows: (1) the subject rested in a supine position for a minimum of 3 minutes, (2) vital signs (BP and pulse rate) were measured while the subject was supine, (3) the subject assumed a standing position for a minimum of 5 minutes, and (4) vital signs (BP and pulse rate) were measured while the subject was standing. Pulse rate and blood pressure data are detailed in Table 7.

Table 7. Pulse Rate and Blood Pressure

		Screening	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8
Supine (Mean)										
Pulse	Placebo	73.6	74.4	73.0	71.0	74.8	74.6	73.1	73.1	75.6
	40-mg	71.4	74.1	74.1	74.7	71.7	72.7	70.4	73.1	70.2
	80-mg	63.9	65.6	65.1	66.7	64.6	68.7	65.5	64.3	68.3
Blood Pressure	Placebo	141.3/77.9	141.4/78.3	139.9/78.4	138.4/77.4	137.8/75.4	140.3/76.9	145.4/79.5	139.8/77.8	136.1/75.1
	40-mg	136.8/75.8	129.7/72.3	128.0/70.5	129.8/72.5	128.4/71.1	124.1/72.0	127.3/73.7	126.7/71.3	130.0/72.2
	80-mg	132.4/69.4	129.8/68.4	122.8/66.7	127.1/66.7	125.1/65.2	124.6/68.9	123.1/66.2	118.4/64.4	120.7/66.9
Standing (Mean)										
Pulse	Placebo		78.1	77.8	74.9	78.1	78.5	76.0	76.1	77.4
	40-mg		75.8	78.6	76.7	75.6	76.6	73.9	76.3	74.3
	80-mg		72.6	72.3	72.9	72.6	72.5	67.6	70.4	72.6
Blood Pressure	Placebo		141.6/81.7	144.3/81.2	139.9/79.4	139.9/80.3	137.7/79.4	143.3/78.4	141.3/78.7	138.2/75.0
	40-mg		129.5/73.1	128.3/72.6	129.4/73.1	124.9/71.0	124.3/71.6	127.6/73.2	122.2/73.2	123.1/68.7
	80-mg		125.4/70.2	123.1/71.9	124.8/69.2	123.7/68.8	119.5/71.9	124.8/70.0	123.3/67.1	117.7/67.9
Orthostatic Changes										
Pulse	Placebo		3.7	4.8	3.9	3.3	3.9	2.9	3.0	1.8
	40-mg		1.8	4.5	2.0	3.9	3.8	3.5	3.2	4.1
	80-mg		6.9	7.3	6.2	7.9	3.8	2.1	6.1	4.3
Systolic BP	Placebo		0.2	4.3	1.6	2.1	-2.6	-2.1	1.5	2.2
	40-mg		-0.3	0.3	-0.4	-3.4	0.3	0.3	-4.5	-6.9
	80-mg		-4.4	0.3	-2.3	-1.4	-5.2	1.6	4.8	-3.0
Diastolic BP	Placebo		3.3	2.8	1.9	4.9	2.5	-1.1	0.9	-0.1
	40-mg		0.8	2.1	0.6	-0.1	-0.4	-0.5	1.9	-3.5
	80-mg		1.8	5.2	2.5	3.5	3.0	3.8	2.7	1.0

A resting 12-lead ECG printout with the subject in supine position was obtained at the time points listed in Table 1-Schedule of Assessments. All ECGs were assessed by the PI or qualified designee for clinical significance of any abnormalities or changes and documented on the ECG source document. Any clinically significant abnormalities that occured after the first dose of sodium nitrite was recorded as AEs on the eCRF. The 12-lead ECG was obtained immediately following vitals, with the exception of the Visit 1 Randomization day ECG which was collected prior to dosing. The ECG data details are provided in Table 8.

Table 8. ECG

	Visit 1	Visit 5	Visit 8
Heart Rate (beats/minute)			
Placebo	72.1 +/- 13.9	71.7 +/- 15.1	73.0 +/- 12.2
40-mg	71.4 +/- 12.7	72.2 +/- 14.8	
80-mg	62.7 +/-10.7	65.5 +/- 11.9	74.3 +/- 16.9
160-mg			64.7+/- 10.0
QTcB interval (msec)			
Placebo	433.2 +/- 33.0	430.9 +/- 24.0	438.6 +/- 35.3
40-mg	415.9 +/- 49.0	430.1 +/- 34.8	
80-mg	422.3 +/- 34.0	411.6 +/- 49.7	423.2 +/- 40.3
160-mg			427.7 +/- 31.9
QTcF interval (msec)			
Placebo	421.2 +/- 31.4	419.7 +/- 22.5	425.4 +/- 33.9
40-mg	404.8 +/- 44.9	417.7 +/- 24.0	
80-mg	419.9 +/- 30.5	406.2 +/- 46.2	409.5 +/- 34.3
160-mg			422.8 +/- 27.9

QTc changes > 60 msec: serious; QTc changes > 30 msec: questionable

Laboratory evaluations were collected at the time points listed in Table 1. All safety clinical laboratory testing was performed by a central laboratory, with the exception of the urine pregnancy test and methemoglobin which was completed on-site. Specimen samples were sent from the investigative site to the central laboratory. A urine pregnancy test was performed at the time points listed in Table 1 if any woman was not surgically sterilized or post-menopausal.

Clinical Labs were performed with subjects fasting and include the following: Urinalysis: Protein dipstick, specific gravity, appearance, pH, glucose, blood, bilirubin, ketones, and microscopic examination. Clinical chemistry panel included: albumin, alkaline phosphatase, serum amylase, ALT, AST, BUN , calcium (serum), serum chloride, CO2, serum creatinine, direct bilirubin, Gamma-GT, glucose, LDH, serum phosphorus, potassium, sodium, total bilirubin, total protein, uric acid, total cholesterol, LDL, HDL, triglycerides, and HbA1c (Screening only). Hematology panel included: WBC, RBC, Hb, Hct, MCV, MCH, MCHC, Platelets, RDW.

Female subjects in this study who were not post-menopausal or sterilized were required to be using of the following methods of birth control: total abstinence defined as sexual inactivity which is consistent with the preferred and usual lifestyle of the subject, periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal were not acceptable, male partner sterilization prior to the female subject's entry into the study; and this male is the sole partner for the subject, double barrier method defined as condom and occlusive cap (diaphragm or cervical/vault caps) plus spermicidal agent (foam/gel/film/cream/suppository) combined with pharmaceutical contraception listed below:

- Oral contraception, either combined or progestogen alone
- Injectable progestogen
- Implants of levonorgestrel
- Estrogenic vaginal ring
- Percutaneous contraceptive patches

- Intrauterine device (IUD) or intrauterine system (IUS) that meets the <1% failure rate as stated on the product label

Any subject who becomes pregnant during the study was not eligible to continue in the study and completed end of study procedures at that time. Male subjects and their partners were expected to use appropriate birth control methods or abstain from sexual intercourse. Male subjects agreed to inform the Investigator immediately if their partner becomes pregnant during the course of the study monitoring period.

Complete pregnancy information, including the outcome of the pregnancy, was collected in the source documents on any female subject or partner of a male subject (if she was willing) who became pregnant during this study monitoring period. In the absence of complications, follow-up will be no longer than 6 to 8 weeks following the delivery date. Any premature terminations, whether elective, therapeutic, or spontaneous, were reported. While pregnancy itself was not considered to be an adverse effect, any pregnancy complications, including a spontaneous termination or elective termination for medical reasons, should be reported as an adverse effect. A spontaneous abortion was considered to be an SAE. Any SAE occurring as a result of a post-study pregnancy and considered reasonably related to the investigational product by the Investigator was reported to the Sponsor.

As defined by the International Conference on Harmonisation (ICH), an AE was any untoward medical occurrence in a patient or clinical investigation subject administered an investigational product, whether or not the event was considered related to the investigational product. An AE was therefore any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of the investigational product and was collected starting when IP was administered. Examples of an AE included conditions newly detected or diagnosed after investigational product administration, including conditions that may have been present but undetected prior to the start of the study, conditions known to have been present prior to the start of the study which worsen after the administration of the investigational product, signs, symptoms, or the clinical sequelae of a suspected drug interaction, and signs, symptoms, or the clinical sequelae of a suspected overdose of either investigational product or a concurrent medication (overdose per se was not reported as an AE). Examples of issues not considered an AE included: medical or surgical procedures (e.g., endoscopy, appendectomy); a condition that leads to a procedure is an AE if it qualifies according to the definitions above, situations where an untoward medical occurrence has not occur (e.g., social, observational, diagnostic, or convenience admission to a hospital), fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not represent a clinically significant exacerbation, and abnormal laboratory or test findings that were not assessed by the PI or a sub-Investigator with appropriate medical training as clinically significant. A summary of adverse events is detailed in Table 9.

Table 9. Summary of Adverse Events

	Placebo	40 mg	80 mg
Overall:			
Number (%) of Subjects with at least one AE	9 (50.0%)	12(63.2%)	14(77.8%)
Number (%) of Subjects with at least one TEAE	9 (50.0%)	12(63.2%)	14(77.8%)
Number of AEs	15	32	40
Number of TEAEs	15	32	39
Number of SAEs	2	0	0
Number of TEAEs by Severity			
Mild	12	26	31
Moderate	3	6	8
Number of TEAEs by Relationship to Study Drug			
Not Related	12	10	9
Possibly Related	3	22	24
Probably Related	0	0	6
Six-Day Dose-Escalation Period Only:			
Number (%) of Subjects with at least on TEAE	2(11.1%)	3(15.8%)	7(38.9%)
Number of TEAEs	2	3	11

Statistical Methods

Demographic data, clinical chemistry, CBCs, biomarkers, and adverse events were summarized in tabular form by dose level and overall. Descriptive statistics were used to summarize the demographic and clinical data, such as ECGs and vitals. Laboratory values above and below the normal limit were flagged, and adverse events presented by SOC, severity and relationship to study treatment.

The primary efficacy analysis was compare to the change from baseline and Day 70 (Visit 6) of FMD between the pooled-drug and placebo treated groups following 10 weeks of treatment using an unpaired t-test. In case of a substantially skewed distribution within the comparison groups, a nonparametric two sample Wilcoxon signed-rank test was used. For dichotomized efficacy endpoints the null hypothesis *H0: rc=rp* versus *H1: rc≠rp* was tested, where *rc* is the proportion of subjects with improved results in BID cohort and *rp* was the proportion of subjects with improved results in the placebo cohort. The differences between groups were tested with chi square test or Fisher exact test. Secondary analyses employed repeated measures ANOVA based on Generalized Estimating Equations to incorporate time, group and interaction. Other confounding variables were included in the baseline covariates framework. Analysis of the secondary endpoints such as 6-minute walk and QoL questionnaires was performed as described for the primary efficacy analysis. All statistical decisions were made before un-blinding.

Additionally, plasma levels of sodium nitrite were tabulated and plotted as a log-dose response curve. Functional parameters were tabulated by dose and overall. Summary statistics were computed and log-dose response curves were prepared for each parameter as appropriate.

A statistical analysis plan was developed to detail the statistical approach, particular contrasts of interest, and additionally include any exploratory or unadjusted analysis of the primary efficacy endpoints by treatment group.

With a total sample size of 50 subjects (n=34 sodium nitrite; n= 16 placebo), the study had ~82% power to detect a difference in the means of sodium nitrite (pooled-groups) compared with placebo for the efficacy endpoint of FMD at the 0.050 two-sided level of significance. Specifically, with approximately 34 subjects in the pooled sodium nitrite group and 16 subjects in the placebo group, the study had 82.19% power to detect a 1.4% difference in FMD responses between sodium nitrite treated subjects compared

with placebo treated subjects after 10 weeks of treatment with 1.6% standard deviations (SD). The sample size was thus empirically determined to be sufficient for this early-stage, clinical study. Accounting for drop-outs, a sample size of up to 60 subjects (20 subjects/group) was sufficient to account for drop-outs as needed to achieve a final sample size of approximately 17 subjects per group. Last observation carried forward (LOCF) was applied to missing data.

Example 2: Clinial Studies of Pain Assessment in Specific Patient Populations

Study Rationale and Summary

As described in Example 1, sodium nitrite was investigated as a new therapy for improving function in subjects with PAD. During the assessment of efficacy, quality of life (QoL) questionnaires were conducted which showed that the group of subjects taking 40 mg of sodium nitrite showed significant improvement in pain. The overall goal of this dose-ranging study is therefore to evaluate the improvement in different areas of pain associated with administration of multiple doses of oral sodium nitrite to particular patient populations (e.g., subjects with PAD, diabetic peripheral neuropathy, or subjects with any of the neuropathic pain described herein).

The primary objective of this clinical study is to assess the efficacy of sodium nitrite in reduction of neuropathic pain and the safety and tolerability of multiple doses of twice daily 40 mg and 80 mg sodium nitrite compared with placebo over a defined treatment period. In this study, multiple assessments of biological pain activity and symptoms associated with pain are made during standardized tests. The assessments include: nerve conduction studies, neurosensory testing, pain inventory, functional status assays, and pain surveys.

The subject endpoints include: brief pain inventories, pain diaries, depression and functional status surveys, and neuropathic pain surveys collected from the subjects participating in the studies. The objective endpoints include: nerve conduction studies, physical exams, two-point discrimination test, neurosensory testing via physical exams. Each subject participating in the study will receive pulse oximetry through the study monthly to demonstrate lack of methemoglobinemia.

The trial type is a randomized, double-blind, placebo-controlled, dose ranging, parallel design multiple dosing study targeted on particular patient populations (e.g., subjects with PAD, diabetic peripheral neuropathy, or subjects with any of the neuropathic pain described herein). The trial may have three arms with approximately ten subjects in each arm. Subjects are assigned to either the placebo or sodium nitrite treatment group in accordance with the randomization schedule generated prior to the start of the study. Subjects are randomized into the study to receive one of the treatment regimens of either placebo, 40 mg BID or 80 mg BID of the investigational product of Example 1 is used in these clinical studies.

Other Embodiments

While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure that come within known or customary practice within the art to which the invention pertains and may be applied to the essential features hereinbefore set forth.

All references, patents, patent application publications, and patent applications cited herein are hereby incorporated by reference to the same extent as if each of these references, patents, patent application publications, and patent applications were separately incorporated by reference herein.

What is claimed is:

CLAIMS

1. A method of treating or reducing pain, said method comprising administering to a subject in need thereof a pharmaceutical composition comprising an effective amount of inorganic nitrite or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable excipient.

2. A method of treating or reducing neuropathic pain, said method comprising administering to a subject in need thereof a pharmaceutical composition comprising an effective amount of inorganic nitrite or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable excipient.

3. A method of treating or reducing diabetic peripheral neuropathy, said method comprising administering to a subject in need thereof a pharmaceutical composition comprising an effective amount of inorganic nitrite or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable excipient.

4. The method of any one of claims 1-3, further comprising monitoring whether the subject experiences reduced pain.

5. The method of claim 4, wherein reduced pain is measured as a decrease in pain intensity, frequency, duration, and/or improvements in quality of life.

6. The method of any of claims 1-5, wherein said subject has type 1 or type 2 diabetes.

7. The method of any of claims 1-6, wherein said subject does not have a condition associated with chronic ischemia.

8. The method of any one of claims 1-7, wherein said subject has a predisposition, is diagnosed with, or has chronic pain.

9. The method of claim 8, wherein said chronic pain is associated with lower back pain, arthritis, headache, multiple sclerosis, fibromyalgia, shingles, nerve damage, or cancer.

10. The method of any one of claims 1-9, wherein said pain is selected from the group consisting of: neuropathic pain, inflammatory pain, nociceptive pain, functional pain, musculo-skeletal pain, and central nervous system pain.

11. The method of claim 10, wherein said neuropathic pain is selected from the group consisting of diabetic peripheral neuropathy, post-herpetic neuralgia, trigeminal neuralgia, phantom limb pain, carpal tunnel syndrome, sciatica, pudendal neuralgia, complex regional pain syndrome, sensory polyneuropathys, mono-neuropathies, and central pain syndrome

12. The method of claim 11, wherein said neuropathic pain is diabetic peripheral neuropathy.

13. A method of treating a mood disorder or a disorder of brain development, said method comprising administering to a subject in need thereof a pharmaceutical composition comprising an effective amount of inorganic nitrite or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable excipient.

14. The method of claim 13, wherein said mood disorder is selected from the group consisting of: major depressive disorders, depressive disorders, bipolar disorders, substance induced mood disorders, alcohol induced mood disorders, and benzodiazepine induced mood disorders.

15. The method of claim 13, wherein said disorder of brain development is selected from the group consisting of: impairment of learning and memory, autistic disorder, Rett syndrome, childhood disintegrative disorder, pervasive developmental disorder-not otherwise specified (PDD-NOS), and Asperger syndrome.

16. The method of any one of claims 1-15, wherein said pharmaceutical composition comprises from about 10 mg to about 100 mg of inorganic nitrite.

17. The method of any one of claims 1-15, wherein said pharmaceutical composition comprises from about 20 mg to about 200 mg of inorganic nitrite.

18. The method of any one of claims 1-17, wherein said inorganic nitrite is $NaNO_2$, or KNO_2.

19. The method of claim 18, wherein said inorganic nitrite is $NaNO_2$.

20. The method of any one of claims 1-19, wherein the pharmaceutical composition is formulated for topical, enteral, or parenteral administration.

21. The method of any one of claims 1-19, wherein said pharmaceutical composition is formulated as a solid dosage form for oral administration.

22. The method of claim 21, wherein said pharmaceutical composition is a tablet or capsule.

23. The method of claim 21 or 22, wherein said pharmaceutical composition comprises a pharmaceutically acceptable excipient for delayed release of the inorganic nitrite, or pharmaceutically acceptable salt thereof, such that, when orally administered to a subject, the inorganic nitrite or pharmaceutically acceptable salt thereof is not substantially released in the stomach of said subject.

24. The method of any one of claims 1-23, wherein the pharmaceutical composition is administered one or more times a day.

25. The method of any one of claims 1-24, wherein the pharmaceutical composition is administered for at least two to twenty days.

26. The method of claim 25, wherein the administration occurs for at least two days, at least three days, at least four days, at least five days, at least six days, at least seven days, at least ten days, or at least fifteen days.

27. The method of any one of claims 1-26, wherein the dose is about 0.5 to about 2000 µg/kg; about 0.5 to about 1000 µg/kg; about 0.5 µg/kg to about 500 µg/kg; about 0.5 µg/kg to about 250 µg/kg; about 0.5 µg/kg to about 100 µg/kg; or about 0.5 µg/kg to about 50 µg/kg.

28. The method of claim 27, wherein the dose is about 165 µg/kg; about 16.5 µg/kg; or about 8.25 µg/kg.

29. The method of any one of claims 1-28, wherein said pharmaceutical composition is administered with a second agent.

30. The method of claim 29, wherein said second agent is selected from the group consisting of: a non-steroidal anti-inflammatory drug (NSAID), a corticosteroid, acetaminophen, an opioid, a muscle relaxant, an anti-anxiety drug, an anti-depressant, an anti-convulsant drug, an antipsychotic, an antiepileptic drug, a selective serotonin reuptake inhibitor (SSRI), a norepinephrin inhibitor, and a mood stabilizer.

Figure 1A

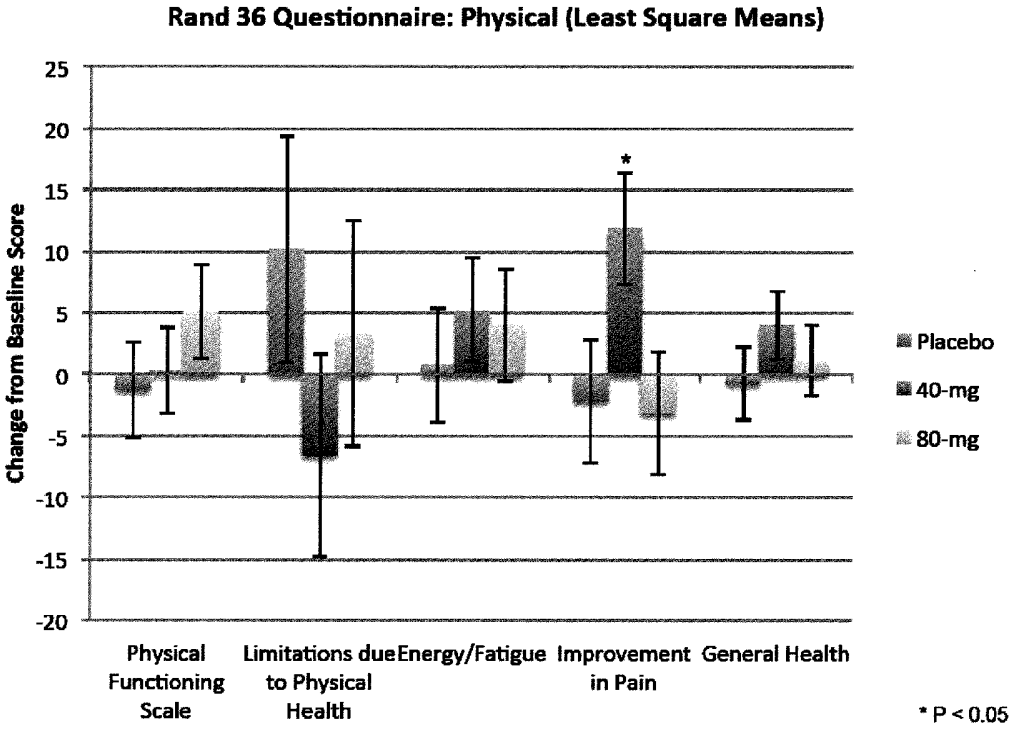


Figure 1B

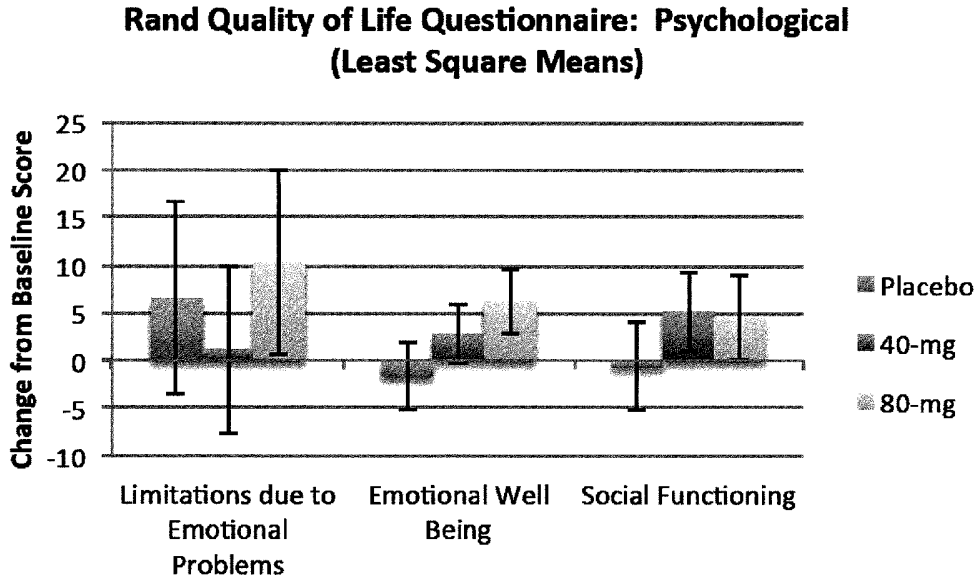


Figure 2A

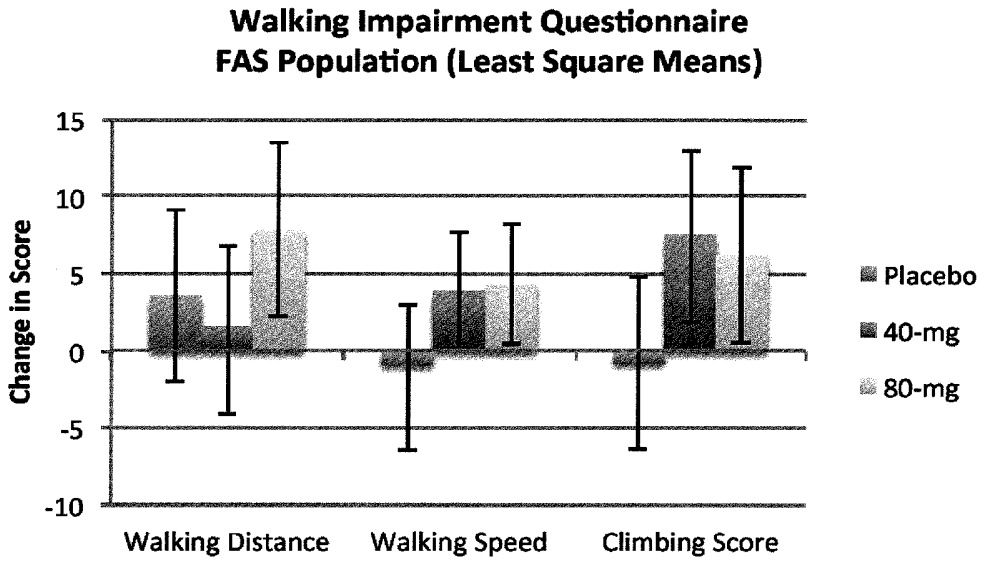
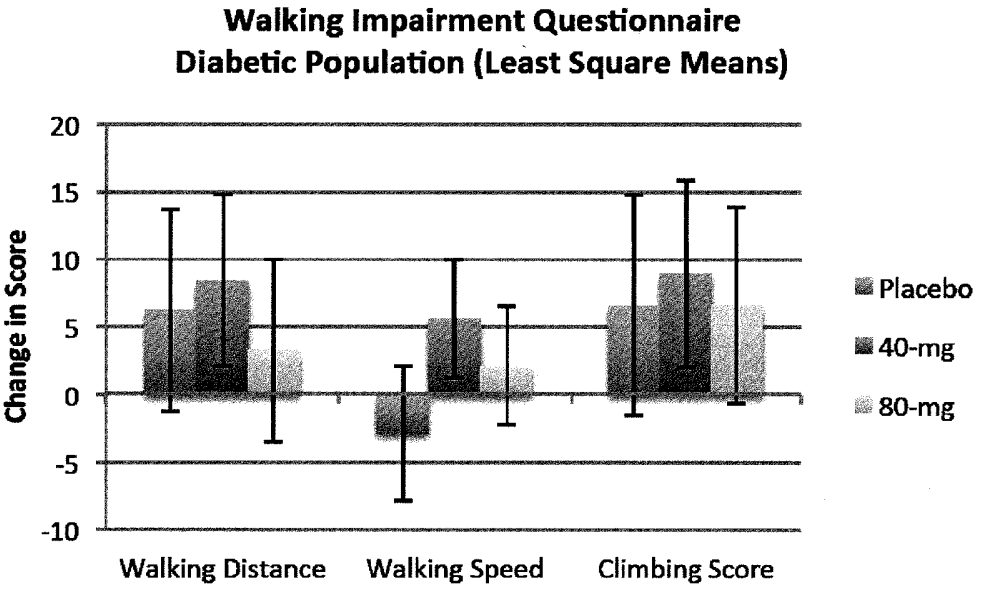


Figure 2B



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Published:

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(54) **Title**: PHARMACEUTICAL FORMULATIONS OF NITRITE AND USES THEREOF

(57) **Abstract**: The present invention relates to pharmaceutical compositions of nitrites such as inorganic nitrites, or any pharmaceutically acceptable salts, solvates, or prodrugs thereof, and the medical use of these compositions. The pharmaceutical compositions, which can be formulated for oral administration, can provide immediate release or extended release of the nitrite ion (NO_2^-)The pharmaceutical compositions of the invention are useful, for example, for modulating brain function, in particular improving mood and/or psychological state, in the treatment of disorders of brain development, and in the treatment and/or reduction of pain.

INTERNATIONAL SEARCH REPORT PCT/US2014/017432 11.08.2014

International application No.
PCT/US 14/17432

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 33/00 (2014.01)
USPC - 424/682; 424/718
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)
USPC - 424/682; 424/718
IPC(8) - A61K 33/00 (2014.01)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC - 424/682; 424/718; 347/100; 106/31.43; 106/31.58; 106/31.75
IPC(8) - D06P 5/30 (2014.01) (keyword delimited)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Patbase; Google Scholar; Freepatentesonline
Search terms used: inorganic potassium sodium lithium nitrite NO2 diabetes neuropathy pain treament monitor intensity frequency duration quality

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 2012/0237617 A1 (Kevil) 20 September 2012 (20.09.2012), para [0011], [0075]	1-4 ----- 5
Y	US 5,692,500 A (Gaston-Johansson) 02 December 1997 (02.12.1997), col 1, ln 54-59; col 3, ln 5-11; col 3, ln 61-67	5
A	US 2011/0086069 A1 (Kevil et al.) 14 April 2011 (14.04.2011), entire document	1-5

☐ Further documents are listed in the continuation of Box C. ☐

* 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
18 July 2014 (18.07.2014)	11 AUG 2014
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

International application No.
PCT/US 14/17432

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.: 6-12, 16-30
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:
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I: Claims 1-5, drawn to a method of treating or reducing pain, said method comprising administering to a subject in need thereof a pharmaceutical composition comprising an effective amount of inorganic nitrite or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable excipient.

Group II: Claims 13-15, drawn to a method of treating a mood disorder or a disorder of brain development, said method comprising administering to a subject in need thereof a pharmaceutical composition comprising an effective amount of inorganic nitrite or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable excipient.

-- see extra 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 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

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.

International application No.
PCT/US 14/17432

Continuation of Box No. III -- Observations where unity of invention is lacking

The inventions listed as Groups I though II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features
Group I includes the special technical feature of a method of treating or reducing pain, not included in the other groups.

Group II includes the special technical feature of a method of treating a mood disorder or a disorder of brain development, not included in the other groups.

Common Technical Features:
The only technical feature shared by Groups I-II that would otherwise unify the groups, is a pharmaceutical composition comprising an effective amount of inorganic nitrite or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable excipient. However, these shared technical features do not represent a contribution over prior art, because the shared technical features are disclosed by US 2012/0237617 A1 to Kevil et al. (hereinafter 'Kevil') 20 September 2012 (20.09.2012).

Kevil discloses a pharmaceutical composition comprising an effective amount of inorganic nitrite or a pharmaceutically acceptable salt thereof (para [0011]; abstract), and a pharmaceutically acceptable excipient (para [0075]).

As the shared feature was known in the art at the time of the invention, it cannot be considered a special technical features that would otherwise unify the groups..

Therefore, Groups I-II lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.

Note: Claims 6-12 and 16-30 are determined to be unsearchable because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a) and are, therefore, not included in any claim group.

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权利要求书2页 说明书32页 附图2页

(54)发明名称

亚硝酸盐的药物制剂及其用途

(57)摘要

本发明涉及亚硝酸盐(例如无机亚硝酸盐)或其任何药学上可接受的盐、溶剂合物或前药的药物组合物和这些组合物的医药用途。可配制用于口服给药的药物组合物可提供立即释放或延缓释放的亚硝酸根离子(NO_2^-)。本发明的药物组合物可用于例如调理脑功能,特别是改善心境和/或心理状态、治疗脑发育障碍和治疗和/或减轻疼痛。

CN 105358160 A

1.一种治疗或减轻疼痛的方法，所述方法包括给予有需要的受试者包含有效量的无机亚硝酸盐或其药学上可接受的盐和药学上可接受的赋形剂的药物组合物。

2.一种治疗或减轻神经性疼痛的方法，所述方法包括给予有需要的受试者包含有效量的无机亚硝酸盐或其药学上可接受的盐和药学上可接受的赋形剂的药物组合物。

3.一种治疗或减轻糖尿病性周围神经病的方法，所述方法包括给予有需要的受试者包含有效量的无机亚硝酸盐或其药学上可接受的盐和药学上可接受的赋形剂的药物组合物。

4.权利要求1-3中任一项的方法，所述方法还包括监测受试者是否感到疼痛减轻。

5.权利要求4的方法，其中疼痛减轻以疼痛强度减弱、频率降低、持续时间缩短和/或生命质量改善来衡量。

6.权利要求1-5中任一项的方法，其中所述受试者患有1型或2型糖尿病。

7.权利要求1-6中任一项的方法，其中所述受试者没有与慢性缺血有关的病况。

8.权利要求1-7中任一项的方法，其中所述受试者具有慢性疼痛易感体质、诊断为慢性疼痛或患有慢性疼痛。

9.权利要求8的方法，其中所述慢性疼痛与下背疼痛、关节炎、头痛、多发性硬化、纤维肌痛、带状疱疹、神经损伤或癌症有关。

10.权利要求1-9中任一项的方法，其中所述疼痛选自：神经性疼痛、炎性疼痛、伤害性疼痛、功能性疼痛、肌肉骨骼疼痛和中枢神经系统疼痛。

11.权利要求10的方法，其中所述神经性疼痛选自糖尿病性周围神经病、带状疱疹后神经痛、三叉神经痛、幻肢痛、腕管综合征、坐骨神经痛、阴部神经痛、复杂性区域疼痛综合征、感觉性多神经病、单一神经病变和中枢疼痛综合征。

12.权利要求11的方法，其中所述神经性疼痛是糖尿病性周围神经病。

13.一种治疗心境障碍或脑发育障碍的方法，所述方法包括给予有需要的受试者包含有效量的无机亚硝酸盐或其药学上可接受的盐和药学上可接受的赋形剂的药物组合物。

14. 权利要求13的方法，其中所述心境障碍选自：重度抑郁症、抑郁症、双相性精神障碍、物质诱发的心境障碍、酒精诱发的心境障碍和苯并二氮杂䓬诱发的心境障碍。

15.权利要求13的方法，其中所述脑发育障碍选自：学习和记忆减退、孤独症、雷特综合征、儿童期崩解症、待分类的广泛性发育障碍(PDD-NOS)和阿斯珀格综合征。

16. 权利要求1-15中任一项的方法，其中所述药物组合物包含约10 mg-约100 mg的无机亚硝酸盐。

17. 权利要求1-15中任一项的方法，其中所述药物组合物包含约20 mg-约200 mg的无机亚硝酸盐。

18.权利要求1-17中任一项的方法，其中所述无机亚硝酸盐是$NaNO_2$或KNO_2。

19.权利要求18的方法，其中所述无机亚硝酸盐是$NaNO_2$。

20.权利要求1-19中任一项的方法，其中配制药物组合物用于局部、肠内或胃肠外给药。

21.权利要求1-19中任一项的方法，其中将所述药物组合物配制成固体剂型用于口服给药。

22.权利要求21的方法，其中所述药物组合物是片剂或胶囊剂。

23.权利要求21或22的方法，其中所述药物组合物包含用于无机亚硝酸盐或其药学上

可接受的盐延缓释放的药学上可接受的赋形剂，使得当口服给予受试者时，所述无机亚硝酸盐或其药学上可接受的盐基本不在所述受试者的胃中释放。

24. 权利要求 1-23 中任一项的方法，其中一天一次或多次给予药物组合物。

25. 权利要求 1-24 中任一项的方法，其中给予药物组合物持续至少 2-20 天。

26. 权利要求 25 的方法，其中所述给药发生至少 2 天、至少 3 天、至少 4 天、至少 5 天、至少 6 天、至少 7 天、至少 10 天或至少 15 天。

27. 权利要求 1-26 中任一项的方法，其中所述剂量为约 0.5- 约 2000 μg/kg、约 0.5- 约 1000 μg/kg、约 0.5 μg/kg- 约 500 μg/kg、约 0.5 μg/kg- 约 250 μg/kg、约 0.5 μg/kg- 约 100 μg/kg 或约 0.5 μg/kg- 约 50 μg/kg。

28. 权利要求 27 的方法，其中所述剂量为约 165 μg/kg、约 16.5 μg/kg 或约 8.25 μg/kg。

29. 权利要求 1-28 中任一项的方法，其中所述药物组合物与第二药剂一起给予。

30. 权利要求 29 的方法，其中所述第二药剂选自：非甾体抗炎药 (NSAID)、皮质类固醇、对乙酰氨基酚、阿片样物质、肌肉松弛药、抗焦虑药、抗抑郁药、抗惊厥药、抗精神病药、抗癫痫药、选择性 5- 羟色胺再摄取抑制剂 (SSRI)、去甲肾上腺素抑制剂和心境稳定剂。

亚硝酸盐的药物制剂及其用途

[0001] 相关申请的交叉引用

本申请要求2013年2月20日提交的美国临时申请号61/767,017的优先权的权益，其通过引用结合到本文中。

[0002] 发明背景

本发明涉及亚硝酸盐的药物组合物和这些组合物的医药用途。

[0003] 一氧化氮(NO)用作神经细胞间的神经递质，在氧化还原信号转导中具有广泛作用。与只将信息从突触前神经元传递到突触后神经元的大多数其它神经递质不一样，小的不带电荷的脂溶性一氧化氮分子可到处扩散，并容易进入细胞中。因此，它可作用于一些附近的神经元，甚至作用于不被突触连接的神经元。同时，NO的短的半寿期意味着所述作用将局限于有限区域，而无需酶促分解或细胞重摄取。NO还与其它自由基、脂质和蛋白质有高度反应性。NO-cGMP级联通过保持长时程增强(LTP)参与学习和记忆。因此，NO是脑中许多过程的重要调节物和介导物，而且NO水平的平衡在维持健康信号转导和脑发育和/或保持心理状态的平衡中至关重要。

[0004] NO的作用还涉及疼痛，然而尚不清楚是NO的抑制还是NO的产生在疼痛的治疗中有益。在一些研究中，提出了若干疼痛相关途径获益于NO的产生。具体地说，在NO存在下是规范化的血流途径，可有助于减轻缺血性疼痛；神经传递途径，其降低滑膜、骨和软组织中神经的兴奋；阿片样物质受体途径，其可刺激机体的正常疼痛减轻途径；和抗炎途径。其它研究提出NO的抑制有益于疼痛治疗。在这些研究中，NO被认为参与激活环加氧酶1(COX-1)并在炎症反应中调节环加氧酶2 (COX-2)表达以增加前列腺素释放，从而诱导周围痛觉过敏和炎症。通过激活N-甲基-D-天冬氨酸(NMDA)受体所产生的NO涉及突触可塑性，这些机制的许多参与中枢敏化，慢性疼痛中的一个普遍问题。某些研究还表明，NO介导镇痛化合物例如阿片样物质和非甾体抗炎药的周围和中枢抗伤害感受作用。

[0005] 因此，持续需要了解NO的生物功能并研究提供NO源用于维持正常脑功能和用于治疗和/或减轻疼痛的治疗策略。

[0006] 发明概述

总的来说，第一方面，本发明的特征在于治疗或减轻疼痛的方法，所述方法包括将包括有效量的无机亚硝酸盐或其药学上可接受的盐和药学上可接受的赋形剂的药物组合物给予有需要的受试者。在一个具体的实施方案中，本发明的特征在于治疗或减轻神经性疼痛的方法，所述方法包括给予上述药物组合物。在一个优选的实施方案中，本发明的特征在于治疗或减轻糖尿病性周围神经病的方法，所述方法包括给予受试者上述药物组合物。

[0007] 第二方面，本发明还包括监测受试者是否感到疼痛减轻，其中疼痛减轻以疼痛强度减弱、频率降低、持续时间缩短和/或生命质量改善来衡量。

[0008] 在一些实施方案中，受试者患有1型或2型糖尿病。在其它实施方案中，受试者没有与慢性缺血有关的病况。在又一个实施方案中，受试者具有慢性疼痛易感体质、诊断为慢性疼痛或患有慢性疼痛。

[0009] 在前述方面的任一个中，慢性疼痛与下背疼痛、关节炎、头痛、多发性硬化、纤

维肌痛、带状疱疹、神经损伤或癌症有关。在一些情况下，疼痛是神经性疼痛、炎性疼痛、伤害性疼痛、功能性疼痛、肌肉骨骼疼痛或中枢神经系统疼痛。在某些实施方案中，神经性疼痛是糖尿病性周围神经病、带状疱疹后神经痛、三叉神经痛、幻肢痛、腕管综合征、坐骨神经痛、阴部神经痛、复杂性区域疼痛综合征、感觉性多神经病、单一神经病变(mono-neuropathies)或中枢疼痛综合征。在一个优选的实施方案中，疼痛是糖尿病性周围神经病。

[0010] 第三方面，本发明的特征在于治疗心境障碍或脑发育障碍的方法，所述方法包括将包括有效量的无机亚硝酸盐或其药学上可接受的盐和药学上可接受的赋形剂的药物组合物给予有需要的受试者。

[0011] 在一些实施方案中，心境障碍选自：重度抑郁症、抑郁症、双相性精神障碍、物质诱发的心境障碍、酒精诱发的心境障碍和苯并二氮杂䓬诱发的心境障碍。在其它实施方案中，脑发育障碍选自：学习和记忆减退、孤独症、雷特综合征(Rett syndrome)、儿童期崩解症、待分类的广泛性发育障碍(PDD-NOS)和阿斯珀格综合征(Asperger syndrome)。

[0012] 在前述方面的任一个中，药物组合物包括约10 mg-约100 mg或约20 mg-约200 mg的无机亚硝酸盐，其中无机亚硝酸盐是$NaNO_2$或KNO_2。在优选的实施方案中，无机亚硝酸盐是$NaNO_2$。在一些方面，药物组合物与第二药剂一起给予，其中第二药剂选自：非甾体抗炎药(NTHE)、皮质类固醇、对乙酰氨基酚、阿片样物质、肌肉松弛药、抗焦虑药、抗抑郁药、抗惊厥药、抗精神病药、抗癫痫药、选择性5-羟色胺再摄取抑制剂(SSRI)、去甲肾上腺素抑制剂和心境稳定剂。

[0013] 在一个实施方案中，一天一次或多次给予药物组合物。在第二个实施方案中，给予药物组合物持续至少2-20天。在第三个实施方案中，给药发生持续至少2天、至少3天、至少4天、至少5天、至少6天、至少7天、至少10天或至少15天。在第四个实施方案中，剂量为约0.5-约2000 μg/kg、约0.5-约1000 μg/kg、约0.5 μg/kg-约500 μg/kg、约0.5 μg/kg-约250 μg/kg、约0.5 μg/kg-约100 μg/kg或约0.5 μg/kg-约50 μg/kg。在一个优选的实施方案中，剂量为约165 μg/kg、约16.5 μg/kg或约8.25 μg/kg。

[0014] 在某些实施方案中，配制药物组合物用于局部、肠内或胃肠外给药。在其它实施方案中，将药物组合物配制成固体剂型用于口服给药。在优选的实施方案中，药物组合物为片剂或胶囊剂。

[0015] 在前述实施方案的任一个中，药物组合物包括用于延时释放无机亚硝酸盐或其药学上可接受的盐的药学上可接受的赋形剂，使得当口服给予受试者时，无机亚硝酸盐或其药学上可接受的盐基本不在受试者胃中释放。

[0016] 所谓“慢性疼痛”意指持续超过3-6个月的疼痛或延续超过预计愈合期的疼痛。慢性疼痛可源于最初的创伤/损伤或感染，或可以是与以下疼痛有关持续性疼痛原因：神经性疼痛(例如糖尿病性周围神经病、带状疱疹后神经痛、三叉神经痛、幻肢痛、腕管综合征、坐骨神经痛、阴部神经痛、复杂性区域疼痛综合征、感觉性多神经病、单一神经病变或中枢疼痛综合征)、头痛、关节痛、背痛、鼻窦痛、肌肉痛、神经痛和累及身体特殊部位(例如肩、骨盆和颈)的疼痛。慢性疼痛还可与下背疼痛、关节炎、头痛、多发性硬化、纤维肌痛、带状疱疹、神经损伤或癌症有关。

[0017] 本文所用术语“延缓释放”是指药物制剂，例如口服给予的制剂，其基本上完整地通过胃，并在小肠和/或大肠（例如结肠）中溶解。在一些实施方案中，活性剂（例如本文所述的亚硝酸盐）的延缓释放因使用口服药物（例如口服剂型）的肠溶包衣引起。

[0018] 本文所用术语药剂的“有效量”是足以发挥有益或所需的结果（例如临床结果）的量，因此，“有效量”取决于施用药剂的情况。

[0019] 可互换使用的术语“延长释放”或“持续释放”是指与同一药物的立即释放制剂相比，在延长的一段时间（例如6-12小时或更长时间）内提供逐步释放药物的药物制剂。优选（但不一定）在延长的一段时间内导致在治疗水平内且落入介于以下峰值血浆浓度范围内的基本不变的药物血液水平：例如0.05-10 μM、0.1-10 μM、0.1-5.0 μM或0.1-1 μM。

[0020] 本文所用术语“配制用于肠内释放”和“肠内制剂”是指能够在胃的高酸性（低pH）环境中提供免于溶解的保护的用于口服给予的药物组合物，例如口服剂型。肠内制剂可通过例如将药物组合物掺入抵抗在胃液中溶解的聚合物中来获得。在一些实施方案中，聚合物具有在约5.0-7.0范围中溶解的最佳pH（“pH敏感聚合物”）。示例性的聚合物包括以商品名Eudragit®（例如Eudragit® L100、Eudragit® S100、Eudragit® L-30D、Eudragit® FS 30D和Eudragit® L100-55）著称的甲基丙烯酸共聚物、醋酸邻苯二甲酸纤维素、醋酸偏苯三酸纤维素、聚醋酸邻苯二甲酸乙烯（例如Coateric®）、羟乙基纤维素邻苯二甲酸酯、羟丙基甲基纤维素邻苯二甲酸酯或虫胶或其水分散体。这些聚合物的水分散体包括醋酸邻苯二甲酸纤维素（Aquateric®）或虫胶（例如MarCoat 125和125N）的分散体。与立即释放制剂相比，肠内制剂降低所给予的剂量释放到胃中的百分比达至少50%、60%、70%、80%、90%、95%或甚至98%。当所述聚合物包覆片剂或胶囊剂时，这种包衣亦称为“肠溶包衣”。

[0021] 本文所用术语“药物组合物”表示含有本文所述化合物（例如无机亚硝酸盐或其任何药学上可接受的盐、溶剂合物或前药）、用药学上可接受的赋形剂配制的、且通常经政府管理机构批准制造或销售的组合物作为用于治疗哺乳动物的疾病的治疗方案的一部分。可以单位剂型配制药物组合物例如用于口服给药（例如片剂、胶囊剂、囊片剂、软胶囊剂或糖浆剂）；用于局部给药（例如作为乳膏剂、凝胶剂、洗剂或软膏剂）；用于静脉内给药（例如作为不含微粒栓子的无菌溶液和在适于静脉内使用的溶剂系统中）；或呈本文所述的任何其它制剂。

[0022] 本文所用“药学上可接受的赋形剂”是指本文所述化合物以外的且具有在患者中无毒和无炎性的性质的任何成分（例如能够悬浮或溶解活性化合物的溶媒）。赋形剂可包括例如：抗粘着剂、抗氧化剂、粘合剂、包衣材料、压片助剂、崩解剂、染料（着色剂）、软化剂、乳化剂、填充剂（稀释剂）、成膜剂或包衣材料、矫味剂、香料、助流剂（促流动剂）、润滑剂、防腐剂、印刷油墨、吸着剂、悬浮剂或分散剂、甜味剂或化合水。示例性的赋形剂包括但不限于：丁基羟基甲苯(BHT)、碳酸钙、磷酸钙（二碱式）、硬脂酸钙、交联羧甲纤维素、交联聚乙烯吡咯烷酮、柠檬酸、交联聚维酮、半胱氨酸、乙基纤维素、明胶、羟丙基纤维素、羟丙基甲基纤维素、乳糖、硬脂酸镁、麦芽糖醇、麦芽糖、甘露糖醇、甲硫氨酸、甲基纤维素、对羟基苯甲酸甲酯、微晶纤维素、聚乙二醇、聚乙烯吡咯烷酮、聚维酮、预胶化淀粉、对羟基苯甲酸丙酯、棕榈酸视黄酯、虫胶、二氧化硅、羧甲基纤维素钠、柠檬酸钠、羟基乙酸淀粉钠、山梨糖醇、淀粉（玉米）、硬脂酸、硬脂酸、蔗糖、滑石、二氧化钛、维生素A、维生素E、维生素C和木糖醇。

[0023] 本文所用术语“药学上可接受的前药”表示在合理医学判断范围内适用于与人和动物的组织接触而又没有过度毒性、刺激性、变态反应等、与合理的受益 / 风险比相称、对其预期用途是有效的及可能时是本发明化合物的两性离子形式的本发明化合物的那些前药。

[0024] 本文所用术语“药学上可接受的盐”表示在合理医学判断范围内适用于与人和动物的组织接触而又无过度毒性、刺激性、变态反应等且与合理受益 / 风险比相称的那些盐。药学上可接受的盐是本领域众所周知的。例如药学上可接受的盐描述于 :Berge 等 , *J. Pharmaceutical Sciences* 66:1-19, 1977 和 *Pharmaceutical Salts: Properties, Selection, and Use*, (编辑 P.H. Stahl 和 C.G. Wermuth), Wiley-VCH, 2008。盐可在本发明化合物的最后的分离和纯化期间原位制备或单独通过使游离碱基团与合适的有机或无机酸反应来制备。代表性的酸加成盐包括乙酸盐、己二酸盐、藻酸盐、抗坏血酸盐、天冬氨酸盐、苯磺酸盐、苯甲酸盐、硫酸氢盐、硼酸盐、丁酸盐、樟脑酸盐、樟脑磺酸盐、柠檬酸盐、环戊烷丙酸盐、二葡糖酸盐、十二烷基硫酸盐、乙磺酸盐、富马酸盐、葡庚糖酸盐、甘油磷酸盐、半硫酸盐、庚糖酸盐、己酸盐、氢溴酸盐、盐酸盐、氢碘酸盐、2- 羟基 - 乙磺酸盐、乳糖酸盐、乳酸盐、月桂酸盐、月桂基硫酸盐、苹果酸盐、马来酸盐、丙二酸盐、甲磺酸盐、2- 萘磺酸盐、烟酸盐、硝酸盐、油酸盐、草酸盐、棕榈酸盐、双羟萘酸盐、果胶酸盐、过硫酸盐、3- 苯丙酸盐、磷酸盐、苦味酸盐、新戊酸盐、丙酸盐、硬脂酸盐、琥珀酸盐、硫酸盐、酒石酸盐、硫氰酸盐、甲苯磺酸盐、十一烷酸盐、戊酸盐等。代表性碱金属盐或碱土金属盐包括钠、锂、钾、钙、镁等,以及无毒铵、季铵和胺阳离子,包括但不限于铵、四甲基铵、四乙基铵、甲胺、二甲胺、三甲胺、三乙胺、乙胺等。

[0025] 本文所用术语“药学上可接受的溶剂合物”或“溶剂合物”意指其中合适溶剂的分子掺入晶格中的本发明的化合物。合适的溶剂在所给予的剂量下是生理上可耐受的。例如溶剂合物可通过从包括有机溶剂、水或其混合物的溶液结晶、重结晶或沉淀来制备。合适的溶剂的实例为乙醇、水 (例如一水合物、二水合物和三水合物)、*N*- 甲基吡咯烷酮 (NMP)、二甲亚砜 (DMSO)、*N,N'*- 二甲基甲酰胺 (DMF)、*N,N'*- 二甲基乙酰胺 (DMAC)、1,3- 二甲基 -2- 咪唑烷酮 (DMEU)、1,3- 二甲基 -3,4,5,6- 四氢 -2-(1H)- 嘧啶酮 (DMPU)、乙腈 (ACN)、丙二醇、乙酸乙酯、苯甲醇、2- 吡咯烷酮、苯甲酸苄酯等。当水是溶剂时,溶剂合物称为“水合物”。

[0026] 本文所用术语“减轻”是指缓解本文所述疾病、病症或病况 (例如疼痛) 的一个或多个症状或病况的治疗。例如在先于易患或之前诊断患有疾病和 / 或病况的受试者的疾病、病症或病况发作的事件之后 (“暴露后预防”) 开始治疗。包括给予本发明的化合物或其药物组合物的治疗可以是急救的、短期的或长期的。给予的剂量在治疗过程中可改变。

[0027] 所谓“心境或心理状态的改善”意指受试者情绪状态的积极变化。

[0028] 所谓“调理脑功能”意指调节或调整脑中 NO 的水平,使得在脑中有发生稳态信号转导。

[0029] 所谓“易感体质或诊断为”意指预先挑选为患有与疼痛相关病况、心境障碍和 / 或心理状态失衡或脑发育障碍的受试者 (例如哺乳动物,包括人和非人) 群体。与疼痛相关病况包括但不限于 :肌肉骨骼疼痛 (在创伤、感染后);由糖尿病、感染、代谢障碍、暴露于毒素、外伤性损伤、脊髓损伤、肿瘤、压迫、炎症引起的疼痛 ;牙痛、外阴切开术疼痛、深部和内脏性疼痛 (例如心脏疼痛、膀胱疼痛或骨盆器官疼痛)、肌肉痛、眼痛、口颌面疼痛 (例如

牙痛、三叉神经痛、舌咽神经痛）、腹部疼痛、妇产科疼痛（例如痛经和阵痛）；与创伤所致神经根损伤、压迫、炎症、有毒化学物质、代谢障碍、遗传病况、感染、血管炎和自身免疫病有关的疼痛；中枢神经系统疼痛例如由脊髓或脑干损伤、脑血管意外、肿瘤、感染、脱髓鞘性疾病（包括多发性硬化）所致疼痛；腰痛、坐骨神经痛和术后疼痛。心境障碍包括但不限于：重度抑郁症、抑郁症、双相性精神障碍、物质诱发的心境障碍、酒精诱发的心境障碍和苯并二氮杂䓬诱发的心境障碍。脑发育障碍包括但不限于：学习和记忆减退、孤独症、雷特综合征、儿童期崩解症、待分类的广泛性发育障碍（PDD-NOS）和阿斯珀格综合征。

[0030] 本文所用术语“前药”表示体内快速转化为上式母体化合物的化合物。前药还包括当给予人时导致体内形成亚硝酸根离子（NO_2^-）或氧化亚氮（NO）的生物等效化合物。全面论述见 T. Higuchi 和 V. Stella, Pro-drugs as Novel Delivery Systems, A.C.S. Symposium Series 第 14 卷以及 Edward B. Roche 编辑, Bioreversible Carriers in Drug Design, American Pharmaceutical Association and Pergamon Press, 1987，其每一个通过引用结合到本文中。优选本发明的化合物的前药是药学上可接受的，例如描述于 EP 1336602A1 的前药，其通过引用结合到本文中。

[0031] 本文所用的和本领域众所周知的“治疗”是用于获得有益或所需的结果（例如临床结果）的方法。有益或所需的结果可包括但不限于不论可检出还是不可检出地，缓解或改善一个或多个症状或病况；减轻疾病、病症或病况的程度；稳定疾病、病症或病况的状态（即不恶化）；防止疾病、病症或病况的传播；延迟或减慢疾病、病症或病况的进展；改善或减轻疾病、病症或病况；和减退（不论部分还是全部）。“治疗”还可意指与如果不接受治疗的预期生存期相比，延长生存期。本文所用术语“治疗”和“医治”还可是指推迟该术语适用的疾病或病况或者所述疾病或病况的一个或多个症状的发作、延迟或逆转其进展或者减轻所述疾病或病况或者所述疾病或病况的一个或多个症状。

[0032] 术语“单位剂型”是指适宜作为用于人类受试者和其它哺乳动物的单位剂量的物理离散单位，各单位含有经计算产生所需治疗作用的预定量的活性物质以及任一种或多种合适的药用赋形剂。

[0033] 本文所用术语“血浆浓度”是指存在于接受治疗的受试者的血浆中的亚硝酸根离子的量（例如在采用下文所述测定法中的兔或在人中测量）。

[0034] 所谓“约”意指所述值的 ±20%。

[0035] 根据发明详述、附图和权利要求书，本发明的其它特征和优势将是显而易见的。

[0036] 附图简述

图 1A-1B表示 RAND 36 问卷的结果。图 1A 表示安慰剂、40 mg 和 80 mg 组的身体生命质量评价结果。图 1B 表示安慰剂、40 mg 和 80 mg 组的心理生命质量评价结果。

[0037] 图 2A-2B表示 WIQ 的结果。图 2A 表示 FAS 群中 WIQ 的结果。图 2B 表示糖尿病患者群中 WIQ 的结果。

[0038] 发明详述

本发明的特征在于治疗和 / 或减轻疼痛、特别是神经性疼痛（例如糖尿病性周围神经病、带状疱疹后神经痛、三叉神经痛、幻肢痛、腕管综合征、坐骨神经痛、阴部神经痛和中枢疼痛综合征）；调理脑功能；改善心境和 / 或心理状态；和治疗脑发育障碍（例如孤独症）的方法。

[0039] 亚硝酸盐

无机亚硝酸盐

本发明的药学上可接受的组合物包括无机亚硝酸盐（例如亚硝酸（HNO_2）的盐或酯）或其药学上可接受的盐。亚硝酸盐可包括而不限于碱金属例如钠、钾的盐；碱土金属例如钙、镁和钡的盐；和有机碱例如胺碱和无机碱的盐。本发明的化合物还包括存在于中间体或最终化合物中的原子的所有同位素。同位素包括具有相同的原子数但不同的质量数的那些原子。例如氢的同位素包括氚和氘。本文所用术语“化合物”是指任何无机亚硝酸盐或其药学上可接受的盐、溶剂合物或前药。所有化合物及其药学上可接受的盐还意指包括溶剂化（例如水化）形式。亚硝酸盐具有化学式NO_2^-，可作为离子存在于水中。亚硝酸钠具有化学式$NaNO_2$，通常溶于水中形成钠离子Na^+和亚硝酸根离子NO_2^-。还应进一步了解，本发明包括亚硝酸盐化合物的所有这类溶剂化形式（例如水合物）。示例性的亚硝酸盐化合物描述于WO 2008/105730，其通过引用结合到本文中。

[0040] 除亚硝酸钠以外，代表性无机亚硝酸盐化合物包括：亚硝酸铵（NH_4NO_2）、亚硝酸钡（$Ba(NO_2)_2$；例如无水亚硝酸钡或亚硝酸钡一水合物）、亚硝酸钙（$Ca(NO_2)_2$；例如无水亚硝酸钙或亚硝酸钙一水合物）、亚硝酸铯（$CsNO_2$）、亚硝酸钴（II）（$Co(NO_2)_2$）、亚硝酸钴（III）钾（$CoK_3(NO_2)_6$；例如亚硝酸钴（III）钾倍半水合物）、亚硝酸锂（$LiNO_2$；例如无水亚硝酸锂或亚硝酸锂一水合物）、亚硝酸镁（$MgNO_2$；例如亚硝酸镁三水合物）、亚硝酸钾（KNO_2）、亚硝酸铷（$RbNO_2$）、亚硝酸银（I）（$AgNO_2$）、亚硝酸锶（$Sr(NO_2)_2$）和亚硝酸锌（$Zn(NO_2)_2$）。

[0041] 可以化学合成领域普通技术人员已知的各种方式制备本发明的化合物。用于制备亚硝酸盐的方法是本领域众所周知的，大量的前体和亚硝酸盐是易于市购可获得的。可使一氧化氮（NO）和二氧化氮（NO_2）的混合物与相应的金属氢氧化物溶液反应，以及通过相应硝酸盐的热分解，来合成碱金属和碱土金属的亚硝酸盐。通过相应的硝酸盐还原可获得其它亚硝酸盐。

[0042] 可采用本领域已知的方法和程序，从容易获得的起始原料制备本发明的化合物。应认识到，在典型或优选的工艺条件（即反应温度、时间、反应物的摩尔比例、溶剂、压力等）给定的情况下，也可采用其它工艺条件，除非另有说明。最适反应条件可随所使用的具体的反应物或溶剂而变化，但是所述条件可由本领域普通技术人员通过常规优化程序确定。

[0043] 合适的药学上可接受的盐包括例如亚硝酸钠、亚硝酸钾或亚硝酸钙。另外其它示例性的盐参见Remington's Pharmaceutical Sciences, 第17版, Mack Publishing Company, Easton, Pa., 1985, 第1418页；Berge等, *J. Pharmaceutical Sciences* 66:1-19, 1977以及*Pharmaceutical Salts: Properties, Selection, and Use*, (编辑P.H. Stahl和C.G. Wermuth), Wiley-VCH, 2008，其每一个通过引用以其整体结合到本文中。

[0044] 药物组合物

当用作药物时，无机亚硝酸盐，例如亚硝酸（HNO_2）的盐例如$NaNO_2$或其药学上可接受的盐、溶剂合物或前药可以药物组合物的形式给予。这些组合物可按制药领域众所周知的方式制备，并可通过各种途径给予，这取决于需要局部治疗还是全身治疗，并取决于待治疗的部位。给药可以是局部、胃肠外、静脉内、动脉内、皮下、肌内、颅内、眶内、经眼、心室内、囊

内、脊柱内、脑池内、腹膜内、鼻内、气雾剂、通过栓剂或口服给药。在一个实施方案中，以美国专利申请号 12/904,791 （通过引用结合到本文中）教导的药物组合物给予无机亚硝酸盐。

[0045] 药物组合物可含有一种或多种药学上可接受的载体。在制备用于本发明的方法的药物组合物中，通常将无机亚硝酸盐、其药学上可接受的盐、溶剂合物或前药与赋形剂混合、通过赋形剂稀释或以例如胶囊、小药囊、纸或其它容器的形式包封在所述载体内。当赋形剂用作稀释剂时，它可以是固体、半固体或液体材料（例如生理盐水），其用作活性成分的溶媒、载体或介质。因此，组合物可呈片剂、散剂、锭剂、小药囊剂、扁囊剂、酏剂、混悬剂、乳剂、溶液剂、糖浆剂和软明胶和硬明胶胶囊剂的形式。如本领域所知，稀释剂的类型可根据预定的给药途径改变。所得组合物可包括其它作用剂，例如防腐剂。

[0046] 本发明的治疗剂可单独或在药学上可接受的赋形剂或载体存在下以混合物给予。根据给药方式和给药途径选择赋形剂或载体。合适的药用载体以及用于药物制剂的药用必需品描述于 *Remington: The Science and Practice of Pharmacy*, 第21版, Gennaro编辑, Lippencott Williams & Wilkins (2005)（本领域的一个众所周知的参考教科书）和 USP/NF（美国药典和国家处方集）。合适的赋形剂的实例为乳糖、葡萄糖、蔗糖、山梨糖醇、甘露糖醇、淀粉、阿拉伯树胶、磷酸钙、藻酸盐、西黄蓍胶、明胶、硅酸钙、微晶纤维素、聚乙烯吡咯烷酮、纤维素、水、糖浆和甲基纤维素。制剂另外可包括：润滑剂例如滑石、硬脂酸镁和矿物油；润湿剂；乳化剂和助悬剂；防腐剂例如苯甲酸甲酯和羟基苯甲酸丙酯；甜味剂和矫味剂。其它示例性的赋形剂描述于 *Handbook of Pharmaceutical Excipients*, 第6版，Rowe 等编辑，Pharmaceutical Press (2009)。

[0047] 药物组合物可包括硝酸盐或其前药或其它治疗剂。示例性的硝酸盐描述于 WO 2008/105730。本文提供可包括在本文所述组合物中的示例性的治疗剂。

[0048] 可通过应用本领域已知方法配制药物组合物，使得在给予患者后提供立即释放、延长释放或延缓释放的活性成分。

[0049] 组合物可以单位剂型配制，各剂量含有例如 0.1-500 mg 的活性成分。例如剂量可含有约 0.1 mg- 约 50 mg、约 0.1 mg- 约 40 mg、约 0.1 mg- 约 20 mg、约 0.1 mg- 约 10 mg、约 0.2 mg- 约 20 mg、约 0.3 mg- 约 15 mg、约 0.4 mg- 约 10 mg、约 0.5 mg- 约 1 mg、约 0.5 mg- 约 100 mg、约 0.5 mg- 约 50 mg、约 0.5 mg- 约 30 mg、约 0.5 mg- 约 20 mg、约 0.5 mg- 约 10 mg、约 0.5 mg- 约 5 mg、约 1 mg from- 约 50 mg、约 1 mg- 约 30 mg、约 1 mg- 约 20 mg、约 1 mg- 约 10 mg、约 1 mg- 约 5 mg、约 5 mg- 约 50 mg、约 5 mg- 约 20 mg、约 5 mg- 约 10 mg、约 10 mg- 约 100 mg、约 20 mg- 约 200 mg、约 30 mg- 约 150 mg、约 40 mg- 约 100 mg、约 40 mg- 约 80 mg 的活性成分或约 50 mg- 约 80 mg 的活性成分。对于制备固体组合物例如片剂，将主要的活性成分与一种或多种药用赋形剂混合形成含有本发明化合物的均质混合物的固体散装制剂组合物。当提及作为均质的这些散装制剂组合物时，活性成分通常均匀分散在组合物中使得组合物可容易地再分成等效的单位剂型，例如片剂和胶囊剂。然后将这种固体散装制剂再分成上述类型的单位剂型。

[0050] *用于口服给药的组合物*

本发明所考虑的药物组合物包括配制用于口服给药的那些（“口服剂型”）。口服剂型可以是例如片剂、胶囊剂、液体溶液剂或混悬剂、散剂或液体或固体晶体的形式，其含有活

性成分与无毒的药学上可接受的赋形剂的混合物。这些赋形剂可以是例如惰性稀释剂或填充剂（例如蔗糖、山梨糖醇、糖、甘露糖醇、微晶纤维素、淀粉包括马铃薯淀粉、碳酸钙、氯化钠、乳糖、磷酸钙、硫酸钙或磷酸钠）；成粒剂和崩解剂（例如纤维素衍生物包括微晶纤维素、淀粉包括马铃薯淀粉、交联羧甲纤维素钠、藻酸盐或藻酸）；粘合剂（例如蔗糖、葡萄糖、山梨糖醇、阿拉伯树胶、藻酸、藻酸钠、明胶、淀粉、预胶化淀粉、微晶纤维素、硅酸镁铝、羧甲基纤维素钠、甲基纤维素、羟丙基甲基纤维素、乙基纤维素、聚乙烯吡咯烷酮或聚乙二醇）；和润滑剂、助流剂和抗粘剂（例如硬脂酸镁、硬脂酸锌、硬脂酸、二氧化硅、氢化植物油或滑石）。其它药学上可接受的赋形剂可以是着色剂、矫味剂、增塑剂、润湿剂、缓冲剂等。

[0051] 用于口服给药的制剂还可作为咀嚼片剂、作为其中活性成分与惰性固体稀释剂（例如马铃薯淀粉、乳糖、微晶纤维素、碳酸钙、磷酸钙或高岭土）混合的硬明胶胶囊剂或作为其中活性成分与水或油介质（例如花生油、液体石蜡或橄榄油）混合的软明胶胶囊剂提供。可采用例如混合器、流化床设备或喷雾干燥装置，在片剂和胶囊剂的情况下按常规方式使用上述成分，制备散剂、颗粒剂和小丸剂。

[0052] 可构建用于口服用途的控释组合物以通过控制活性药物物质的溶出和/或扩散释放活性药物。可进行许多策略的任一种以获得相对于时间概况的控释和目标血浆浓度。在一个实例中，通过适当选择不同的制剂参数和成分获得控释，包括例如不同类型的控释组合物和包衣材料。因此，将药物与合适的赋形剂一起配制成药物组合物，使得在给药时以受控方式释放药物。实例包括单一或多单位片剂或胶囊剂组合物、油溶液剂、混悬剂、乳剂、微囊剂、微球剂、纳米粒剂、贴剂和脂质体。在某些实施方案中，组合物包括生物可降解的、pH 和/或温度敏感的聚合物包衣材料。

[0053] 可通过对化合物的片剂、胶囊剂、小丸剂或颗粒制剂适当包衣，或通过将化合物掺入合适的基质中，来实现溶出或扩散控释。控释包衣材料可包括上述包衣物质的一种或多种和/或例如虫胶、蜂蜡、glycowax、蓖麻蜡、巴西棕榈蜡、硬脂醇、单硬脂酸甘油酯、二硬脂酸甘油酯、棕榈酸硬脂酸甘油酯、乙基纤维素、丙烯酸树脂、dl- 聚乳酸、醋酸丁酸纤维素、聚氯乙烯、聚乙酸乙烯酯、乙烯基吡咯烷酮、聚乙烯、聚甲基丙烯酸酯、甲基丙烯酸甲酯、2- 羟基甲基丙烯酸酯、甲基丙烯酸酯水凝胶、1,3 丁二醇、乙二醇甲基丙烯酸酯和/或聚乙二醇。在控释基质制剂中，基质材料还可包括例如水合甲基纤维素、巴西棕榈蜡和硬脂醇、carbopol 934、硅酮、三硬脂酸甘油酯、丙烯酸甲酯 - 甲基丙烯酸甲酯、聚氯乙烯、聚乙烯和/或卤化碳氟化合物。

[0054] 其中可掺入本发明的化合物和组合物用于口服给药的液体形式包括水性溶液剂、适当调味的糖浆剂、水性或油性混悬剂和用食用油（例如棉籽油、芝麻油、椰子油或花生油）调味的乳剂以及酏剂和类似的药用溶媒。

[0055] *包衣材料*

可将配制用于口服递送的药物组合物（例如本发明的片剂或胶囊剂）包衣或以另外的方式化合以提供具有延缓或延长释放优势的剂型。可改动包衣材料以预定方式（例如为了获得控释制剂）释放活性药物物质，或例如可通过使用肠溶包衣（例如 pH 敏感的聚合物（“pH 控释”）、具有缓慢或 pH 依赖性的溶胀、溶出或蚀解速率的聚合物（“时间控制释放”）、通过酶降解的聚合物（“酶控制释放”或“生物可降解释放”）和形成被压力增加破坏的坚固层的聚合物（“压力控制释放”）），将其改动以在穿过胃后才释放活性药物物质。可

用于本文所述药物组合物的示例性的肠溶包衣包括糖包衣、薄膜包衣（例如以羟丙基甲基纤维素、甲基纤维素、甲基羟乙基纤维素、羟丙基纤维素、羧甲基纤维素、丙烯酸酯共聚物、聚乙二醇和/或聚乙烯吡咯烷酮为基础），或基于甲基丙烯酸共聚物、醋酸邻苯二甲酸纤维素、邻苯二甲酸羟丙基甲基纤维素、醋酸琥珀酸羟丙基甲基纤维素、聚醋酸乙烯邻苯二甲酸酯、虫胶和/或乙基纤维素的包衣材料。此外，可使用时间延迟材料例如单硬脂酸甘油酯或二硬脂酸甘油酯。

[0056] 例如片剂或胶囊剂可包含内剂量和外剂量组分，后者呈遍布在前者上的覆盖形式。两种组分可通过用作抵抗在胃中崩解并允许内组分完整穿过十二指肠或在释放时延迟的肠衣层分隔开。

[0057] 理想的是，在使用肠溶包衣时，大量的药物在下胃肠道中释放。

[0058] 除实现延缓或延长释放的包衣材料以外，固体片剂组合物可包括经改动以保护组合物免遭有害化学变化（例如在活性药物物质释放前的化学降解）的包衣材料。可按以下文献所述类似方式，将包衣材料用于固体剂型：*Encyclopedia of Pharmaceutical Technology,* 第5和6卷，编辑Swarbrick和Boyland, 2000。

[0059] *用于结肠药物释放的制剂*

在一些实施方案中，可使用靶向结肠的药物递送系统。示例性的方法包括但不限于：

(a) 药物与载体共价连接形成在胃和小肠中稳定的并在被肠微生物菌群酶促转化时在大肠中释放药物的前药；这些前药的实例包括偶氮缀合物、环糊精缀合物、糖苷缀合物、葡糖醛酸缀合物、葡聚糖缀合物、多肽和聚合物缀合物；

(b) 将完整分子递送至结肠的方法，例如具有在中性至碱性pH下释放药物的pH敏感聚合物的包衣材料，或具有在结肠被细菌降解时释放药物的生物可降解的聚合物的包衣材料；

(c) 将药物包埋在响应pH或生物降解时释放药物的生物可降解的基质和水凝胶中；

(d) 其中一旦复层制剂穿过胃时，药物在3-5小时延迟时间（相当于小肠的通过时间）后释放的时间释放系统；

(e) 使用氧化还原敏感聚合物，其中偶氮和二硫化合物聚合物的组合在响应结肠的氧化还原电位时提供药物释放；

(f) 使用选择性黏附于结肠粘膜的生物黏附聚合物慢慢释放药物；和

(g) 其中药物因渗透压所致通过半透膜释放的渗透控制的药物递送。

[0060] *给药途径*

如本领域技术人员所知，且与待治疗的具体疾病或病况有关，可根据所选的给药途径以各种形式将本文所述组合物给予患者。可通过例如局部、肠内或胃肠外应用，给予用于本文所述方法的组合物。局部应用包括但不限于表皮、吸入、灌肠、滴眼剂、滴耳剂和通过体内粘膜应用。肠内应用包括口服给药、直肠给药、阴道给药和胃饲管。胃肠外给药包括静脉内、动脉内、囊内、眶内、心脏内、皮内、经气管、表皮下、关节内、囊下、蛛网膜下、脊柱内、硬膜外、胸骨内、腹膜内、皮下、肌内、跨上皮、经鼻、肺内、鞘内、直肠和局部模式的给药。胃肠外给药可以是在选择的一段时间内通过连续输注。

[0061] 对于静脉内或鞘内递送或直接注射，组合物必须是无菌的和流动的以致组合物可通过注射器递送。除水以外，载体可以是等渗的缓冲盐水溶液、乙醇、多元醇（例如甘油、丙

二醇和液体聚乙二醇等）及其合适的混合物。可通过例如使用包衣材料例如卵磷脂、通过在分散体的情况下保持所需粒度和通过使用表面活性剂，来保持适当的流动性。在许多情况下，优选在组合物中包括等渗剂，例如糖、多元醇例如甘露糖醇或山梨糖醇和氯化钠。可通过在组合物中包括延迟吸收的作用剂（例如一硬脂酸铝或明胶），产生注射用组合物的长期吸收。

[0062] 给药途径的选择可取决于是达到局部作用还是全身作用。例如对于局部作用，组合物可配制用于局部给药，并直接施用于需要其作用的位置。对于全身的长期作用，组合物可配制用于肠内给药，并通过消化道给予。对于全身即时作用和/或短期作用，组合物可配制用于胃肠外给药，并通过消化道以外的途径给予。

[0063] *胃肠外给药*

在本发明的范围内的还有来自生物可降解聚合物的胃肠外贮库系统。将这些系统注射或植入肌肉或皮下组织，并在几天到几个月的延长的时间内释放所掺入的药物。聚合物的特性和装置的结构两者可控制可以是连续的或脉动的释放动力学。基于聚合物的胃肠外贮库系统可归类为植入剂或微粒剂。前者是注射到皮下组织的圆柱形装置，而后者定义为范围为 10-100 μm 的球状颗粒。采用挤出、压制或注射成型制备植入剂，而对于微粒剂，通常采用相分离方法、喷雾干燥技术和水包油包水乳剂技术。形成微粒剂的最常用的生物可降解聚合物是乳酸和/或乙醇酸例如聚（乙醇酸）和聚（L-乳酸）的聚酯（PLG/PLA 微球剂）。特别有价值的是原位形成贮库系统，例如热塑性糊剂和通过固化、通过冷却或因溶胶凝胶转变所致形成的胶凝系统、交联系统和通过两亲性脂质形成的有机凝胶。用于上述系统的热敏聚合物的实例包括N-异丙基丙烯酰胺、泊洛沙姆（环氧乙烷和环氧丙烷嵌段共聚物，例如泊洛沙姆 188 和 407）、聚（N-乙烯基己内酰胺）、聚（silo 乙二醇），聚磷腈衍生物和 PLGA-PEG-PLGA。

[0064] *给药方案*

用于调理脑功能，特别是改善心境和/或心理状态、治疗脑发育障碍和治疗和/或减轻疼痛的本发明方法通过给予无机亚硝酸盐足以导致改善心境和/或心理状态、治疗和/或减轻疼痛和治疗脑发育障碍的一段时间和量来进行。

[0065] 给予组合物的量和频率可根据例如给予的是什么、患者的状况和给药方式而变化。在治疗应用中，可将组合物给予患有疼痛（例如神经性疼痛、神经病、糖尿病性周围神经病）的患者，其量足以缓解或至少部分缓解疼痛的症状（例如不适、酸痛、绷紧或僵硬）及其并发症（例如疲劳、失眠、免疫系统虚弱、抑郁、焦虑、紧张、易怒或失能）。剂量可能取决于例如疼痛进展的类型和程度（例如通过世界卫生组织的“疼痛阶梯（Pain Ladder）”准则测定）、疼痛的严重性（例如急性、亚急性或慢性）、具体患者的年龄、体重和一般状况、所选组合物的相对生物功效、赋形剂的配方、给药途径和主治临床医生的判断等变量。有效剂量可自来源于体外或动物模型试验系统的剂量反应曲线推算。有效剂量是通过例如改善疼痛的指征或症状或减慢其进展产生所需临床结果的剂量。

[0066] 每剂无机亚硝酸盐的量可变化。例如受试者可接受约 0.1 μg/kg-约 10,000 μg/kg。一般而言，以这样的量给予亚硝酸盐使得峰值血浆浓度范围为 150 nM-250 μM。示例性的剂量可介于约 0.1-约 2000 μg/kg、约 0.5-约 1000 μg/kg、约 0.5-约 2000 μg/kg、约 100-约 1500 μg/kg、约 0.5 μg/kg-约 500 μg/kg、约 0.5 μg/kg-约 250 μg/kg、

约 0.5 μg/kg- 约 100 μg/kg、约 0.5 μg/kg- 约 50 μg/kg、约 100- 约 350 μg/kg、约 340- 约 750 μg/kg 或约 750- 约 1000 μg/kg 之间。示例性的剂量可为约 8.25 μg/kg、约 10 μg/kg、约 16.5 μg/kg、约 20 μg/kg、约 30 μg/kg、约 50 μg/kg、约 100 μg/kg、约 165 μg/kg、约 200 μg/kg、约 500 μg/kg、约 750 μg/kg、约 1000 μg/kg、约 1250 μg/kg、约 1500 μg/kg、约 1750 μg/kg 或约 2000 μg/kg。示例性的峰值血浆浓度的范围可为 0.05-10 μM、0.1-10 μM、0.1-5.0 μM 或 0.1-1 μM。峰值血浆浓度可保持 2-14 小时、4-14 小时、6-14 小时、6-12 小时或 6-10 小时。

[0067] 治疗频率也可改变。可以每天一次或多次（例如一次、两次、三次、四次或更多次）或每这么多小时（例如约每 2、4、6、8、12 或 24 小时）治疗受试者。优选每 24 小时 1 或 2 次给予药物组合物。治疗时程可具有不同的持续时间，例如 2、3、4、5、6、7、8、9、10 或更多天、2 周、1 个月、2 个月、4 个月、6 个月、8 个月、10 个月或超过 1 年。例如治疗可以是一天两次持续 3 天、一天 2 次持续 7 天、一天 2 次持续 10 天。治疗周期可以例如一周一次、一月两次或每月一次的间隔重复，治疗周期被其中不给予治疗的时间分隔开。治疗可以是单次治疗或可以持续长达受试者的生命期（例如许多年）。

[0068] 药盒

本文所述的任何药物组合物可与一套说明书一起使用，即构成药盒。药盒可包括使用药物组合物作为本文所述疗法的说明书。例如说明书可提供使用本发明的化合物调理脑功能，特别是改善心境和 / 或心理状态、治疗脑发育障碍和治疗和 / 或减轻疼痛的给药和治疗方案。

[0069] 治疗方法

本发明提供亚硝酸盐（例如无机亚硝酸盐）或其药学上可接受的前药的营养和药物组合物，尤其在保持心理状态的平衡和减轻疼痛（例如慢性疼痛）中用于预防性和治疗性营养补充两者。具体地说，本发明涉及可用于治疗患有以下疼痛的患者的亚硝酸盐（例如无机亚硝酸盐）或其药学上可接受的前药的新的组合物：急性疼痛、亚急性疼痛或慢性疼痛（例如持续超过 3-6 个月的疼痛或延续超过预计愈合期的疼痛和 / 或源于最初的创伤 / 损伤或感染的疼痛，或可以是与神经性疼痛有关的持续原因的疼痛（例如糖尿病性周围神经病、带状疱疹后神经痛、三叉神经痛、幻肢痛、腕管综合征、坐骨神经痛、阴部神经痛、复杂性区域疼痛综合征、感觉性多神经病、单一神经病变或中枢疼痛综合征、头痛、关节痛、背痛、鼻窦痛、肌肉痛、神经痛和累及身体特殊部位（例如肩、骨盆和颈）的疼痛），和 / 或与下背疼痛、关节炎、头痛、多发性硬化、纤维肌痛、带状疱疹、神经损伤或癌症有关的疼痛。本发明还涉及可用于治疗患有心境障碍和 / 或心理状态失衡和脑发育障碍的患者的亚硝酸盐（例如无机亚硝酸盐）或其药学上可接受的前药的新的组合物。

[0070] *疼痛*

在下面实施例中描述的临床试验后，发现接受本发明的组合物的患者也报告了疼痛减轻。因此，在优选的实施方案中，假设本发明的组合物可用于治疗或减轻全身疼痛和神经性疼痛。

[0071] *神经性疼痛*

神经性疼痛可根据其来源呈多种形式，并且可根据持续时间描述为急性、亚急性或慢性。急性疼痛可持续约几个小时到少于 30 天。亚急性疼痛可持续 1-6 个月，慢性疼痛被描

述为持续超过 3-6 个月的疼痛或延续超过预计愈合期的疼痛。在神经性疼痛中，如果开始的损伤由于神经完全或部分神经断裂或神经丛创伤而产生，则疼痛可描述为周围神经病性的。周围神经病可产生于外伤性损伤、感染、代谢障碍、糖尿病和 / 或暴露于毒素。或者，在中枢神经系统病变（例如脊髓损伤或脑血管事件）后，神经性疼痛被描述为中枢神经病性的。本发明的方法包括给予本文所述组合物以治疗神经性疼痛。神经性疼痛的类型包括但不限于 :糖尿病性周围神经病、带状疱疹后神经痛、三叉神经痛、幻肢痛、腕管综合征、坐骨神经痛、阴部神经痛、复杂性区域疼痛综合征、感觉性多神经病、单一神经病变和中枢疼痛综合征。

[0072] *神经病*

本文所述组合物可用于治疗神经病，具体地说，糖尿病性周围神经病。神经病可能具有许多原因，例如持续损伤或暴露于毒素或慢性疾病（例如帕金森多发性硬化、自身免疫病和糖尿病），其中糖尿病为最大的风险因子。在糖尿病患者中，对于神经病发生通常需要许多年，因为神经损伤由于长期暴露于高血糖水平的损害作用下而随时间产生。受试者患糖尿病越久，发生神经病的风险越高。可将本文所述组合物预防性地给予患有糖尿病的受试者以防止或降低发生糖尿病性周围神经病的风险或治疗性地给予以治疗糖尿病性周围神经病。

[0073] 虽不希望受理论约束，但是本文所述的亚硝酸盐的组合物可通过其对参与糖尿病并发症的山梨糖醇 - 醛糖还原酶途径的间接作用，特别有益于糖尿病受试者。在有高的高血糖状态的糖尿病受试者中，醛糖还原酶对葡萄糖的亲和力增加，这导致较高水平的山梨糖醇和较低的 NADPH。NADPH 特别起促进一氧化氮 (NO) 和谷胱甘肽产生的作用，其导致管扩张。NADPH 氧化成 NADP+ 也是防止形成活性氧类别所必需的。糖尿病受试者中较低的 NADPH 水平抑制 NO 产生，并且单独导致神经元细胞死亡和疼痛。因此，补充 NO 供应的能力在某些情况下可改善糖尿病受试者的神经性疼痛的症状。

[0074] *炎性疼痛*

炎性疼痛是由组织损伤或炎症引起的一种疼痛形式（例如在手术后疼痛或类风湿性关节炎中）。在周围神经损伤后，通常以慢性形式在损伤部位远端遭受症状，并且其特征在于感觉过敏（对天然刺激的敏感性增强）、痛觉过敏（对有害刺激物异常敏感）、异常性疼痛（与对正常无害触觉刺激的超敏反应有关的广泛触痛）和 / 或自发性灼痛或刺痛。在炎性疼痛中，症状至少最初在损伤或发炎组织部位是明显的，并且通常伴有关节炎相关疼痛、肌肉骨骼疼痛和手术后疼痛。不同类型的疼痛可同时存在或疼痛可在自然的病程中从炎性转化成神经病性，如在带状疱疹后神经痛中一样。

[0075] *伤害性疼痛*

伤害性疼痛是在响应有害刺激物（例如针刺或在创伤或手术期间）时体验到的疼痛。伤害性疼痛可分成浅表和深层的，而深层疼痛可分成深层躯体性和内脏性的。浅表疼痛通过激活皮肤或浅表组织中的伤害感受器而引起。深层躯体性疼痛通过刺激韧带、肌腱、骨、血管、筋膜和肌肉中的伤害感受器引发，并且是钝性的、疼痛的、定位不明的疼痛。内脏性疼痛起源于内脏（器官）。内脏性疼痛可以是定位明确的，但常常极难于找到，且若干内脏区域当受损或发炎时产生牵涉性痛，其中感觉位于远离病理或损伤部位的区域。

[0076] *其它类型的疼痛*

功能性疼痛是指其中没有明显的神经系统的外周病理或病变的情况。这种特殊形式的疼痛由神经系统功能异常产生，和这样的痛疼所表征的病况包括纤维肌痛、紧张型头痛和肠易激综合征。

[0077] 与慢性疼痛有关的常见病况包括但不限于背部损伤（例如椎间盘突出或椎间盘膨出、椎管狭窄、压缩骨折、软组织损害、损伤性骨折和结构畸形）、头痛（例如肌紧张性头痛、眼疲劳性头痛、偏头痛、丛集性头痛）、关节痛（例如骨关节炎、类风湿性关节炎和重复性劳损）、纤维肌痛和癌症（例如白血病、脑癌、膀胱癌、乳腺癌、宫颈癌、结肠直肠癌、子宫内膜癌、食管癌、头颈癌、肝癌、肺癌、淋巴瘤、卵巢癌、胰腺癌、前列腺癌、肾癌、皮肤癌、胃癌、睾丸癌、甲状腺癌和尿路上皮癌）相关性疼痛。

[0078] 本文所述药物组合物和方法可用于治疗、减轻或预防不论是急性还是慢性的不同形式的疼痛，即炎性疼痛、伤害性疼痛、功能性疼痛和神经性疼痛。可能与疼痛有关的示例性病况包括例如软组织、关节、骨的炎症和/或损害（例如急性创伤、骨关节炎或类风湿性关节炎）、肌筋膜疼痛综合征（纤维肌痛）、头痛（包括丛集性头痛、偏头痛和紧张性头痛）、心肌梗死、心绞痛、缺血性心血管疾病、中风后疼痛、镰状细胞性贫血、周围血管闭塞性疾病、癌症、皮肤或关节的炎性病况、糖尿病性神经病和源于手术或外伤性损伤的急性组织损害（例如烧伤、撕裂伤或骨折）。

[0079] 本发明还可用于治疗或减轻肌肉骨骼疼痛（在创伤、感染和运动后）、由脊髓损伤、肿瘤、压迫、炎症引起的神经性疼痛、牙痛、外阴切开术疼痛、深部和内脏性疼痛（例如心脏疼痛、膀胱疼痛或骨盆器官疼痛）、肌肉痛、眼痛、口颌面疼痛（例如牙痛、三叉神经痛、舌咽神经痛）、腹部疼痛、妇产科疼痛（例如痛经和阵痛）、与创伤所致神经根损伤、压迫、炎症、有毒化学物质、代谢障碍、遗传病况、感染、血管炎和自身免疫病有关的疼痛、中枢神经系统疼痛例如由脊髓或脑干损伤、脑血管意外、肿瘤、感染、脱髓鞘性疾病（包括多发性硬化）所致疼痛、腰痛、坐骨神经痛和术后疼痛。

[0080] *功效评价*

可在任何标准疼痛动物模型中针对功效测试本文所述组合物。不同的模型测试正常动物对强烈或有害刺激的敏感性（生理或伤害性疼痛）。这些试验包括对热、机械或化学刺激的反应。热刺激常常包括应用热刺激（通常42-55℃之间变化），包括例如：对尾的辐射热（甩尾试验）、对后爪跖面的辐射热(Hargreaves试验）、热板试验和后爪或尾浸入热水中。还可采用浸入冷水、丙酮蒸发或冷板试验来测试冷痛响应性。涉及机械刺激的试验通常测量引起后爪对分级强度单丝von Frey毛的缩回反射的阈值或对爪持续压迫刺激的阈值（例如Ugo Basile analgesiometer)。还可测量对标准针刺的反应的持续时间。当使用化学刺激时，测量对化学刺激物（例如辣椒素、芥子油、缓激肽、ATP、福尔马林、乙酸）施加或注射到皮肤、肌肉、关节或内部器官（例如膀胱或腹膜）中的反应。具体地说，可在动物模型例如用作1型糖尿病周围神经病模型的链脲佐菌素诱发的糖尿病大鼠和小鼠（参见例如Tesch等，*Nephrology*. 12(3):261-266,2007)、用作2型糖尿病周围神经病模型的瘦蛋白缺陷型(ob/ob)小鼠模型（参见例如Drel等，*Diabetes*. 55(12):3335-3343, 2006)、非肥胖糖尿病(NOD)小鼠模型、自发性诱发的Ins2 Akita小鼠模型、Db/Db瘦蛋白受体缺陷型小鼠模型、WBN/Kob自发性糖尿病大鼠模型、SDT脂肪大鼠模型、高脂肪饮食C5BL/6J小鼠模型和猕猴PDN模型（参见例如Islam, *J Diabetes Res*. 2013: 149452, 2013)中，研

究在给予本发明的组合物时糖尿病性周围神经病的疼痛的评价。

[0081] 此外，不同的试验通过测量疼痛神经途径的周围或中枢组分兴奋性的改变评价疼痛敏化。在这一方面，可通过重复热刺激以及施用或注射致敏化学制品（例如前列腺素、缓激肽、组胺、5-羟色胺、辣椒素或芥子油），诱导外周敏化（即高阈值伤害感受器的阈值和响应性的变化）。可通过有害刺激（例如热）、化学刺激（例如注射或施用化学刺激物）或感觉纤维的电激活诱导中枢敏化（即由外周疼痛纤维的活性诱导的中枢神经系统的神经元兴奋性的改变）。

[0082] 还可采用经开发以测量外周炎症对疼痛敏感性的作用的各种疼痛试验来研究本文所述组合物的功效(Stein等，*Pharmacol. Biochem. Behav.* (1988) 31: 445-451；Woolf等，*Neurosci.* (1994) 62: 327-331)。另外，各种试验利用外周神经系统病变评价周围神经性疼痛。一个这样的实例是“轴突切开疼痛模型”(Watson, *J. Physiol.* (1973) 231:41)。其它类似的试验包括涉及节段脊神经结扎的SNL试验(Kim和Chung *Pain* (1992) 50: 355)；涉及部分神经损伤的Seltzer模型(Seltzer, *Pain* (1990) 43: 205-18)；保留性神经损伤(SNI)模型(Decosterd和Woolf, *Pain* (2000) 87:149)；慢性压迫损伤(CCI)模型(Bennett (1993) *Muscle Nerve* 16: 1040)；涉及中毒性神经病变例如糖尿病（链佐星模型）、吡哆醇神经病、泰素、长春新碱和其它抗肿瘤药诱导的神经病变的试验；涉及神经缺血的试验；周围神经炎模型（例如外周神经应用的CFA）；使用HSV感染的带状疱疹后神经痛模型和压迫模型。

[0083] 慢性疼痛被描述为影响脑结构和功能的疾病。磁共振成像研究显示甚至在涉及疼痛进程相关区域的静止期间的异常解剖学和功能连接性。持续疼痛还显示引起一旦疼痛消退是可逆的灰质丢失。因此，可利用神经可塑性的度量来评价本文所述组合物的功效。可利用脑电图(EEG)来测量与未接受组合物的受试者相比，接受组合物的受试者的相对β活性、α活性和δ活性的变化。

[0084] 神经病影响运动纤维或大的感觉纤维，传统工具例如肌电图学和神经传导研究可用于测定将本文所述组合物给予受试者的功效。也可采用定量感觉测试来测定治疗的功效。定量感觉测试包括施用受控的机械、热或化学刺激。受试者报告了他们对刺激的感受，并指出变疼痛的点，这允许评价受试者对不同类型的刺激的感觉阈值。测量组合物的有效性的其它体内测定法包括单丝测试和神经传导速度测试。皮肤活检也可用于监测对疗法或疾病进程的反应。皮肤活检需要使用局部麻醉从机体任何位置采集的表皮的少量样品。将活检样品用针对PGP9.5（一种panaxonal标志物）的抗体免疫标记，使得皮肤中的小的感觉神经末梢可使用光学显微镜观察并计数。皮肤活检允许定量测量表皮中的感觉神经末梢，因为它们作为可以计数的各个神经末梢存在。可获得显示皮肤神经末梢的正常密度和提供用于试验受试者比较的点的规范数据。其它离体功效测试包括组织的组织病理或活检以寻找轴突的萎缩变化、髓鞘形成的轴突营养不良（即脱髓鞘）和大的髓鞘化纤维数目的减少。

[0085] 在所有的上述试验中，例如可根据行为、电生理学、神经化学或成像技术对结果度量进行评价以检测神经活动的变化。在所有的上述试验中，还可通过测定疼痛药理性质和非药理性质例如疼痛强度（为标准化疼痛评分中的度量）、模式（例如持续的、间歇的）、位置、辐射、频率、时机和持续时间、对生命质量的影响（睡眠、功能、食欲和心境），来评价疼

痛减轻的改善。

[0086] *心境障碍和心理状态失衡*

脑中的NO信号转导可调理多个过程例如不同形式的可塑性（长时程增强和长时程压抑、LTP和LTD），调节节律性活动，包括肠活动、呼吸节律、昼夜节律、运动和丘脑皮质波动(thalamocortical oscilliation)。本文所述组合物是将NO递送到特定部位的有效的NO供体化合物，因此这些组合物还可通过保持NO水平的平衡，用于调理脑功能，特别是改善心境和/或心理状态，并可用于治疗心境障碍。

[0087] 术语心境障碍是指在受试者中观察到的基础的或纵向的情绪状态。两类心境障碍是普遍公认的；界线基于究竟是否存在躁狂发作还是轻躁发作。因此，存在抑郁症，其中最已知的和研究最深入的是重度抑郁症(MDD)和双相型障碍(BD)，其特征在于躁狂症或轻躁狂的间歇性发作，通常与抑郁发作交织。还有MDD和BD的抑郁形式，其较不严重，被称为心境恶劣障碍（相对于MDD）和循环情感性障碍（相对于BD）。

[0088] 其它类型的抑郁症包括但不限于：非典型抑郁症、忧郁型抑郁症、精神病性重度抑郁症、紧张型抑郁症、产后抑郁症、季节性情感障碍和待分类的抑郁症(DD-NOS)。双相性精神障碍包括：双相I型、双相II型、循环型障碍和待分类的双相性精神障碍(BD-NOS)。心境障碍还可归类为物质诱发的。这些物质诱发的心境障碍包括：酒精诱发的心境障碍、苯并二氮杂䓬诱发的心境障碍和兴奋剂诱发的心境障碍（例如苯丙胺、脱氧麻黄碱和可卡因）。

[0089] *脑发育障碍*

NO还通过调解LTP的具体形式参与学习和记忆机制。因此，本文所述组合物还可用于治疗脑发育障碍，例如：学习和记忆减退、孤独症、雷特综合征、儿童期崩解症、待分类的广泛性发育障碍(PDD-NOS)和阿斯珀格综合征。孤独症是特征在于社交相互作用和沟通受损和特征在于局限性重复行为的神经发育障碍。

[0090] 孤独症通过改变神经细胞与其突触如何连接和组构，而影响脑的信息加工。孤独症是3个公认的孤独症谱系障碍(ASD)之一，其它两种是阿斯珀格综合征（其缺乏认知发展和语言的延迟）和广泛性发育障碍。因为孤独症是由神经功能障碍引起的，所以本发明的组合物还可用于治疗孤独症和相关神经发育障碍。

[0091] 雷特综合征，最初名为大脑萎缩性血氨过多，是几乎专门累及女性但也存在于男性患者中的脑灰质的神经发育障碍。临床特征包括手和足小，头生长速度减慢（包括一些中的小头畸形）。重复刻板的手部动作（例如手绞拧和/或重复把手放入口中）也是常见的临床特征。雷特综合征受试者易患胃肠道紊乱，高达80%有癫痫发作，且通常没有语言能力。约50%的受累个体不能走动。脊柱侧弯、生长障碍和便秘是雷特综合征的非常常见的特征，并且可能是难以解决的。雷特综合征被列于广泛性发育障碍的大类别下。

[0092] 儿童期崩解症(CDD)，亦称为海勒综合征(Heller's syndrome)和解体性精神病(disintegrative psychosis)，是一种特征在于语言、社交功能和运动技能的迟发性(>3岁）发育延迟的罕见病况。CDD具有与孤独症的某些类似性，有时被认为是其低功能形式，但在技能的倒退或技能的一系列倒退之前常注意到十分正常发育的明显时期。许多儿童在障碍变得明显时已经有些迟缓，但这些迟缓在低龄儿童中不总是明显的。该倒退可发生的年龄是变化的，并且可能为2-10岁，这种开始的定义主要取决于看法。一些儿童描述幻觉或显得对幻觉起反应，但大多数明显的症状是明显获得的技能丧失。

[0093] *组合疗法/治疗*

本发明的组合物和方法也可与用于治疗疼痛、心境障碍和/或心理状态失衡或脑发育障碍的本领域已知的其它医疗法联合使用，所述其它医疗法包括非甾体抗炎药(NSAID)、皮质类固醇、对乙酰氨基酚、阿片样物质、肌肉松弛药、抗焦虑药、抗抑郁药、抗惊厥药、抗精神病药、心境稳定剂、锂和5-羟色胺重摄取抑制剂(SSRI)。本发明的组合物和方法还可与其它形式的治疗联合使用，包括但不限于：认知行为疗法、音乐疗法、艺术疗法、小组疗法、精神疗法、身体运动、宠物疗法、沟通疗法、教育疗法和家庭疗法。具体治疗的选择可变化，并且将取决于疼痛的严重性、心境障碍或脑发育障碍、受试者的一般健康状况和主治临床医生的判断。

[0094] 对于神经性疼痛的治疗，本发明的组合物可在给予以下药物之前、同时或之后使用：三环抗抑郁药(TCA)，例如阿米替林；选择性5-羟色胺重摄取抑制剂(SSRI)或去甲肾上腺素抑制剂，例如度洛西汀、米那普仑和文拉法辛；抗癫痫药(AED)，例如加巴喷丁、普瑞巴林、托吡酯和左乙拉西坦；及其它神经性疼痛药，例如去甲替林、丁氨苯丙酮、地昔帕明、非甾体抗炎药、阿片样物质(例如可待因、氢可酮、氢吗啡酮、美沙酮、吗啡、羟考酮)、利多卡因、麦芽酚镓(gallium maltolate)和大麻素。

[0095] 本发明组合物还可与一种或多种其它的活性成分组合配制，其可包括药剂例如NSAID(例如阿司匹林、布洛芬、酮洛芬、酮咯酸氨丁三醇和奈普生)、皮质类固醇(例如泼尼松龙、甲泼尼龙、氢化可的松、安西奈德、醋酸氟轻松、氟尼缩松、泼尼卡酯、倍他米松和曲安奈德)、对乙酰氨基酚、阿片样物质(例如吗啡、芬太尼、羟考酮、可待因)、肌肉松弛药(例如卡立普多、环苯扎林和地西泮)、抗焦虑药(例如度洛西汀、氟西汀、阿普唑仑、依他普仑和劳拉西泮)、抗抑郁药(例如地昔帕明、阿米替林、阿戈美拉汀、依托哌酮和苯乙肼)、抗惊厥药(例如碳酸锂、柠檬酸锂、托吡酯、奥卡西平和丙戊酸)、抗精神病药(例如阿立哌唑、氯氮平、利培酮、阿塞那平和奥氮平)和SSRI(例如西酞普兰、帕罗西汀、氟伏沙明和舍曲林)。

[0096] 在一个实施方案中，上述化合物的任一种可与无机亚硝酸盐(例如亚硝酸钠)一起配制或与无机亚硝酸盐(例如亚硝酸钠)一起给予患有疼痛(例如糖尿病性神经病或其它神经性疼痛)的患者。当共同给予时，两种化合物彼此适宜在24小时内(例如在12小时、8小时、4小时、2小时、1小时、30分钟、15分钟内或基本上同时)给予。

[0097] 在一些实施方案中，组合物还包括无机硝酸盐；在其它实施方案中，组合物不包括无机硝酸盐。例如本发明组合物可包括比率介于1-5至1-100亚硝酸盐：硝酸盐，例如1-5、1-10、1-30、1-50、1-70或1-100亚硝酸盐：硝酸盐的无机亚硝酸盐和硝酸盐。

实施例

[0098] 在下文所述实施例中使用下列术语的缩略语和定义的列表。

缩略语	术语
ABI	踝肱指数
ACS	急性冠状动脉综合征
AUC	曲线下面积
AE	不良事件
BID	一天两次
CBC	全血计数

CFR	美国联邦法规
CHF	充血性心力衰竭
CNS	中枢神经系统
C_{max}	最大血浆药物浓度
C_{tau}	给药间隔期间的平均药物浓度
DBP	舒张期血压
DLT	剂量限制性毒性
ECG	心电图
eCRF	电子病例报告表
EDC	电子数据采集
FDA	食品与药物管理局
FMD	血流介导的血管扩张
G6PD	葡萄糖 -6 磷酸脱氢酶
HbA1c	血红蛋白 A1c
ICF	知情同意书
IL-6	白介素 -6
IP	研究产品
LOCF	末次观测值结转
MDRD	肾疾病研究中的饮食改进
MetHb	正铁血红蛋白
NO	一氧化氮
NYHA	纽约心脏协会
PAD 、	外周动脉疾病
PD	药效学
PI	首席研究员
PK	药代动力学
QoL	生命质量
RAND 36	RAND 36 项简明健康状况调查表
SAE	严重不良事件
SBP	收缩期血压
SD	标准偏差
SICAM	可溶性胞间粘附分子
SOC	系统器官分类
TIA	短暂性脑缺血发作
VCAM	血管细胞粘着蛋白
WIQ	行走受损问卷

[0099] 实施例 1:2a期临床研究

研究基本原理和详情

研究了作为用于改善患有 PAD 的受试者功能的新疗法的亚硝酸钠。该剂量范围研究的总体目的是评价患有 PAD 的受试者中多剂量口服亚硝酸钠的安全性、药代动力学、耐受性和潜在生物活性。如上文详述,PAD 的主要病理生理学与下肢血流量的限制有关,导致运动耐受性受限和生命质量下降。PAD 的共同特性是内皮功能障碍、NO 生物利用度降低和 NO 存储耗尽,一种当 PAD 和代谢疾病（例如糖尿病）共同存在时可变严重的结果。亚硝酸钠是在体内存在和代谢的无机盐。已知亚硝酸钠在生理浓度下引起血管扩张。

[0100] 该早期临床研究的主要目的是评价与 10 周治疗期内的安慰剂相比,一天两次 40 mg 和 80 mg 亚硝酸钠的多剂量的安全性和耐受性。该研究的第二目的是评价亚硝酸钠的药代动力学,并表明亚硝酸钠对生物活性测量和步行距离和跛行症状的功能测量的药效作用。最后,表征并评价了亚硝酸钠的剂量、血浆浓度和药效作用之间的关系。在该研究中,

在动脉反应性和跛行限制性运动的标准测试期间进行了生物活性和走动功能的多项评价。药效学评价包括:肱动脉血流介导的血管扩张(FMD)、6分钟步行试验、所选的目标生物标志物、生命质量问卷(WIQ和RAND 36)。

[0101] 主要终点包括:临床安全性和耐受性数据包括自发AE报告、ECG、生命体征、护理/医生观察和临床实验室值。次要终点包括血流介导的血管扩张反应、6分钟步行试验期间涵盖的最大距离、亚硝酸钠的血浆药代动力学(包括但不限于AUC、C_{max}、C_{tau})和与在该研究中进行的药效学评价的关系和生命质量(WIQ和RAND 36)。此外,探查性药效学/生物标志物终点包括炎症、氧化应激、代谢功能、血管生成的标志物或动脉粥样硬化疾病的其它标志物的变化,在数据允许时(例如亚硝酸钠、亚硝酸盐、硝酸盐、可溶性胞间粘附分子(SICAM)、血管细胞粘着蛋白(VCAM)、F2-异前列烷和白介素-6(IL-6))。

[0102] 试验类型是针对患有PAD的受试者的随机双盲安慰剂对照剂量范围平行设计多剂量研究。受试者至少35岁,但不大于85岁。如果受试者遭受跛行,则受试者还有1个月的稳定的PAD症状史。按照研究开始之前产生的随机方案,将受试者分配到安慰剂或亚硝酸钠治疗组。借助通过电子数据采集(EDC)的交互式网络响应系统(interactive web response system,IWRS)使受试者随机进入研究中以接受安慰剂、40 mg BID或80 mg BID的治疗方案之一。由于这是一项双盲研究,所以受试者、研究人员和现场人员均不知情。TheraVasc和CPC也不知情。在医疗意外的情况下或万一出现严重医疗情况时,当由于临床管理或受试者健康必需了解研究产品时,管理受试者的研究人员或其它医生可揭秘受试者的治疗代码。研究人员在揭盲前做出各种努力以联系CPC医学监视以讨论选择办法。如果出于任何原因盲法终断,且研究人员在揭盲前无法联系CPC,则研究人员必须在揭盲事件后在不显示受试者的研究治疗分配的情况下尽可能快地通知CPC,除非信息对留在研究中的受试者的安全性是重要的。此外,研究人员应在适当数据采集工具中记录揭秘受试者的盲法治疗分配的日期和原因。如果需要向一个或多个管理机构发送调整报告,则报告明确受试者的治疗分配。适用时,根据相关条例、CPC政策或两者,将调整报告副本送交研究人员。

[0103] *研究产品(IP)*

亚硝酸钠胶囊剂,剂量规格为40 mg和80 mg/胶囊,保存在受控的室温(20-25℃,68-77℉)下。还供应匹配的安慰剂胶囊剂,并保存在受控的室温下。在按照表1所述就诊方案分装的50个计数瓶中提供TV1001。将IP保存在安全条件下。Bilcare,Global Clinical Supplies标记、贮存和分配亚硝酸钠和匹配的安慰剂。如下描述分配并给予IP。表2描述了研究药物的详情。

表 1. 评价方案

就诊名称	筛选	就诊 1 随机化	就诊 2	就诊 3	安全性就诊[1]	就诊 4	就诊 5	打电话 1	打电话 2	就诊 6	就诊 7	安全性就诊[1]	结束就诊 8	跟踪打电话	提前终止
时间(天)	-21 至 -14 天	第 0 天	第 1 天	第 4 天	第 7 天	第 14 天	第 28 天	第 42 天	第 58 天	第 70 天	第 71 天	第 71+1 天	就诊 7 后 8 天	就诊 8 后 7 天或提前终止	n/a
容许差异			±4 小时	±1 天	±1 天	±2 天	±2 天	±2 天	±2 天	±2 天	±1 天	±1 天	±1 天	±1 天	n/a
知情同意书	X														
人口统计学资料	X														
医学和药物史	X	X													
体检	X												X		X
生命体征[2]	X	X	X	X	X	X	X			X	X	X	X		X
12-导联 ECG		X					X						X		X
临床安全实验室	X		X	X		X	X			X	X		X		X
Mcl-Hb 实验室[3]			X	X	X	X	X			X	X	X	X		X
PK 样品			X	X		X	X			X	X		X		X
7 个时间点内的 PK 和 Mcl-Hb[3]		X													
尿妊娠试验	X	X				X							X		X
PD 生物标志物		X								X					X
踝肱指数(ABI)	X														
FMD[4]		X								X					X
QoL (WIQ, RAND 36)		X								X					X
6 分钟步行试验		X								X					X
分配的研究药物		X				X	X				X				
不良事件		X	X	X	X	X	X	X	X	X	X	X	X	X	X
同期治疗		X	X	X	X	X	X	X	X	X	X	X	X	X	X

评价纳入/排除标准	X	X														
评价研究终止标准		X	X	X	X	X	X	X			X	X	X			

1 如果 Met-Hb 为 8%或更大，才需要这次就诊。

2 生命体征在第 1 剂的 IP 之前为仰卧的，第 1 剂后为体位的。

3 在基线(给药前)和给药后在 15 分钟±5 分钟、30 分钟±5 分钟、1 小时±10 分钟，2 小时±10 分钟，4 小时±10 分钟和 6 小时±10 分钟时重复抽血。

4 可在就诊 1 停止前 7 天和就诊 6 停止前 5 天进行 FMD。

[0104] 表 2. 研究药物

研究药物	TV1001	安慰剂
形式	胶囊剂	胶囊剂
可获得的单位剂量规格	40 和 80 mg	匹配的 40 和 80 mg
给药途径	口服给予	口服给予
供应商	TheraVasc Inc.	TheraVasc Inc.
生产商	UPM Pharmaceuticals 6200 Seaforth Street, Baltimore, MD 21224	UPM Pharmaceuticals 6200 Seaforth Street, Baltimore, MD 21224

在每次研究就诊时，指导受试者退回未使用的研究药物和空包装；清点所有退回的胶囊剂，并以合适的形式记录。顺从性计算为服用的胶囊剂数目除以预期的胶囊剂数目。如果受试者服用比预期少的胶囊剂，则现场人员应劝告受试者有关 IP 顺从性的重要性。研究人员负责收回并适当保存研究药物，并且负责保持记录产品场地递送、场地库存、分发产品给每个受试者，并在研究结束时将产品退回 TheraVasc 或指定人员。按照指示将所有使用、未使用和部分使用的药物包装退回 TheraVasc 或指定人员。

[0105] 如果有安全性参数的显著改变或被视为与用研究药物治疗有关的重大 AE （即接受活性药物的受试者相对于安慰剂的安全性概况失衡），则停止研究。出于下列原因以及出于可能未列出的其它安全性原因，根据责任研究人员和现场研究团队的意见，使个别受试者退出。万一一个或多个受试者退出，则招募其它受试者以确保适当数目的受试者完成组群。个别受试者退出的具体原因包括但不限于：

● 在任何 SOC 或心脏监测结果中具有一种严重不良事件模式的受试者，由研究人员和 / 或发起人确定。

[0106] ● 在研究参与中任一次正铁血红蛋白值≥ 15% 的受试者。

[0107] ● 具有正常基线血压经历以下任一种的受试者：持续超过 24 小时的至 160 mm Hg 收缩期和 / 或 90 mmHg 舒张期的血压升高、持续超过 24 小时的从基线血压升高 30 mm Hg 收缩期和 / 或 15 mm Hg 舒张期、任何血压的症状性升高。

[0108] ● 具有在基线稳定升高的血压的具有以下任一种的受试者：持续超过 24 小时的至 180 mm Hg 收缩期和 / 或 100 mmHg 舒张期的血压升高、持续超过 24 小时从基线血压升高 20 mm Hg 收缩期和 / 或 10 mm Hg 舒张期、血压的任何症状性升高。

[0109] ● 在有或没有 10 搏动 / 分钟 (BPM) 脉动增加和存在症状的情况下经历基线血压下降≥ 20 mm Hg 收缩期的受试者。

[0110] 跟踪发生需要介入的高血压或低血压的任何受试者直到解决，优选直到撤除任何介入疗法。

[0111] 在该研究的地方没有数据监测委员会 (DMC)，安全性由指定的研究医学专家监督。组成筹划指导委员会，其包括发起方的 CEO、两名具有临床试验经验的临床医生、一名医疗管理专家和一名具有亚硝酸钠及其生物作用专长的研究人员。CPC 向委员会提供有关各地的受试者招募的每月现状报告、本地活动的监管报告和有关试验的其它非安全性信息。由瓶装药盒分配人以盲法方式提供有关分配到各地的药盒数、退回的瓶子和在 IP 的随机化和分配中引起的任何问题的类似报告，确保不向委员会提供有关实际随机化的信息。委员

会应讨论报告，如果注意到任何方案偏差或未遵从研究人员协议或整体研究计划，则立即采取行动以纠正这种偏差，保证顺从性或停止向研究人员发送研究药物，终止研究人员参与研究，要求将所有研究药物退回发起人，并通知FDA。委员会监测各地的受试者增加，需要时停止不能招募受试者的地点，并增加其它地点。委员会在收到可能影响受试者安全性的任何信息的2个历日内会面。委员会与CPC讨论所有安全性信息，并根据21 CFR 312.32条款按需要向FDA和所有的参与临床研究人员报告有关药物安全性的任何信息。委员会按照21 CFR 312.33起草有关研究进展的年报。对于该研究，未计划期中分析。

[0112] *研究就诊*

研究就诊包括以下组分：

筛选

该就诊在就诊1—随机化的14-21天内进行。在进行任何研究专项评价之前，取得签名的知情同意书(ICF)。进行以下筛选评价：(1)知情同意，(2)人口统计学资料，(3)病史和药物史，(4)体检，(5)仰卧生命体征，(6)临床安全性实验室，(7)尿妊娠试验，(8)踝肱指数，和(9)评价纳入/排除标准。

[0113] *就诊1-随机化*

这被视为研究的第0天。在该就诊中，随机分配受试者，并给予第1剂研究药物。进行下列评价：(1)更新病史和药物史，(2) 12导联ECG，(3)尿妊娠试验，(4) FMD(可在就诊1前7天内进行)，(5)生命质量问卷(WIQ和RAND 36)，(6) 6分钟步行试验，(7)评价纳入/排除标准，(8)分配的研究药物(研究药物的给药发生在诊所。受试者留在诊所地点以进行安全性跟踪直到最后一次PK采样完成)，(8) PK采样(给药前和给药后：15、30分钟±5分钟和1、2、4、6小时±10分钟)，(9) MetHb采样(给药前和给药后：15、30分钟±5分钟和1、2、4、6小时±10分钟)，(10) PD生物标志物，(11)体位生命体征，(12)不良事件/同期治疗评价(在给予第1剂后捕获不良事件)，和(13)评价研究停止标准。

[0114] *就诊2(第1天)*

在就诊1第1剂给药的时间后1天(24小时) +/- 4小时进行该就诊。在PK采样前30分钟(+/- 10分钟)，受试者必须在诊所服用早晨剂量的研究药物。进行下列评价：(1)给予早晨剂量的研究药物，(2)临床安全性实验室，(3) PK采样，(4) MetHb采样，(4)体位生命体征，(5)不良事件/同期治疗评价，和(6)评价研究停止标准。

[0115] *就诊3(第4天)*

该就诊在就诊1后4 +/- 1天进行。在PK采样前30分钟(+/- 10分钟)，受试者必须在诊所服用早晨剂量的研究药物。进行下列评价：(1)给予早晨剂量的研究药物，(2)临床安全性实验室，(3) PK采样，(4) MetHb采样，(5)体位生命体征，(6)不良事件/同期治疗评价，(7)评价研究停止标准(如果受试者不符合停止标准但的确遇到MetHb增加至8%或更高，则如下所述安排在第7天的任选的安全性就诊)。

[0116] *任选的安全性就诊(第7天)*

如果受试者在就诊3的MetHb为8%或更高，才进行该就诊。应在就诊1 +/- 1天后7天进行。在MetHb采样前30分钟(+/- 10分钟)，受试者必须在诊所服用早晨剂量的研究药物。进行下列评价：(1)给予早晨剂量的研究药物，(2) MetHb采样，(3)体位生命体征，(4)不良事件/同期治疗评价，和(5)评价研究停止标准。

[0117] *就诊 4（第 14天 ）*

该就诊在就诊 1 后 14 +/- 2 天进行。在 PK 采样前 30 分钟 (+/- 10 分钟),受试者必须在诊所服用早晨剂量的研究药物。进行下列评价 :(1) 给予早晨剂量的研究药物,(2) 临床安全性实验室,(3) PK 采样,(4) MetHb 采样,(5) 尿妊娠试验,(6) 体位生命体征,(7) 不良事件 / 同期治疗评价,(8) 评价研究停止标准,(9) 研究药物顺应性,和 (10) 分配的研究药物。

[0118] *就诊 5（第 28天 ）*

该就诊在就诊 1 后 28 +/- 2 天进行。在 PK 采样前 30 分钟 (+/- 10 分钟),受试者必须在诊所服用早晨剂量的研究药物。进行下列评价 :(1) 给予早晨剂量的研究药物,(2) 临床安全性实验室,(3) PK 采样,(4) MetHb 采样,(5) 体位生命体征,(6) 12 导联 ECG,(7) 不良事件 / 同期治疗评价,(8) 评价研究停止标准,(9) 研究药物顺应性,和 (10) 分配的研究药物。

[0119] *打电话 1*

在就诊 1 后 42 +/- 2 天给受试者打电话。询问受试者有关任何不良事件和同期治疗的变化。

[0120] *打电话 2*

在就诊 1 后 56 +/- 2 天给受试者打电话。询问受试者有关任何不良事件和同期治疗的变化。

[0121] *就诊 6（第 70天 ）*

该就诊在就诊 1 后 70 +/- 2 天进行。在 PK 采样前 30 分钟 (+/- 10 分钟),受试者必须在诊所服用早晨剂量的研究药物。进行下列评价 :(1) 给予早晨剂量的研究药物,(2) 临床安全性实验室,(3) PK 采样,(4) MetHb 采样,(5) PD 生物标志物,(5) 体位生命体征,(6) FMD (可在就诊 6 前 5 天内进行),(7) 生命质量问卷 (WIQ 和 RAND 36),(8) 6 分钟步行试验,(9) 不良事件 / 同期治疗评价,(10) 评价研究停止标准,和 (11) 研究药物顺应性。

[0122] *就诊 7（第 71天 ）*

该就诊在就诊 6 后 1 天 + 1 天进行。在 PK 采样前 30 分钟 (+/- 10 分钟),受试者必须在诊所服用早晨剂量的研究药物 (剂量递增)。进行下列评价 :(1) 分配的研究药物,(2) 给予早晨剂量的研究药物 (在分配和给予研究药物时,按所述指导受试者从 1 粒胶囊剂 BID 增加到 2 粒胶囊剂 BID。受试者留在诊所进行 1 ½ 小时给药后观察),(3) 临床安全性实验室,(4) PK 采样,(5) MetHb 采样 (受试者留在诊所直到可获得结果),(6) 体位生命体征,(7) 不良事件 / 同期治疗评价,(8) 评价研究停止标准 (如果受试者不符合停止标准但遇到 MetHb 增加至 8% 或更高,则在任选的安全性就诊 (就诊 7 + 1) 中,如下述安排在第 70 +2 天的安全性就诊,(9) 安全性评价 (恰在受试者离开之前),(10) MetHb 结果的评价,和 (11) 坐姿生命体征 - 脉搏率和 BP。

[0123] *任选的安全性就诊 (就诊 7 + 1)*

只有受试者在就诊 7 时的 MetHb 为 8% 或更高才进行该就诊。在就诊 7 后 1+1 天进行。在 MetHb 采样前 30 分钟 (+/- 10 分钟),受试者必须在诊所服用早晨剂量的研究药物。进行下列评价 :(1) 给予早晨剂量的研究药物,(2) MetHb 采样,(3) 体位生命体征,(4) 不良事件 / 同期治疗评价,和 (5) 评价研究停止标准。

[0124] *就诊 8—终止 (就诊 7 + 6)*

在就诊 7 后 6 +/- 1 天进行该就诊。在 PK 采样前 30 分钟 (+/- 10 分钟),受试者必须在诊所服用早晨剂量的研究药物。这将是最后的剂量和研究就诊。进行下列评价 :(1) 体检,(2) 临床安全性实验室,(3) PK 采样,(4) MetHb 采样,(5) 尿妊娠试验,(6) 体位生命体征,(7) 12 导联 ECG,(8) 不良事件 / 同期治疗评价,和 (9) 研究药物顺应性。

[0125] *跟踪打电话*

在就诊 8 后 7 +/- 1 天给受试者打电话。如果该受试者提前终止研究,则在提前终止就诊 +/- 1 天后 7 天给受试者打电话。询问受试者有关任何不良事件和同期治疗的变化。

[0126] *提前终止就诊 (ET)*

在受试者在就诊 6 前出于任何原因必须提前从研究参与中退出的情况下,做出各种努力以完成提前终止就诊。在 PK 采样前 30 分钟 (+/- 10 分钟),受试者必须在诊所服用早晨剂量的研究药物,除非受试者出于安全性退出和应立即停止服用 IP。进行下列评价 :(1) 适用时,给予早晨剂量的研究药物,(2) 体检,(3) 临床安全性实验室,(4) PK 采样,(5) MetHb 采样,(6) PD 生物标志物,(7) 体位生命体征,(8) 12 导联 ECG,(9) FMD (可在 ET 就诊前 5 天内进行),(10) 生命质量问卷 (WIQ 和 RAND 36),(11) 6 分钟步行试验,(12) 尿妊娠试验,(13) 不良事件 / 同期治疗评价,和 (14) 研究药物顺应性。此外,如果提前终止发生在就诊 6 之后但在就诊 8 的合适就诊窗之前,则完成就诊 8 所需要的所有程序。

[0127] *受试者的选择和退出*

纳入标准包括年龄介于 35 和 85 岁的受试者。受试者必须是男性或绝经后、绝育或使用合适节育的女性。合适的节育必须是总体节欲 (total abstinence)、男性配偶绝育或与使用口服避孕药、注射孕激素、左炔诺孕酮植入剂、雌激素阴道环、经皮避孕贴剂或子宫内装置 (IUD) 配对的双屏障方法。外周动脉疾病 (PAD) 史通过医疗图表或静息时踝肱指数≤ 0.90 证实。如果受试者接受针对心脏风险因素的医学标准治疗,则受试者在筛选前必须处于稳定治疗持续至少 1 月。如果包括在该方案中,则上个月不显著改变例如西洛他唑、己酮可可碱、他汀类或血管紧张素转化酶 (ACE) 抑制剂的治疗 ;监督下的运动康复训练 ;参与正式戒烟计划或戒烟处方药物,并且预期在研究持续时间内不改变。如果受试者遇到跛行症状,则受试者在筛选前必须有稳定的下肢症状持续至少 1 个月 (例如跛行症状无改变)。要求受试者提供书面知情同意和签名知情同意书记载的意愿。

[0128] 排除标准包括患有非动脉粥样硬化 PAD (例如 Buerger 血管炎)、下肢手术或经皮血管重建的受试者、筛选前最近 6 个月内移植失败的证据或其它外周血管手术程序、治疗期内预期的下肢血管重建、心肌梗死、不稳定心绞痛、筛选前 3 个月内的脑血管事件或短暂性脑缺血发作 (TIA)、糖尿病控制差 (HgA1c > 10.0)、尽管治疗但高血压控制差 (收缩期血压 (SBP) ≥ 160 mmHg 或舒张期血压 (DBP) ≥ 100 mmHg)、现有医疗方案中的收缩期血压≤ 100 mmHg、对亚硝酸钠或相关化合物超敏反应和记录为 eGFR < 30 mL/ 分钟 /1.73 m^2 的肾功能不全 (肾疾病研究中的饮食改进 MDRD)。排除标准还包括是妊娠或哺乳妇女的受试者、预期寿命 < 6 个月的人、研究人员的意见认为与该研究有关的风险可能增加的慢性病、研究人员的意见认为受研究治疗或参与妨碍的需要积极的抗肿瘤疗法的活动性恶性肿瘤 (虽然允许稳定的基细胞皮肤癌,并允许只用激素疗法治疗的癌症)、活动性感染 (即全身性或骨髓炎)、NYHA CHF III 或 IV 类、因急性冠状动脉综合征 (ACS)、心肌梗死 (MI)、充

血性心力衰竭 (CHF) 或中风近期住院 (< 30 天)、在筛选前 1 年内的最新 (< 30 天) 冠状动脉血管重建事先已用血管生成因子或干细胞疗法治疗、在筛选前超过 1 个月内参与另一项 PAD 临床试验、具有暴露的肌腱、肌肉或骨或诊断为严重腿缺血 (CLI)、在筛选前 3 个月内事前已进行截肢或实施计划的可能限制行走的截肢术 (尽管允许小趾)。排除标准还包括其进行 6 分钟步行试验的能力受跛行以外的症状限制、诊断为酒精或其它物质滥用、患有正铁血红蛋白血症史 (metHb ≥ 15%)、不能说英语 (因需要给予标准化英语语言问卷)、有贫血证据或慢性溶血性病况 (包括镰状细胞疾病) 史、长期使用抗偏头痛药 (例如 Imitrex 或舒马曲坦) 和在筛选时葡萄糖 -6- 磷酸盐脱氢酶 (G6PD) 缺乏症阳性筛选的受试者。还排除长期服用下列药物的受试者 :别嘌醇、PDE-5 抑制剂、镇静性三环类抗抑郁药、镇静性抗组胺药、麦啶和相关麻醉性中枢神经系统 (CNS) 抑制药和硝酸盐。

[0129] 退出标准允许受试者在他 / 她自己的要求下在任何时间从研究中退出。如果在研究中继续不是受试者的最大兴趣是研究人员的意见,则受试者也可在研究人员的要求下退出。如果他或她符合上述停止标准,则受试者退出。万一受试者出于任何原因退出研究,应做适当努力跟踪受试者以确定其退出研究的原因,并进行上述提前终止就诊。打电话、确认信和提供交通协助被视为适当努力。表 3 提供受试者退出概要。

[0130] 表 3. 受试者退出

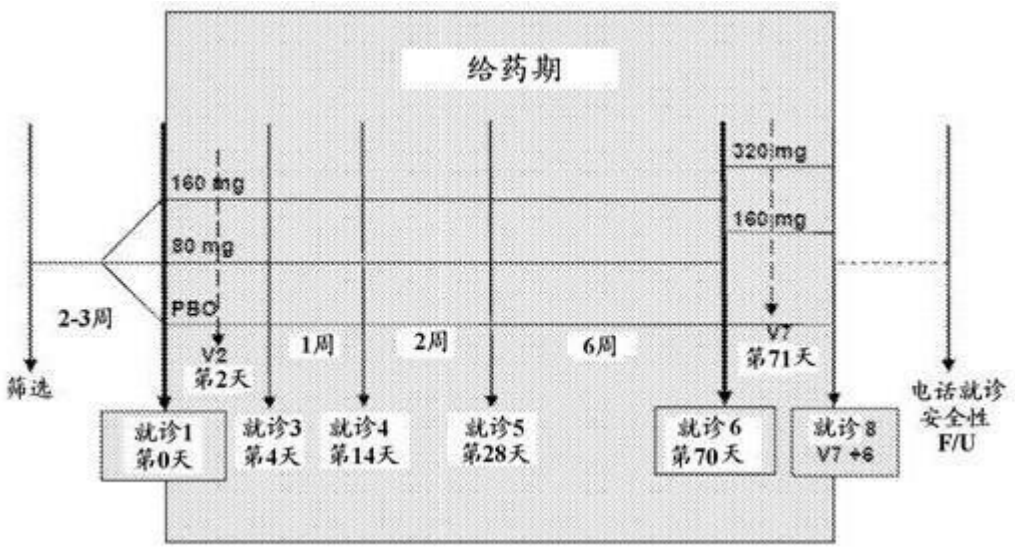
受试者分配概要

	安慰剂 n=18	40 mg n=19	80 mg n=18
完成研究的受试者	15(83.3%)	17(89.5%)	15(83.3%)
在完成前退出的受试者	3(16.7%)	2(10.5%)	3(16.7%)
退出原因:			
不良事件	0(0.0%)	1(5.3%)	2(11.1%)
符合退出—新的低血压	1(5.6%)	0(0.0%)	1(5.6%)
受试者要求—精力不足	0(0.0%)	1(5.3%)	0(0.0%)
受试者要求—拒绝继续	1(5.6%)	0(0.0%)	0(0.0%)
受试者要求—无利益	1(5.6%)	0(0.0%)	0(0.0%)

受试者的治疗

3 个给药组是下表 4 所示的安慰剂、40 mg BID 和 80 mg BID 亚硝酸钠。所有剂量以一天两次口服剂量给予 10 周。在 10 周给药期和完成功效评价后当天,各治疗组的受试者进入 6 天剂量增加期 (剂量倍增)。40 mg 亚硝酸钠 BID 组的受试者的剂量增加到 80 mg 亚硝酸钠 BID 达 6 天,80 mg 亚硝酸钠 BID 中的受试者的剂量增加到 160 mg 亚硝酸钠 BID 达 6 天。安慰剂受试者 BID 服用安慰剂胶囊剂数倍增。在 6 天剂量增加期结束时停止所有研究药物。

[0131] 表 4. 给药组



禁止长期服用 Imitrex（舒马曲坦）、别嘌醇、PDE-5 抑制剂、镇静性三环类抗抑郁药、镇静性抗组胺药、麦啶和相关麻醉性 CNS 抑制药和硝酸盐的受试者参与该研究。

[0132] 在每次研究就诊时，指导受试者退回未使用的研究药物；清点所有退回的胶囊剂，并以适当的形式记录。顺从性计算为服用的胶囊剂数目除以预期的胶囊剂数目。通过用瓶中的胶囊剂数目 50 减去剩余的胶囊剂数目，计算服用的胶囊剂的数目。如果受试者服用比预期少的胶囊剂，则现场人员劝告受试者有关 IP 顺从性的重要性。在研究结束时，研究人员负责收回并适当保存研究药物，以及保持递送到场的产品、场地库存、分发产品给每个受试者和将产品退回 TheraVasc 或指定人员的记录。按照指示，将所有使用、未使用和部分使用的药物包装退回 TheraVasc 或指定人员。

[0133] *功效评价*

功效参数包括：(1) 血流介导的血管扩张 (FMD)、6 分钟步行试验、药代动力学 (PK)、生物标志物 / 药效学 (PD) 标志物和生命质量 (QoL) 问卷。

[0134] 对本发明重要的是有关生命质量的反应。生命质量通过以下两个问卷测量：WIQ 和 RAND 36。按相同顺序给予两个问卷：先是 WIQ，接着是 RAND 36。WIQ 是测量基于社区的步行的疾病专用工具。问卷由 4 个分量表（疼痛严重性、距离、速度和楼梯）组成。WIQ 由研究人员或指定人员口头给予受试者。RAND 36 是测量一般健康状况问题的工具。研究人员指导受试者自己完成 RAND 36。研究人员不会试图向受试者解释问题。如果受试者不理解具体问题，则研究人员告知受试者尽他的或她的最大能力解释问题的含义，并提供对受试者显得是最准确的答案。不允许家庭成员或其它个体替受试者回答问题或完成问卷。所有问卷直接在书面源文件页上完成。因此，研究协调人检查所有问卷以确保每个问题只有一个回答、每个问题都有回答和任何必需的更正都经研究人员（或指定人员）或受试者用首字母签名并注明日期。RAND 36 身体和心理评价的结果详见图 1A-B。RAND 36 显示在 40 mg 组中生命质量评价改善和疼痛评价显著改善的趋势。WIQ 评价的结果详见图 2A-B。WIQ 显示步行距离的评价无改变和步行速度和爬楼梯改善的趋势。

[0135] *安全性评价*

评价了下列安全性参数：病史和药物史、同期治疗使用、身体检查、生命体征、12 导联 ECG、临床化学法、CBC、尿分析和不良事件。针对没有手术绝育的女性的怀孕可能性完成

尿妊娠测试。在给予各剂量水平的亚硝酸钠的第 1 剂后，进行了急性不良事件（即血压下降、眩晕）的评价。剂量限制性毒性（DLT）定义为 3 级和临床上显著的血液学事件，特别是 MetHb。

[0136] 总的来说，在治疗组中未观察到严重的不良反应。观察到剂量依赖性低血压作用，证明了治疗的血液动力学作用。此外，甚至在 160 mg 剂量增加时，也不涉及正铁血红蛋白水平。

[0137] 在筛选就诊时获得人口统计信息（表 5）和完整医疗史（表 6）。记录了任何进行性疾病和筛选前 5 年的病史和过去 1 个月的药物史。在随机化之前与受试者回顾病史和药物史以确保所有数据迄今正确和完整。

[0138] 表 5. 人口统计数据

		安慰剂 n=18	40-mg n=19	80-mg n=18
在知情通知书时的年龄(岁)		64.9 +/- 8.98	65.3 +/- 8.86	67.9 +/- 9.99
性别	男性	13(72.2%)	15(78.9%)	13(72.2%)
	女性	5(27.8%)	4(21.1%)	5(27.7%)
人种/种族				
	黑人或非洲裔美国人	5(27.8%)	6(31.6%)	8(44.4%)
	白人	12(66.7%)	12(63.2%)	10(55.6%)
	其它	1(5.6%)	1(5.3%)	0(0.0%)
体重(kg)		88.07 +/- 27.24	79.32 +/- 13.53	88.99 +/- 16.70
身高(cm)		173.18 +/- 13.29	172.01 +/- 9.87	172.18 +/- 9.95
筛选 BMI (kg/m2)		29.32 +/- 8.31	26.71 +/- 2.99	30.01 +/- 5.03
筛选时标记肢的 ABI		0.56 +/- 0.15	0.62 +/- 0.20	0.69 +/- 0.17
糖尿病诊断		10(55.6%)	14(73.7%)	14(77.8%)
筛选时的 Hb A1c (% Hb)		6.97 +/- 1.48	6.99 +/- 1.27	6.71 +/- 0.94

表 6. 病史背景

	安慰剂 N=18	40-mg N=19	80-mg N=18
最近 5 年的 PAD	18(100%)	19(100%)	18(100%)
最近 5 年的外周血管重建	8(44.4%)	2(10.5%)	8(44.4%)
最近 5 年的冠状动脉疾病	6(33.3%)	5(26.3%)	7(38.9%)
心绞痛	2(11.1%)	0	4(22.2%)
心肌梗死	0	2(10.5%)	2(11.1%)
最近 5 年的冠状动脉血管重建	1(5.6%)	0	4(22.2%)
充血性心力衰竭	1(5.6%)	0	1(5.6%)
最近 5 年的脑血管疾病	2(11.1%)	3(15.8%)	5(27.8%)
缺血性中风	0	1(5.3%)	1(5.6%)
TIA，小中风	1(5.6%)	0	1(5.6%)
高血压	16(88.9%)	18(94.7%)	16(88.9%)
血脂异常	15(83.3%)	18(94.7%)	16(88.9%)
1 型糖尿病	0	1(5.3%)	0
2 型糖尿病	10(55.6%)	12(63.2%)	12(66.7%)
深静脉血栓形成/肺栓塞	0	0	2(11.1%)
支架/气囊/分流术	5(27.8%)	0	1(5.6%)

在筛选就诊时测量了 ABI 评价，以评价按照纳入标准受试者是否合适。只有在受试者在仰卧位休息至少 10 分钟后才进行 ABI 评价。ABI 定义为较高的两足收缩血压（足背和胫后肌）和较高的两臂收缩压之间的比率。介于 5 和 10 MHz 之间的连续波多普勒用来测量每条腿的足背和胫后动脉两者以及每只臂的肱动脉的收缩压。较高的两臂血压和两腿较高的两踝血压用于计算。计算两腿的 ABI。ABI 在至少一肢中必须小于 0.90 才具有研究资格。

[0139] 现场人员记录受试者在随机化进入研究后服用的任何药物，包括处方药、营养补充剂和非处方药物及其用作同期治疗的原因。如果受试者需要用列为禁止的同期治疗的任何药物治疗，则他或她应退出参与研究，并完成 ET 就诊。

[0140] 在筛选时进行全面身体检查，包括身高、体重和下列系统的评价：一般外貌；眼；耳、鼻和喉；头颈；胸肺；心血管；腹部；肌肉骨骼；淋巴；皮肤；神经和四肢。在就诊 8 或提前终止时，跟踪体检评价体重和上述系统的任何变化。在就诊 8 留意到的任何显著变化作为不良事件记录，除非 PI 或指定人员另有说明。

[0141] 在筛选就诊时测量仰卧生命体征。在获取生命体征测量值前，受试者以仰卧位休息最少 3 分钟。生命体征包括 BP 和脉搏率。在所有研究就诊中在第 1 剂 IP 给药后记录体位生命体征，包括仰卧和站立测量的血压和脉搏率。测量如下进行：(1) 受试者以仰卧位休息最少 3 分钟，(2) 在受试者为仰卧的同时测量生命体征 (BP 和脉搏率)，(3) 受试者采取立姿最少 5 分钟，和 (4) 在受试者站立时测量生命体征 (BP 和脉搏率)。脉搏率和血压数据详见表 7。

[0142] 表 7. 脉搏率和血压

		筛选	就诊 1	就诊 2	就诊 3	就诊 4	就诊 5	就诊 6	就诊 7	就诊 8
仰卧(均值)										
脉搏率	安慰剂	73.6	74.4	73.0	71.0	74.8	74.6	73.1	73.1	75.6
	40-mg	71.4	74.1	74.1	74.7	71.7	72.7	70.4	73.1	70.2
	80-mg	63.9	65.6	65.1	66.7	64.6	68.7	65.5	64.3	68.3
血压	安慰剂	141.3/77.9	141.4/78.3	139.9/78.4	138.4/77.4	137.8/75.4	140.3/76.9	145.4/79.5	139.8/77.8	136.1/75.1
	40-mg	136.8/75.8	129.7/72.3	128.0/70.5	129.8/72.5	128.4/71.1	124.1/72.0	127.3/73.7	126.7/71.3	130.0/72.2
	80-mg	132.4/69.4	129.8/68.4	122.8/66.7	127.1/66.7	125.1/65.2	124.6/68.9	123.1/66.2	118.4/64.4	120.7/66.9
站立(均值)										
脉搏率	安慰剂		78.1	77.8	74.9	78.1	78.5	76.0	76.1	77.4
	40-mg		75.8	78.6	76.7	75.6	76.6	73.9	76.3	74.3
	80-mg		72.6	72.3	72.9	72.6	72.5	67.6	70.4	72.6
血压	安慰剂		141.6/81.7	144.3/81.2	139.9/75.4	139.9/80.3	137.7/79.4	143.3/78.4	141.3/78.7	138.2/75.0
	40-mg		129.5/73.1	128.3/72.6	129.4/73.1	124.9/71.0	124.3/71.6	127.6/73.2	122.2/73.2	123.1/68.7
	80-mg		125.4/70.2	123.1/71.9	124.8/69.2	123.7/68.8	119.5/71.9	124.8/70.0	123.3/67.1	117.7/67.9
直立变化										
脉搏率	安慰剂		3.7	4.8	3.9	3.3	3.9	2.9	3.0	1.8
	40-mg		1.8	4.5	2.0	3.9	3.8	3.5	3.2	4.1
	80-mg		6.9	7.3	6.2	7.9	3.8	2.1	6.1	4.3
收缩 BP	安慰剂		0.2	4.3	1.6	2.1	-2.6	-2.1	1.5	2.2
	40-mg		-0.3	0.3	-0.4	-3.4	0.3	0.3	-4.5	-6.9
	80-mg		-4.4	0.3	-2.3	-1.4	-5.2	1.6	4.8	-3.0
舒张 BP	安慰剂		3.3	2.8	1.9	4.9	2.5	-1.1	0.9	-0.1
	40-mg		0.8	2.1	0.6	-0.1	-0.4	-0.5	1.9	-3.5
	80-mg		1.8	5.2	2.5	3.5	3.0	3.8	2.7	1.0

在表 1- 评价方案中所列时间点上获取仰卧位受试者的静息 12 导联 ECG 输出结果。由 PI 或有资格的指定人员评价所有 ECG 的任何异常情况或改变的临床显著性，并记录在 ECG 源文件上。记录在第 1 剂亚硝酸钠后出现的任何临床显著性异常情况作为 eCRF 上的 AE。在生命体征后立即获取 12 导联 ECG，在给药前采集的就诊 1 随机化日 ECG 除外。表 8 提供了 ECG 数据详情。

[0143] 表 8. ECG

	就诊1	就诊5	就诊8
心率(次/分钟)			
安慰剂	72.1 +/- 13.9	71.7 +/- 15.1	73.0 +/- 12.2
40-mg	71.4 +/- 12.7	72.2 +/- 14.8	
80-mg	62.7 +/-10.7	65.5 +/- 11.9	74.3 +/- 16.9
160-mg			64.7+/- 10.0
QTcB间期(msec)			
安慰剂	433.2 +/- 33.0	430.9 +/- 24.0	438.6 +/- 35.3
40-mg	415.9 +/- 49.0	430.1 +/- 34.8	
80-mg	422.3 +/- 34.0	411.6 +/- 49.7	423.2 +/- 40.3
160-mg			427.7 +/- 31.9
QTcF间期(msec)			
安慰剂	421.2 +/- 31.4	419.7 +/- 22.5	425.4 +/- 33.9
40-mg	404.8 +/- 44.9	417.7 +/- 24.0	
80-mg	419.9 +/- 30.5	406.2 +/- 46.2	409.5 +/- 34.3
160-mg			422.8 +/- 27.9

QTc改变> 60 msec: 严重; QTc改变> 30 msec: 可疑

在表 1 所列时间点上收集实验室评价。所有安全性临床实验室测试通过中心实验室进行,现场完成的尿妊娠试验和正铁血红蛋白除外。将样本样品从研究现场送往中心实验室。如果任何女性未经手术绝育或不是绝经后,则在表 1 所列时间点上进行尿妊娠试验。

[0144] 对受试者空腹进行临床实验室,包括 :尿分析 :蛋白质试纸、比重、外观、pH、葡萄糖、血液、胆红素、酮和显微镜检查。临床化学组包括 :白蛋白、碱性磷酸酶、血清淀粉酶、ALT、AST、BUN、钙 (血清)、血清氯化物、CO2、血清肌酸酐、直接胆红素、γ -GT、葡萄糖、LDH、血清磷、钾、钠、总胆红素、总蛋白质、尿酸、总胆固醇、LDL、HDL、甘油三酯和 HbA1c (仅筛选)。血液组包括 :WBC、RBC、Hb、Hct、MCV、MCH、MCHC、血小板、RDW。

[0145] 在该研究中不是绝经后或绝育的女性受试者要求采用以下节育方法 :定义为与受试者优选和平常的生活方式一致的性生活不活跃的总体节欲、周期性禁欲 (例如日历法、排卵法、症状体温法、排卵后法) 且体外排精法是不可接受的、在女性受试者进入研究前男性配偶绝育且该男性是受试者的唯一配偶、定义为与下列避孕药组合的避孕套和闭塞帽 (子宫帽或宫颈 / 宫颈穹窿帽) 加杀精子剂 (泡沫剂 / 凝胶剂 / 膜剂 / 乳膏剂 / 栓剂) 的双屏障方法 :

- 口服避孕药,结合孕激素或仅孕激素
- 注射孕激素
- 左炔诺孕酮植入剂
- 雌激素阴道环
- 经皮避孕贴剂
- 符合产品标签所表明的 <1% 失效率的子宫内装置 (IUD) 或子宫内系统 (IUS)

在研究期间怀孕的任何受试者在该研究中无资格继续,此时结束研究程序。男性受试者和他们的配偶理应采取合适的节育方法或避免性交。如果男性受试者的配偶在研究监测期的进程中怀孕,则他们同意立即通知研究人员。

[0146] 将有关任何女性受试者或男性受试者的配偶 (如果她愿意) 在该研究监测期内怀

孕的完整的妊娠信息（包括妊娠结果）收集在源文件中。在没有并发症时，跟踪应不长于在分娩日期后 6-8 周。报告任何早产终止，不论选择的、治疗性的还是自发的。虽然妊娠本身不被视为不良反应，但任何妊娠并发症，包括自发终止或出于医学原因的选择终止，都应报告为不良反应。自然流产被视为 SAE。向发起人报告因研究后妊娠所致发生的和研究人员认为与研究产品相当有关的任何 SAE。

[0147] 按国际协调会议（International Conference on Harmonisation，ICH）的定义，AE 是在给予研究产品的患者或临床研究受试者中的任何不利的医学事件，不论事件是否被视为与研究产品有关。因此 AE 是暂时与使用研究产品有关的且在给予 IP 时开始收集的任何不利的和非预期的病征（包括异常实验室结果）、症状或疾病（新的或恶化的）。AE 的实例包括在给予研究产品后新近检出或诊断的病况，包括已知可能已存在但在开始研究前未检出的病况；已知在研究开始前已存在而在给予研究产品后恶化的病况；疑似药物相互作用的病征、症状或临床后遗症；和疑似研究产品或同期药物过量的病征、症状或临床后遗症（过量本身不报告为 AE）。不视为 AE 的问题的实例包括：医学或外科手术（例如内镜检查、阑尾切除术）；如果按照上述定义它符合要求的话，则导致手术的病况是 AE；其中不利医学事件未发生的情况（例如社交、观察、诊断或便利住院）；未表现出临床显著恶化的在开始研究前存在或检出的先存在的疾病或病况的波动；和 PI 或具有适当医学训练的助理研究人员未评价为临床显著的异常实验室或试验结果。不良事件的概要详见表 9。

[0148] 表 9. 不良事件概要

	安慰剂	40 mg	80 mg
总体:			
具有至少1个AE的受试者的数目(%)	9(50.0%)	12(63.2%)	14(77.8%)
具有至少1个AE的受试者的数目(%)	9(50.0%)	12(63.2%)	14(77.8%)
AE的数目	15	32	40
TEAE的数目	15	32	39
SAE的数目	2	0	0
根据严重性的TEAE的数目			
轻微	12	26	31
中度	3	6	8
根据与研究药物的关系的TEAE的数目			
无关	12	10	9
可能有关	3	22	24
大概有关	0	0	6
仅6天剂量增加期:			
具有至少1个TEAE的受试者的数目(%)	2(11.1%)	3(15.8%)	7(38.9%)
TEAE的数目	2	3	11

统计方法

通过剂量水平和整体以表格形式概括人口统计数据、临床化学、CBC、生物标志物和不良事件。应用描述统计学概括人口统计和临床数据，例如 ECG 和生命体征。对高于或低于正常限度的实验室值标记，通过 SOC、严重性和与研究治疗的关系表现不良事件。

[0149] 应用非配对 t 检验，主要功效分析比较了在 10 周治疗后合并药物和安慰剂治疗组间 FMD 的基线和第 70 天（就诊 6）的变化。在比较组内基本不对称分布的情况下，应用非参数两样品 Wilcoxon 符号秩和检验。对于二分功效终点，检验了虚假设 $H0: rc=rp$ 相对于 $H1: rc \neq rp$，其中 rc 为 BID 组中具有改进结果的受试者比例，rp 是安慰剂组中具有改进结果的受试者比例。组间差异用 x 方检验或费希尔精确检验（Fisher exact test）检验。次

要分析采用基于广义估计方程的重复测量 ANOVA 以并入时间、组和相互作用。其它混淆变量包括在基线协变量框架中。按照主要功效分析所述，进行次要终点（例如 6- 分钟步行和 QoL 问卷）的分析。在揭盲前进行所有统计决策。

[0150] 另外，对亚硝酸钠的血浆水平制表并作图作为对数剂量反应曲线。通过剂量和总体对功能参数制表。计算汇总统计，适当时对各参数制作对数剂量反应曲线。

[0151] 展开统计学分析计划以详细描述统计学方法，特别是目标对比，且另外包括治疗组的主要功效终点的任何初步或未调整分析。

[0152] 以 50 名受试者（n=34 名亚硝酸钠；n= 16 名安慰剂）的总样品大小，该研究对于 FMD 的功效终点具有约 82% 效能检出亚硝酸钠（合并组）与安慰剂相比的均值的差异，两侧水平显著性为 0.050。具体地说，在合并的亚硝酸钠组中约 34 名受试者和安慰剂组中 16 名受试者的情况下，该研究具有 82.19% 效能检出在 10 周治疗后亚硝酸钠治疗的受试者与安慰剂治疗的受试者相比的 FMD 反应中的 1.4% 差异，其标准差 (SD) 为 1.6%。因此对于该早期临床研究，凭经验确定该样品大小足够。对于退出，至多 60 名受试者 (20 名受试者 / 组）的样品大小足以满足根据需要的退出以达到每组约 17 名受试者的最终样品大小。将末次观测值结转法 (LOCF) 应用于遗漏数据。

[0153] 实施例 2:特定患者群中疼痛评价的临床研究

研究基本原理和概要

如实施例 1 中所述，研究了作为用于改善患有 PAD 的受试者功能的新疗法的亚硝酸钠。在功效评价期间，进行了生命质量 (QoL) 问卷，其表明服用 40 mg 亚硝酸钠的受试者组显示疼痛显著改善。因此该剂量范围研究的总体目的是评价与将多剂量的口服亚硝酸钠给予特殊患者群（例如患有 PAD、糖尿病性周围神经病的受试者或本文所述任何神经性疼痛的受试者）有关的不同疼痛区域的改善。

[0154] 该临床研究的主要目的是评价亚硝酸钠在减轻神经性疼痛中的功效和多剂量的一天两次 40 mg 和 80 mg 亚硝酸钠与安慰剂相比在指定治疗期内的安全性和耐受性。在该研究中，在标准测试中进行了生物疼痛活性和与疼痛有关的症状的多项评价。评价包括：神经传导研究、感觉神经测试、疼痛调查表、功能状态测定和疼痛调查。

[0155] 主观终点包括：从参与研究的受试者收集的简明疼痛调查表、疼痛日记、抑郁和功能状态调查和神经性疼痛调查。客观终点包括：神经传导研究、体检、两点分辨测试、通过体检的感觉神经测试。参与研究的各受试者将在整个研究中每月一次接受脉搏血氧测定以表明没有正铁血红蛋白血症。

[0156] 试验类型是靶向特殊患者群（例如患有 PAD、糖尿病性周围神经病的受试者或本文所述任何神经性疼痛的受试者）的随机双盲安慰剂对照剂量范围平行设计多剂量研究。试验可具有 3 组，各组约 10 名受试者。按照研究开始之前产生的随机方案，将受试者分配到安慰剂或亚硝酸钠治疗组。使受试者随机进入研究以接受在这些临床研究使用的实施例 1 的安慰剂、40 mg BID 或 80 mg BID 研究产品的治疗方案之一。

[0157] 其它实施方案

虽然结合其具体的实施方案描述了本发明，但应了解能够具有其它修改，且本申请意在包括总体上遵循本发明的原则、包括在本发明所属领域内已知或常规的实践范围内并且可适用于上文阐述的基本特征的本公开内容的这样的偏离的本发明的任何变化、用途或改

动。

[0158] 本文引用的所有参考文献、专利、专利申请公开文本和专利申请均通过引用结合到本文中，程度就像这些参考文献、专利、专利申请公开文本和专利申请通过引用分别予以结合一样。

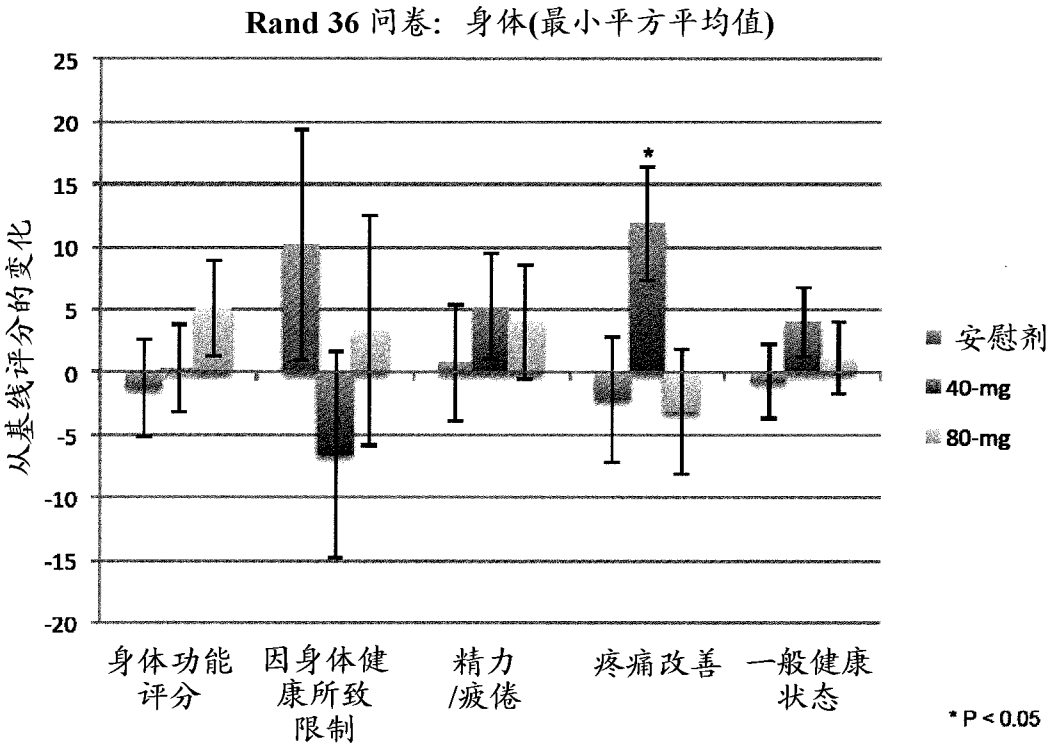

Rand 36 问卷: 身体(最小平方平均值)
从基线评分的变化
25
20
15
10
5
0
-5
-10
-15
-20
*
身体功能评分
因身体健康所致限制
精力/疲倦
疼痛改善
一般健康状态
安慰剂
40-mg
80-mg
* P < 0.05

图 1A

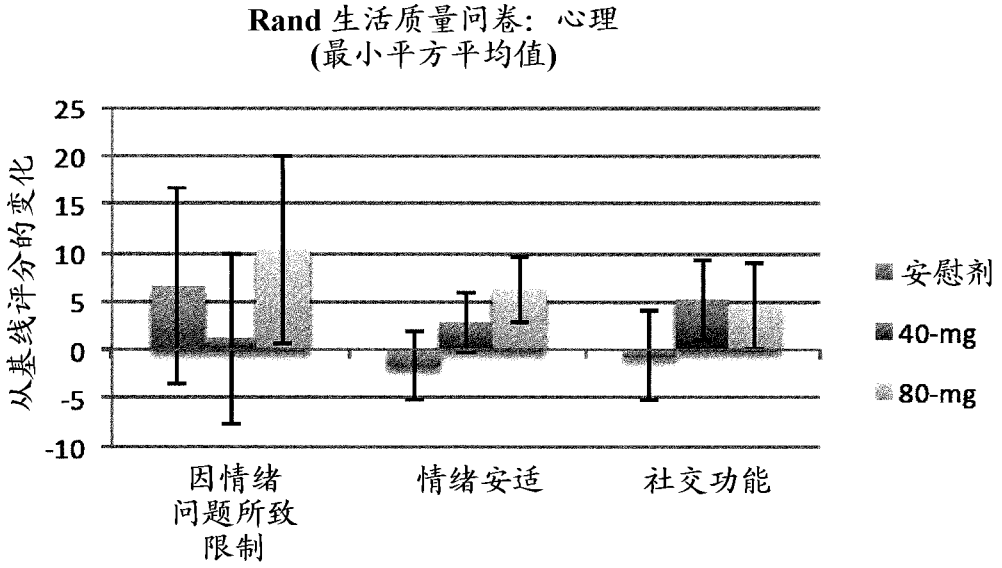

Rand 生活质量问卷: 心理
(最小平方平均值)
从基线评分的变化
25
20
15
10
5
0
-5
-10
因情绪问题所致限制
情绪安适
社交功能
安慰剂
40-mg
80-mg

图 1B

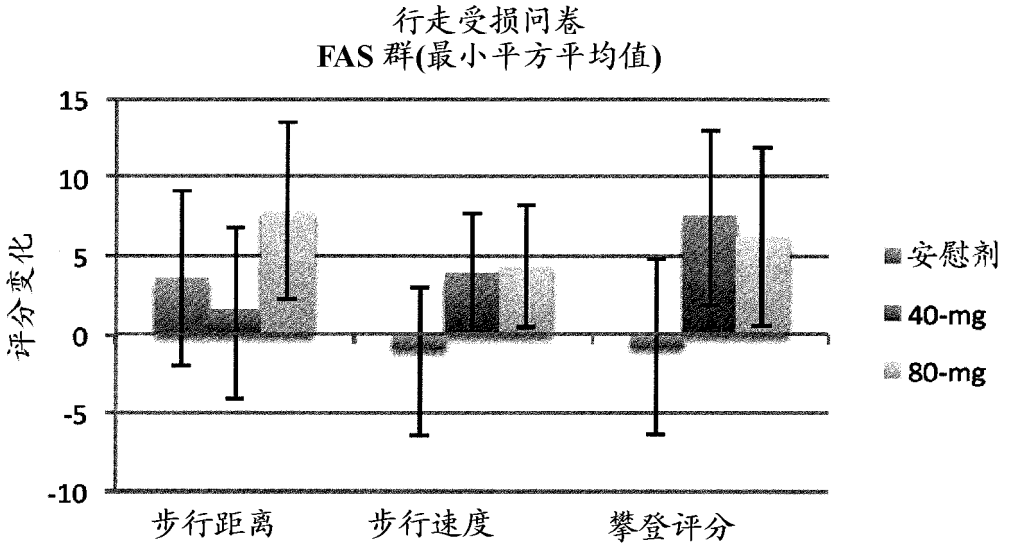

行走受损问卷
FAS 群(最小平方平均值)
15
10
5
0
-5
-10
评分变化
步行距离
步行速度
攀登评分
安慰剂
40-mg
80-mg

图 2A

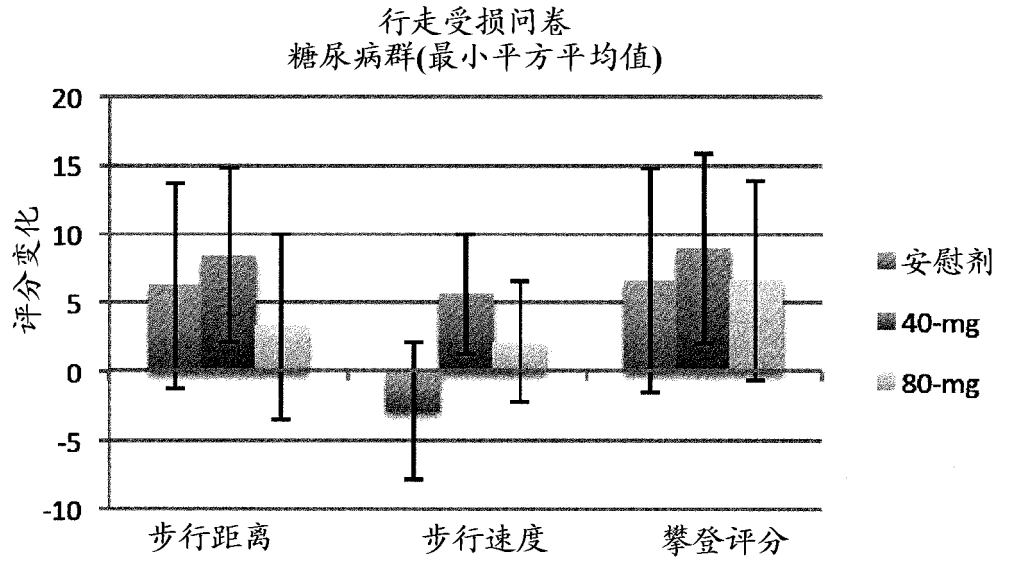

行走受损问卷
糖尿病群(最小平方平均值)
20
15
10
5
0
-5
-10
评分变化
步行距离
步行速度
攀登评分
安慰剂
40-mg
80-mg

图 2B