Improved Pharmaceutical Botulinum Toxin Compositions

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

TECHNICAL FIELD OF THE INVENTION

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

BACKGROUND OF THE INVENTION

Botulinum neurotoxin, a toxin isolated from a strain of *Clostridium botulinum*, a deadly toxin at higher concentrations and quantities, has been used as a valuable therapeutic for the treatment of many neuromuscular diseases (*e.g.*, dystonia, hemifacial spasm, bruxism, spasticity, cerebral palsy, torticollis), as well as sensory disorders and cutaneous disorders (myofacial pain, migraine, tension headaches, neuropathy, hyperhydrosis).

Prior to this invention, the *in vivo* binding of albumin to botulinum toxin has never been identified as important to clinical effectiveness of botulinum-toxin-based pharmaceuticals. By enhancing regional sequestration of the neurotoxin and facilitating saturation of neurotoxin receptors on neural tissues, high-concentration-albumin formulations improve the clinical effectiveness of botulinum toxin and reduce side effects such as those resulting from diffusion of the botulinum toxin from the site of administration. There has been no prior suggestion that altering the formulation of botulinum toxin by increasing its concentration relative to the neurotoxin could enhance the effectiveness for the treatment of human disease. The existing botulinum toxin preparations currently available for clinical practice are BOTOX®, DYSPORT®, MYOBLOC®. The present invention identifies the mechanism and provides compositions of improved utility of botulinum-toxin-based pharmaceuticals by increasing the concentration of a sequestration agent and other viscous agents to enhance sequestration and improve the effectiveness where other available botulinum toxin preparations have failed.

In recent years, Borodic *et al.* have characterized the regional effect of botulinum toxin using muscle fiber morhphometrics, cholinesterase staining, and cutaneous wrinkling

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from depression of facial muscle tone. (Borodic (1992) Botulinum A toxin for (expressionistic) ptosis overcorrection after frontalis sling. *Ophthalmic Plastic and Reconstructive Surg.* 8(2): 137-142; incorporated herein by reference in its entirety).

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

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

Formulation	Essential Blepharospasm	Torticollis
BOTOX®	50 U[1]	200 U
DYSPORT®	200 U	600-1,200 U
MYOBLOC®	3,000-5,000 U	10,000-15,000 U

[1]Units (U) are LD_{50} units determined using 20-30 g Swiss-Webster mice, as described herein.

A. Complications Associated with Conventional Botulinum-Toxin Formulations.

Beyond effective dose requirements, substantial differences in the complication rate have been noted at therapeutic quantities of different botulinum preparations. Side effects such as those resulting from diffusion of the botulinum toxin from the site of administration appear to be dependent on the formulation of botulinum toxin. For instance, dysphagia rates (difficulty swallowing) is a well-known complication of botulinum toxin administration when used for the treatment of cervical dystonia. (Borodic *et al.* (1990) Botulinum A toxin for the treatment of spasmodic torticollis. *Dysphagia and Regional Toxin Spread. Head & Neck*, 12: 392-398; incorporated herein by reference in its entirety). Differences in the rate of this

complication between formulations has been well appreciated when reviewing prior art literature between 1984-1995. Furthermore differences in the rate of ptosis have been reported when comparing various immunotypes and different preparations of the same immunotype (see Table 1). It has become well accepted that this complication is the result of diffusion of botulinum toxin away from the injections sites, a property which is in conflict with the clinical goal of containing the denervating or biologic effect to a specific target region.

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

Complication	BOTOX®	DYSPORT®[2]	MYOBLOC®[3]
Ptosis[1]	<2%	12-15%	30-40%
Dysphagia	<2%	14-21%	10-17%

[1]Nussgens *et al.* (1997) Comparison of two botulinum-toxin preparations in the treatment of essential blepharospasm. *Graefes Arch Clin Exp Ophthalmol* 235(4): 197-199.

[2]Phase 3 Studies 1998-1989 for Oculinum Meta-analysis of clinical studies on Dysphagia and Botulinum 1995 at NIH (Borodic).

[3]Lew *et al.* (1997) Botulinum toxin type B: a double-blind, placebo-controlled, safety and efficacy study in cervical dystonia. *Neurology* 49(3): 701-707.

In 1991, Borodic *et al.* demonstrated a histological model demonstrating a histochemical and morphologic diffusion gradient from point injections of botulinum toxin. (Borodic *et al.* (1991) Botulinum toxin: Clinical and scientific aspects. *Ophthalmology Clinics of North America* 4: 491-503; incorporated herein by reference in its entirety). The gradient was further demonstrated to be dose dependent over single muscle strips and capable of crossing fascial planes. The diffusion model was further demonstrated on the facial wrinkling pattern of the human forehead. (Borodic *et al* (1992) Botulinum toxin for spasmodic torticollis, multiple vs. single point injections per muscle. *Head and Neck* 14: 33-37). Diffusion was thereafter used to explain the mechanism for dysphagia after surface injections of botulinum injection for the human neck and ptosis (drooping eyelid complication) after periocular injections for the treatment of essential blepharospasm. Ptosis results from diffusion of neuromuscular blocking activity from the lid edge to the muscular portion of the upper eyelid retractor, which lies in the upper orbital space. Dysphagia results from diffusion of neuromuscular weakening effect from the sternomastoid muscle, targeted for treatment of torticollis, to peripharygeal musculature which generates the force for effective swallowing. From both histological models and clinical experience, diffusion appears to be directly related to the quantity of toxin given in LD_{50} units, that is, the greater

the LD_{50} units used, the greater the diffusion from a point injection. From literature summary from the 1980's and early 1990's, dysphagia is more common with use of DYSPORT® than BOTOX® at effective doses. Recently, from studies done at European centers, the differences in dysphagia rates have been confirmed (Ranoux *et al.* (2002) Respective potencies of DYSPORT® and BOTOX®: a double blind, randomized, crossover study in cervical dystonia. *J. Neurol. Neurosurg. Psychiatry* 72: 459-462). Differences in ptosis rates for the treatment of blepharospasm have also been observed comparing BOTOX® with DYSPORT® with BOTOX® demonstrating less common incidence of this complication (Nussgens *et al.* (1997) Comparison of two botulinum-toxin preparations in the treatment of essential Blepharospasm. *Graefes Arch Clin Exp Ophthalmol.* 235(4): 197-199). Major differences in the ptosis complication have been reported when using botulinum toxin type B for the treatment of glabellar and forehead wrinkles when compared to botulinum type A (BOTOX®). (Holck *et al.* Comparison of High Dose Botulinum Toxin Type B to Botulinum Type A in the Treatment of Lateral Canthal Rhytides American Society of Ophthalmic Plastic and reconstructive Surgeons Annual Meeting , Anaheim, CA 11-14-03).

B. Sequestration.

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

BOTOX® and DYSPORT® are derived from different strains of Clostridial species. BOTOX® is derived from the Hall strain of *Clostridium botulinum* originally maintained by the University of Wisconsin, whereas DYSPORT® is derived from British Microbiology Collection. Immunological cross reactivity exists between the products as both products were derived from immunotype A strains. Despite similar immunotypes, the clinical responses between BOTOX® and DYSPORT® may be explained by the differences in the

excipients used in each formulation. The difference in human serum albumin concentrations between BOTOX® and DYSPORT® are outlined in Table 3.

Table 3. Human Serum Albumin content of various pharmaceutical formulations of botulinum toxin.

Formulation	Albumin[1]	LD_{50}/μg albumin
BOTOX®	500 μg	0.2
DYSPORT®	125 μg	5.0

[1]Albumin is represented in mg per 100 LD_{50} units of botulinum toxin. Other differences exist including the presence of stabilizing sugars, Lactose is used in DYSPORT® and not used in BOTOX®.

The albumin discrepancy between BOTOX® and DYSPORT® is almost identical to the difference in dose requirements observed between BOTOX® and DYSPORT® in multiple clinical studies. The correlation between the albumin ratio/clinical potency ratio is further strengthened by changes in pharmacological properties of DYSPORT® when albumin is added to the vials using a mouse hemidiaphram animal model. Wohlfahrt *et al.* noted using this model that adding albumin to one vials of DYSPORT® brought biologic activity higher using the mouse hemi-diaphragm model. (Biglalke *et al* (2001) Botulinum A toxin: DYSPORT® improvement of biological availability. *Exp. Neurol.* 168(1): 162-170). The authors suggested the increased biologic activity resulted from increased stability as measured with the mouse LD_{50} bioassay afforded by the albumin concentration increase. (Biglalke *et al* (2001) Botulinum A toxin: DYSPORT® improvement of biological availability. *Exp. Neurol.* 168(1): 162-170). The authors explained the differences of albumin on the LD_{50} bioassay without reference to mechanism of action in tissues or pharmacolgic-pharmacokinetic importance, that is, *in vivo* albumin binding, enhanced sequestration, and improvement in therapeutic effects. The same authors further observed in a rat-diaphragm preparation, that the addition of albumin to the BOTOX® preparation could not substantially increase regional denervative effects and did not advocate any changes in formulation. The findings of these researchers concluded that there was an effect of the albumin concentration on the LD_{50} measurements however, there work did not demonstrate any increased potency of BOTOX® on regional denervation or that DYSPORT® could be enhance to give any greater denervation potency over BOTOX®. There work was limited by

the in vitro nature of their experiments, that is, using a non blood perfused animal dissection of a motor nerve (phrenic nerve) and diaphragm muscle, which fails to accounts for dilutions and tissue fluid flow capable of washing injected toxin away from targeted tissue prior to binding with the nerve axon terminal receptors. The real time application requires an in vivo analysis of the effects of albumin on regional denervation as outlined in the following experiments. Their work did identify reasons for differences in LD_{50} as measured by the mouse lethality assay. The conclusion were no improvements in potency or effectiveness could be made over existing BOTOX® preparation and is directly contrary to the conclusion derived herein. (Hanover Germany International Botulinum Toxin Meeting 2002).

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

SUMMARY OF THE INVENTION

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

In another embodiment, the present invention provides a composition comprising botulinum toxin and a sequestration agent, wherein the sequestration agent is present in an

amount between 550 and 900,500 μg sequestration agent per 100 LD_{50} units botulinum toxin, wherein the albumin may be formulated as a solid albumin particle.

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

In another embodiment, the compositions of the present invention comprise a botulinum toxin that consists essentially of fractionated-light-chain botulinum toxin. In yet another embodiment, the botulinum toxin consists essentially of a mixture of hybrid and chain-translocated forms of botulinum toxin. In a further embodiment, the botulinum toxin consists essentially of chimeric forms of botulinum toxin. Although the present invention may utilize any botulinum toxin, botulinum toxin fragment that retains neurotoxic activity, botulinum toxin chimeras and hybrids, chemically-modified botulinum toxin, and specific activities well known to those of ordinary skill in the art, in one embodiment the botulinum toxin is purified to a specific activity greater than or equal to about 20 LD_{50} units per nanogram botulinum toxin.

The present invention provides compositions of botulinum toxin and a sequestration agent wherein the ratio of LD_{50} units of botulinum toxin to μg sequestration agent is less than or equal to about 0.2 for botulinum toxin type A and is less than or equal to about 10 for botulinum toxin type B.

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

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

collagen, epinephrine or hyaluronate. In a more preferred embodiment, the sequestration agent is hyaluronate. In the most preferred embodiment, the sequestration agent is albumin.

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

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

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

In one embodiment, the present invention provides methods for producing denervation in a subject suffering from blepharospasm comprising administering between 10-200 LD_{50} units of a composition of the present invention, as described herein. In another embodiment, the present invention provides methods for producing denervation in a subject suffering from torticollis. Preferably, the effective amount of a composition of the present invention is between 10 and 3000 LD_{50} units.

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

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

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

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

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

The present invention also provides a method of treating back pain or arthritic pain in a subject in need thereof, comprising administering an effective amount of a composition of

the present invention, as disclosed herein. Preferably, the effective amount is between 5 and 4,000 LD_{50} units.

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

The present invention provides a method of treating a hyperhyrosis syndrome in a subject in need thereof, comprising administering an effective amount of a composition of the present invention, as disclosed herein. Preferably, the effective amount is between 5 and 4,000 LD_{50} units.

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

The present invention also provides a use of any of the compositions disclosed herein for the treatment of a neuromuscular disease, pain, inflammatory conditions, facial wrinkles, rhytides, cosmetic alteration of lip and brow, and the like. The present invention also provides a use of any of the compositions disclosed herein for the preparation of a medicament for the treatment of a neuromuscular disease, pain, inflammatory conditions, facial wrinkles, rhytides, cosmetic alteration of lip and brow, and the like.

DETAILED DESCRIPTION OF THE INVENTION

The present invention describes a method and composition to enhance the clinical effectiveness of botulinum-toxin preparation for clinical use by means of increasing sequestration of botulinum neurotoxin molecules in the region of the human or mammalian body targeted for therapy through the use of a sequestration agent or "molecular anchor". Enhanced sequestration using higher concentration of macromolecules such as proteins (*e.g.*, albumin, collagen and the like), and/or lipids and/or polysaccharides (*e.g.*, hyaluronate, and the like) can be useful to provide a molecular anchor to neurotoxin molecules preventing

diffusion away from the injection point, causing maximal saturation of botulinum neurotoxin receptors, thereby achieving greater efficacy with the amount of neurotoxin used to achieve desired clinical effects. The sequestration agent enhances containment of regional denervation, and enhances clinical outcomes. The increased sequestration allows for better delivery to nerve ending, with enhanced uptake and augmentation of denervative and other biologic effects. The invention requires a sequestration agent added to a formulation of neurotoxin which binds to the neurotoxin, prevents dissemination of the neurotoxin and demonstrates improvement in clinical response in patients who were previously treated without the carrier molecule at preferred concentrations. The sequestration agent may be an existing excipient at significantly higher concentrations than previously used (such as human serum albumin), or a material that has not been previously used to stabilize botulinum toxin (such as sodium hyaluronate). The sequestration agent must bind to the botulinum toxin molecule and prevents its diffusion so that the neurotoxin may react with the nerve-terminal ending or any neural structure so that effectiveness of the therapy is improved.

A. Definitions.

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

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

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

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

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

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

As used herein, "subject" means a mammal.

B. Albumin.

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

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

is dose dependent. Saturation of zinc binding on albumin enhances the denervating effect of botulinum toxin.

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

MYOBLOC® is formulated at an acidic pH <6.0 which provides for increased stability and stability of the liquid formulation at room temperature. Unfortunately, the acidic pH has an adverse side effect on the structure and probably tissue carrying properties of the human serum albumin in this biologic drug' s formulation. At varying pH, the isomerization of albumin can be considerable as well as the tertiary configuration of the albumin protein and physical properties (see Peters (1996) All about Albumin. Academic Press, New York; incorporated herein by reference in its entirety). Alterations in physical properties (via changes in binding of botulinum toxin and dynamics of botulinum toxin molecular release in tissues) can be used to explain some of the considerable differences in dose requirements comparing BOTOX® and MYOBLOC® in clinical practice. With higher pH, type B formulation, similar histological effects can be seen with equivalent LD_{50} units (see Borodic *et al.* (1993) Botulinum B Toxin as an Alternative to Botulinum A Toxin, A Histological study. *Ophthalmic Plastic and Reconstructive Surgery* 9(3): 182-190).

Although other proteins (*e.g.* gelatin, lactalbumin, lysozyme), lipids and carbohydrates may serve as effective sequestration agents, albumin, including encapsulated albumin and solid microspheres is the preferred protein sequestration agent, in part, because of its low immunogenicity.

C. Sequestration.

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

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

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

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

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

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

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

Open-Lable Trials	15 patients each receiving 5-15 LD_{50} units	100% demonstrated decreased involuntary movement	No ptosis complication
Double-Blind Placebo Controlled Trials	30 patients (ratio 1:1 treatment/control) each receiving 15 LD_{50} units	1. Degree of involuntary movements significantly better than controls. 2. Subjective parameters significantly better than controls	No ptosis complications

Prior literature has indicated that existing BOTOX® preparations require 20 LD_{50} units to achieve favorable results for this indication. (Borodic *et al.* (1993) Botulinum Toxin for aberrant facial nerve regeneration. Dose response relationships. *Plastic and Reconstructive Surgery*, (91)6: 1042-1045. 1993). Furthermore, there has been a 20% incidence of ptosis (a diffusion complication) associated with the use of botulinum toxin for involuntary blepharospasm, based on a 100 patient study on BOTOX® for the treatment of blepharospasm and using comparable LD_{50} doses (see new batch approval study from Allergan Pharmaceuticals, 1998; incorporated herein by reference in its entirety). Comparing the incidence of this complication in the high-albumin study shown above with the BOTOX® equivalency study (19/99, compared to 0/30, $P<0.01$, Chi Square), it appears that the high-

albumin type A botulinum toxin composition required fewer LD_{50} units to achieve acceptable therapeutic results (reduction in effective amount of toxin) and was associated with limited diffusion into the orbit which frequently results in ptosis. The decreased incidence of this complication indicated sequestration of the effects of botulinum toxin was enhanced by the higher albumin content.

EXAMPLES

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

Example 1: Treatment of blepharospasm.

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

The albumin content of the BOTOX® was altered by adding 5,000 μg human serum albumin to a vial of BOTOX® (100 LD_{50} units). The resulting composition has an albumin concentration of 2,750 μg/cc (0.018 LD_{50}/μg albumin). Administration of 60 LD_{50} units of the high-albumin preparation produced a nearly complete resolution of symptoms. The high-albumin concentration was clinically effective even when used in subsequent administrations (4 injection cycles) for over two years.

Example 2: Treatment of hemifacial spasm.

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

The albumin content of the BOTOX® was increased by adding human serum albumin sufficient to achieve a concentration of 5,250 μg/cc (0.00952 LD_{50}/μg albumin).

Administration of 30 LD_{50} units of the high-albumin preparation proved highly effective and substantially relieved the clinical symptoms.

Example 3: Treatment of hemifacial spasm.

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

The albumin content of BOTOX® was increased by adding human serum albumin sufficient to achieve a concentration of 5,250 μg/cc (0.00952 LD_{50}/μg albumin). Administration of 40 LD_{50} units of the high-albumin preparation proved highly effective and substantially relieved the clinical symptoms.

Example 4: Treatment of benign essential blepharospasm.

The subject is a 72-year-old university president who was diagnosed with benign essential blepharospasm. Four prior injections of the standard BOTOX® preparation failed to achieve any significant improvement. The subject was referred for possible surgical removal of muscle and nerve to weaken muscles necessary for eyelid closure. Instead, a high-albumin preparation of botulinum toxin was administered to the usual injections sites that are specific for benign essential blepharospasm. The high-albumin preparation was produced by adding 12,250 μg/cc (0.004 LD_{50}/μg albumin). Administration of 60 LD_{50} units of the high-albumin preparation achieved excellent results when the administration of the conventional BOTOX® formulation had failed. Three months after the initial administration of the high-albumin botulinum toxin preparation, 40 LD_{50} units of a high-albumin preparation comprising 25,000 μg albumin per 100 LD_{50} units (0.002 LD_{50}/μg albumin) were administered and produced greater than 80% relief of the clinical symptoms of blepharospasm.

Example 5: Treatment of blepharospasm.

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

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

Example 6: Treatment of blepharospasm.

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

Human serum albumin and 0.5 cc Healon® (hyaluronate) were both added to a 100 LD_{50} units of botulinum toxin type A (BOTOX®). The high-albumin preparation produced contained 25,500 μg albumin per 100 LD_{50} units (0.005 LD_{50}/μg albumin). Administration of 60 LD_{50} units reduced the clinically-observed involuntary-eyelid contractions.

Example 7: Treatment of essential blepharospasm.

The subject was a 66-year-old female with essential blepharospasm. Repeated treatment with BOTOX® (type A), using a range between 40 to 300 LD_{50} units, produced no therapeutic benefit. Botulinum toxin type B (MYOBLOC®) was administered at a dose of 10,000 LD_{50} units within the periocular region and also failed to produce any relief. Bilateral-facial neurectomy also failed to produce any substantial relief of symptoms. Additional surgical procedures to remove muscles necessary for eyelid closure were similarly ineffective.

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

Example 8: Treatment of severe chronic blepharospasm.

The subject is an 83-year-old male with severe chronic blepharospasm. The subject had developed ptosis, a diffusion side effect, after repeated treatments with therapeutic doses of conventional botulinum toxin formulations. The emergence of ptosis complicated the

treatment of this subject by requiring lower doses of botulinum toxin. The lower dosing proved less effective.

The patient received an a high-albumin formulation of botulinum toxin that was produced by mixing 25,000 μg human serum albumin 100 LD_{50} units of BOTOX®. The high-albumin preparation contained 12,750 μg albumin per cc (0.004 LD_{50}/μg albumin). Using the high-albumin preparation, 60-70 LD_{50} units were administered with excellent clinical results and no evidence of ptosis after the therapy. The enhanced sequestration of much higher concentrations of botulinum toxin depressed the spread of the neurotoxin into the muscles within the eye socket.

Example 9: Treatment of essential blepharospasm.

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

A high-albumin botulinum toxin composition (20,000 μg albumin per cc; 0.0025 LD_{50} BOTOX®/μg albumin) was administered to the subject with a resultant clinical improvement of the blepharospasm and no diffusion-related side effects (ptosis).

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

Example	Albumin Concentration (μg/cc)	High-Albumin Preparation (LD_{50}/μg albumin/cc)	BOTOX® (LD_{50}/μg albumin/cc)	DYSPORT® (LD_{50}/μg albumin/cc)	MYOBLOC® (LD_{50}/μg albumin/cc)
1	2,750	0.0180	0.2	5	10
2	5,250	0.0095	0.2	5	10
3	5,250	0.0095	0.2	5	10
4	12,500	0.0040	0.2	5	10
	25,000	0.0020			
5	50,250	0.0001	0.2	5	10
6*	10,200	0.0050	0.2	5	10

7	25,000	0.0020	0.2	5	10
8	12,500	0.0040	0.2	5	10
9	20,000	0.0025	0.2	5	10

LD_{50}/mcg albumin/cc for BOTOX®, DYSPORT®, MYOBLOC® given for direct comparison

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

After quantitating the biologic effect by dilution of purified botulinum toxin, a quantity of albumin is added to the lyophilized material in a quantity sufficient to exceed 500 mg per 100 LD_{50}. The increased albumin binds to botulinum toxin and enhances sequestration of the injected neurotoxin providing for better saturation of neurotoxin receptors and improved clinical effect.

Example 11: Preparation of a high-albumin composition of botulinum toxin further comprising hyaluronate.

After quantitating the biologic effect by dilution of purified botulinum toxin, a quantity of albumin is added to the lyophilized material in a quantity sufficient to exceed 500 μg per 100 LD_{50} units. Additionally, another sequestration agent, which further enhances sequestration, is added to keep the botulinum neurotoxin from diffusing away from the injections site. Such a sequestration agent includes but is not limited to a diluted solution of sodium hyaluronate. The increased albumin non-covalently binds to botulinum toxin and an enhances the sequestration of the neurotoxin providing better saturation of neurotoxin receptors and, consequently, an improved clinical effect.

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

After quantifying the denervating effect of a botulinum neurotoxin by dilution of a purified botulinum toxin, albumin is mixed with the lyophilized botulinum neurotoxin in a quantity sufficient to exceed 500 μg albumin per 100 LD_{50} units. Additionally, another physical agent, which further enhances sequestration, is added to keep botulinum neurotoxin from diffusing away from the injections field. Such an agent would be a diluted mixture of animal or human collagen. The increased albumin non-covalently binds to botulinum toxin and an enhances to the sequestration of the neurotoxin proving better saturation of neurotoxin receptors and improved clinical effect.

Example 13: Preparation of a high-albumin composition of botulinum toxin comprising a recombinantly-produced botulinum toxin-albumin fusion protein.

Botulinum toxin is produced as a fusion protein with albumin thereby producing an albumin molecule that is covalently linked to a botulinum toxin. The fusion protein is tested using the mouse LD_{50} bioassay to determine the effective amount. The regional denervation rabbit ptosis bioassay and mouse hindlimb bioassay may be used to confirm the effective amount of a composition comprising the fusion protein. A clinical-dose-escalation study would be further used to confirm and refine effective amount.

1. A composition comprising botulinum toxin and a sequestration agent, wherein the sequestration agent is present in an amount between 550 and 550,000 μg sequestration agent per 100 LD_{50} units botulinum toxin.

2. The composition of claim 1, wherein the sequestration agent is present in an amount between 550 and 5,500 μg sequestration agent per 100 LD_{50} units botulinum toxin.

3. The composition of claim 1, wherein the sequestration agent is present in an amount between 5,500 and 13,000 μg sequestration agent per 100 LD_{50} units botulinum toxin.

4. The composition of claim 1, wherein the sequestration agent is present in an amount between 13,000 and 50,500 μg sequestration agent per 100 LD_{50} units botulinum toxin.

5. The composition of claim 1, wherein the sequestration agent is present in an amount between 50,500 and 505,000 μg sequestration agent per 100 LD_{50} units botulinum toxin.

6. The composition of claim 1, wherein the sequestration agent is formulated as encapsulated microspheres in an amount between 50,500 and 90,500 μg sequestration agent per 100 LD_{50} units botulinum toxin.

7. The composition of claim 1, wherein the botulinum toxin is selected from the group consisting of: botulinum toxin types A, B, C, D, E, F and G.

8. The composition of claim 7, wherein the botulinum toxin is botulinum toxin type A from Hall strain *Clostridium botulinum*.

9. The composition of claim 1, wherein the botulinum toxin comprises fractionated-light-chain botulinum toxin.

10. The composition of claim 1, wherein the botulinum toxin consists essentially of fractionated light chain botulinum toxin.

11. The composition of claim 1, wherein the botulinum toxin consists essentially of a mixture of hybrid and chain-translocated forms of botulinum toxin.

12. The composition of claim 1, wherein the botulinum toxin consists essentially of chimeric forms of botulinum toxin.

13. The composition of claim 1, wherein the botulinum toxin has a specific activity greater than or equal to 20 LD_{50} units per nanogram botulinum toxin.

14. The composition of claim 1, wherein the ratio of LD_{50} units of botulinum toxin to μg sequestration agent is less than or equal to 0.2 for botulinum toxin type A.

15. The composition of claim 1 wherein the ratio of LD_{50} units of botulinum toxin to μg sequestration agent is less than or equal to 10 for botulinum toxin type B.

16. The composition of claim 1 further comprising a pharmaceutically acceptable carrier.

17. The composition of claim 1, wherein the botulinum toxin is noncovalently bound to the asequestration agent.

18. The composition of claim 1, wherein the botulinum toxin is covalently bound to the sequestration agent.

19. A composition of claim 1 further comprising zinc or a zinc salt.

20. The composition of claim 1, wherein the sequestration agent is albumin.

21. The composition of claim 1, wherein albumin is present in an amount between 550 and 5,500 μg albumin per 100 LD_{50} units botulinum toxin.

22. The composition of claim 1, wherein albumin is present in an amount between 5,500 and 13,000 μg albumin per 100 LD_{50} units botulinum toxin.

23. The composition of claim 1, wherein albumin is present in an amount between 13,000 and 50,500 μg albumin per 100 LD_{50} units botulinum toxin.

24. The composition of claim 1, wherein albumin is present in an amount between 50,500 and 505,000 μg albumin per 100 LD_{50} units botulinum toxin.

25. The composition of claim 1, wherein albumin is formulated as encapsulated microspheres in an amount between 50,500 and 90,500 μg albumin per 100 LD_{50} units botulinum toxin.

26. The composition of claim 1, wherein the albumin is formulated as a solid albumin particle.

27. The composition of claim 1, wherein the albumin is recombinantly produced.

28. The composition of claim 20 further comprising one or more additional sequestration agents.

29. The composition of claim 1, wherein the sequestration agent is selected from the group consisting of: proteins, lipids and carbohydrates.

30. The composition of claim 29, wherein the sequestration agent is collagen.

31. The composition of claim 29, wherein the sequestration agent is hyaluronate.

32. The composition of claim 29, wherein the sequestration agent is epinephrine.

33. A method of localized denervation in a subject in need thereof, comprising administering an effective amount of the composition of claim 1.

34. The method of claim 33, wherein the subject suffers from a neuromuscular disease associated with increased muscle tone or involuntary movement.

35. The method of claim 34, wherein the neuromuscular disease associated with increased muscle tone with involuntary movement is blepharospasm or torticollis.

36. The method of claim 35, wherein the neuromuscular disease associated with increased muscle tone with involuntary movement is blepharospasm.

37. The method of claim 36, wherein the effective amount is between 10-200 LD_{50} units.

38. The method of claim 35, wherein the neuromuscular disease associated with increased muscle tone with involuntary movement is torticollis.

39. The method of claim 38, wherein the effective amount is between 10 and 3000 LD_{50} units.

40. A method of treating a condition selected from the group consisting of facial wrinkles, rhytides and cosmetic alteration of lip and brow, in a subject in need thereof, comprising administering an effective amount of the composition of claim 1.

41. The method of claim 40, wherein the effective amount is between 2.5 and 400 LD_{50} units.

42. A method of treating human headache disorders in a subject in need thereof, comprising administering an effective amount of the composition of claim 1.

43. The method of claim 42, wherein the effective amount is between 5 and 1,000 LD_{50} units.

44. A method of treating human migraine headache disorders in a subject in need thereof, comprising administering an effective amount of the composition of claim 1.

45. The method of claim 44, wherein the effective amount is between 5 and 1,000 LD_{50} units.

46. A method of treating human inflammatory conditions in a subject in need thereof, comprising administering an effective amount of the composition of claim 1.

47. The method of claim 46, wherein the effective amount is between 5 and 4,000 LD_{50} units.

48. A method of treating myopathic or neuropathic pain in a subject in need thereof, comprising administering an effective amount of the composition of claim 1.

49. The method of claim 48, wherein the effective amount is between 5 and 4,000 LD_{50} units.

50. A method of treating back pain or arthritic pain in a subject in need thereof, comprising administering an effective amount of the composition of claim 1.

51. The method of claim 50, wherein the effective amount is between 5 and 4,000 LD_{50} units.

52. A method of treating gastrointestinal spasm and strictures in a subject in need thereof, comprising administering an effective amount of the composition of claim 1.

53. The method of claim 52, wherein the effective amount is between 5 and 4,000 LD_{50} units.

54. A method of treating a hyperhyrosis syndrome in a subject in need thereof, comprising administering of the composition of claim 1.

55. The method of claim 54, wherein the effective amount is between 5 and 4,000 LD_{50} units.

56. A method of producing the composition of claim 1, comprising mixing albumin to botulinum toxin in a weight to weight ratio which exceeds 100 μg albumin to 1 ng of botulinum toxin.

57. A method of producing the composition of claim 1, comprising freeze drying or flash drying a mixture of a botulinum toxin and albumin in a weight to weight ratio of 100 μg albumin to 1 ng of botulinum toxin.

58. A method of producing the composition of claim 1, comprising lyophilizing a mixture of a botulinum toxin and albumin in a weight to weight ratio of 100 μg to 1 ng of botulinum toxin.

59. A composition comprising botulinum toxin and a sequestration agent, wherein the sequestration agent is present in an amount between 550 and 900,500 μg sequestration agent per 100 LD_{50} units botulinum toxin.

60. The composition of claim 59, wherein the albumin is formulated as a solid albumin particle.