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Solicitud de Patente para COMPOSICIONES
FARMACÉUTICAS MEJORADAS DE TOXINA
BOTULÍNICA

Solicitante: Botulinum Toxin Research Associates, Inc.

Darío Cárdenas Navas, identificado como aparece al pie de mi firma, atentamente me dirijo a ese Despacho dando respuesta al Oficio No. 15.018 notificado por fijación en lista de fecha 28 de Noviembre de 2008.

En virtud de lo dispuesto en Artículo 45 de la Decisión 486 de la Comunidad Andina, atentamente solicito se me conceda una prórroga para la presentación del documento de cesión el cual se encuentra aún en trámite de legalización.

Para tal efecto, adjunto el recibo oficial correspondiente a tal solicitud de prórroga.

Atentamente,

Darío Cárdenas Navas
c.c. 17.066.629 de Bogotá
t.p. 1.250 C.S.J.

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4

123

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2

Proteins and Enzymes

J. ELLIS BELL

EVELYN T. BELL

Department of Biochemistry
University of Rochester

PRENTICE-HALL, INC.

Englewood Cliffs, New Jersey / 07632

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the amount of protein when it is not pure, the method can be used to follow the protein activity and to calculate a "specific activity." If one makes assumptions about the number of binding sites for ligand, it can also be used to quantitate the protein.

Structural Proteins

The determination of the "activity" of structural proteins represents the most difficult aspect of protein purification: frequently, activity measurements are only possible by immunoassay using antibody to the purified material.

The problems may best be illustrated by reference to the purification of laminin, a glycoprotein obtained from basement membranes. In essence, laminin was discovered as an impurity in collagen preparations from tumor cells: It was purified by standard approaches to give a "pure" protein by protein chemistry criteria—antibodies were made, and, via immunofluorescence studies, an *in vivo* localization of the laminin was achieved. Subsequently, immunoassay techniques were used to follow the purification.

In other instances, assays of biological function may be employed to follow purification, as has been used for example with interleukin-II, a T-lymphocyte growth factor required for the stimulation and growth of responding T cells, which is produced by a particular subset of T cells.

Finally, it is sometimes possible to "purify" a protein based on some particular chemical property—a unique cofactor or unusual amino acid composition, for example, the hydroxyproline content of collagen. Clearly, there are no firm rules for the assay of structural proteins, and definition of activity has to be rather pragmatic.

SPECIFIC ACTIVITY

Finally, we come to a definition of specific activity. Depending on the state of knowledge concerning the molecular weight of the protein, the specific activity can be defined by one of the equations

$$\text{specific activity} = \frac{\text{activity}}{\text{mg protein}} \quad (1-1)$$

$$\text{specific activity} = \frac{\text{activity}}{\text{mol protein}} \quad (1-2)$$

In either instance, as the purity of the protein preparation increases, the apparent specific activity increases, reaching a defined value for the pure protein. As indicated earlier and considered in more detail in Chap. 2, it is often considered that a protein is pure when a constant specific activity is reached in a purification. If the specific activity of the pure protein is known, the apparent specific activity can be used to calculate the percent purity at a given point in a purification scheme.

Calculation of Specific

From the example can be calculated if the reaction mixture is known. If this rate was achieved, can calculate the specific

If the molecular weight of the enzyme can be calculated in units. Assume that the concentration of

$$70,000 \text{ g/liter} = 1$$

$$4 \text{ } \mu\text{g/ml} = \frac{1}{25}$$

Since the rate is expressed in $4 \text{ } \mu\text{g/ml}$ solution. The

In this type of calculation for the enzyme. In these calculations the dimer molecular weight is calculated on the basis of active sites per molecule: $55.4/2 = 27.7 \text{ min}^{-1}$.

Finally, even when conditions as described earlier affect the enzyme activity, the activity would be given by the maximum-rate substrate

Before discussing fractional generalities that can be the goal is to obtain, in study, with little regard for initially to screen a starting material with which has been selected it is necessary

increases, the apparent protein. As indicated considered that a protein ification. If the specific activity can be used to a scheme.

Before discussing fractionation procedures for proteins (Chap. 2), there are several generalities that can be made concerning sources of proteins for purification. Often the goal is to obtain, in pure form, a particular enzyme (or other activity) for further study, with little regard to the starting material. In such a case it is extremely helpful initially to screen a variety of species and tissues within a species to obtain a starting material with the highest initial specific activity. Once a starting tissue has been selected it is necessary to establish whether the protein is soluble (i.e., in the

12/128

PROTEÍNAS Y ENZIMAS

J. ELLIS BELL

EVELYN T. BELL

Departamento de Bioquímica
Universidad de Rochester

PRENTICE-HALL INC

Englewood Cliffs, New Jersey / 07632

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129

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14. Cap. 1. Conceptos básicos de purificación de proteínas y caracterización

...la cantidad de proteína cuando no es pura, el método puede ser empleado para seguir la actividad de la proteína y para calcular la "actividad específica". Si se hacen suposiciones en cuanto al número de sitios de unión para el ligando, esto se puede usar para cuantificar la proteína.

Proteínas Estructurales

La determinación de la "actividad" de proteínas estructurales representa el aspecto de mayor dificultad de la purificación de la proteína: con frecuencia, las

41

130

mediciones de la actividad son posibles únicamente mediante inmunoensayos empleando anticuerpo para el material purificado.

Los problemas pueden ser ilustrados de una mejor manera haciendo referencia a la purificación de laminina, una glicoproteína obtenida a partir de membrana basal. En esencia, la laminina fue descubierta como una impureza en preparaciones de colágeno de células tumorales: fue purificada mediante aproximaciones estándar para proporcionar una proteína “pura”, según los criterios químicos de proteínas, se utilizaron anticuerpos y mediante estudios de inmunofluorescencia se logró una localización in vivo de la laminina. En consecuencia, se emplearon técnicas de inmunoensayo para seguir la purificación.

En otros casos, se pueden utilizar ensayos biológicos para seguir la purificación, como se han usado por ejemplo con la interleucina II, un factor de crecimiento linfocitario T requerido para la estimulación y crecimiento de células T de respuesta, el cual es producido por un subconjunto particular de células T.

Finalmente, algunas veces es posible “purificar” una proteína con base en alguna propiedad química particular, un único cofactor o composición de aminoácidos inusual, por ejemplo, el contenido de hidroxiprolina del colágeno. Claramente, no existen reglas sólidas para el ensayo de proteínas estructurales y la definición de la actividad tendrá que ser más que pragmática.

ACTIVIDAD ESPECÍFICA

Finalmente, llegamos a la definición de actividad específica. Dependiendo del estado de conocimiento con relación al peso molecular de la proteína, la actividad específica puede ser definida mediante una de las ecuaciones:

$$\text{Actividad específica} = \frac{\text{actividad}}{\text{mg de proteína}} \quad (1-1)$$

$$\text{Actividad específica} = \frac{\text{actividad}}{\text{mol de proteína}} \quad (1-2)$$

En cada caso, cuando la pureza de la preparación de proteína se incrementa, la actividad específica aparente se incrementa, alcanzando un valor definido para la proteína pura. Como se indicó anteriormente y se consideró detalladamente en el capítulo 2, a menudo se considera que una proteína se encuentra pura cuando se alcanza una actividad específica constante en una purificación. Si la actividad específica de la proteína es conocida, la actividad específica aparente puede ser usada para calcular el porcentaje de pureza a un punto dado en un esquema de purificación.

Cálculo de Actividades Específicas

A partir del ejemplo de la deshidrogenasa alcohol discutida anteriormente, se puede calcular la *actividad específica* si la concentración de la enzima o la cantidad de enzima en la mezcla de reacción son conocidas. En el ejemplo, podemos calcular la tasa de 0.00316 mM/min. Si esta tasa fue alcanzada con una concentración de proteína, por ejemplo, de 4 µg/ml, se puede calcular la actividad específica,

$$0.00316/4 = 0.00079 \text{ mM}/\mu\text{g/ml/min}$$

Si el peso molar de la deshidrogenasa alcohol es conocido, la molaridad de la enzima puede ser calculada y la actividad específica expresada en términos de unidades mucho más simples. Asumiendo que el peso molecular es de 70,000, entonces 4µg/ml da una concentración molar de

$$\begin{aligned} 70,000 \text{ g/litro} &= 1 \text{ M}, \quad 70,000 \text{ mg/ml} = 1 \text{ M}, \quad 1 \text{ mg/ml} = 1/70 \times 10^{-4} \text{ M} \\ 4 \mu\text{g/ml} &= 4/70 \times 10^{-7} \text{ M} = 5.7 \times 10^{-8} \text{ M} \end{aligned}$$

Dado que la tasa es expresada en unidades milimolar, esto se convierte en $5.7 \times 10^{-5} \text{ mM}$ para una solución de 4µg/ml. La *actividad específica* es por lo tanto

$$\frac{0.00316}{(5.7 \times 10^{-5}) \text{ min}^{-1}} = 55.4 \text{ min}^{-1}$$

En este tipo de cálculo se debe tener mucho cuidado para definir “molécula” de la enzima. En estos cálculos se empleó un peso molecular de 70,000: Este es

el peso molecular del *dímero* (el dímero contiene *dos* sitios activos). La actividad específica calculada con base de la totalidad del peso molecular puede ser dividida por el número de sitios activos por molécula para dar la actividad específica por sitio de unión: en este ejemplo, $55.4/2 = 27.7 \text{ min}^{-1}$.

Finalmente, incluso cuando las actividades se miden con concentraciones de sustrato de saturación, como se describió anteriormente, la temperatura y el pH deben ser definidos y si las sales amortiguadoras afectan la actividad de la enzima, estas también deben definirse. En consecuencia, la actividad específica por sitio activo estaría dada como 27.7 min^{-1} a 25°C , pH 7.0 en solución amortiguadora de fosfato. Si las concentraciones de sustrato a la tasa máxima no son usadas, también deben ser dadas.

FUENTES DE PROTEÍNA

Antes de discutir los procedimientos de fraccionamiento para proteínas (Capítulo 2), existen varias generalidades que pueden ser realizadas en cuanto a las fuentes de proteínas para purificación. A menudo el objetivo es obtener en una forma pura una enzima particular u otra actividad para estudio adicional, con poca estimación del material de partida. En tal caso, resulta extremadamente beneficioso inicialmente seleccionar una variedad de especies y tejidos dentro de una especie para obtener un material de partida con la actividad específica inicial más alta. Una vez el tejido de partida ha sido seleccionado, es necesario establecer si la proteína es soluble, esto es.....

133



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(54) **PHARMACEUTICAL COMPOSITIONS COMPRISING BOTULINUM TOXIN AND HUMAN SERUM ALBUMIN**

PHARMAZEUTISCHE ZUSAMMENSETZUNGEN ENTHALTEND BOTULINUMTOXIN UND HUMANES SERUM-ALBUMIN

COMPOSITIONS PHARMACEUTIQUES RENFERMANT DE LA TOXINE BOTULINIQUE ET DE L'ALBUMINE SERIQUE HUMAINE

(84) Designated Contracting States: AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IT LI LU MC NL PT RO SE SI SK TR (30) Priority: 20.12.2002 US 435901 P (43) Date of publication of application: 16.11.2005 Bulletin 2005/46 (73) Proprietor: Botulinum Toxin Research Associates, Inc. Quincy, Massachusetts 02169 (US)	(72) Inventor: BORODIC, Gary, E. Canton, MA 02021 (US) (74) Representative: Kador & Partner Corneliusstrasse 15 80469 München (DE) (56) References cited: WO-A-01/58472 WO-A-94/00481 WO-A-97/35604
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EP 1 594 523 B1

75

134

Description

[0001] This invention relates to improved pharmaceutical compositions comprising botulinum neurotoxin and an human serum albumin as sequestration agent. The invention further provides pharmaceutical compositions and the use of said compositions for the treatment of a variety of neuromuscular diseases.

BACKGROUND OF THE INVENTION

[0002] 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, hyperhidrosis).

[0003] 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 receptor 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 human serum albumin to enhance sequestration and improve the effectiveness where other available botulinum toxin preparations have failed.

[0004] In recent years, Borodic *et al.* have characterized the regional effect of botulinum toxin using muscle fiber morphometrics, cholinesterase staining, and cutaneous wrinkling from depression of facial muscle tone. (Borodic (1992) Botulinum A toxin for (expressionistic) ptosis overcorrection after frontalis sling. Ophthalmic Plastic and Reconstructive Surg. 8(2): 137-142; incorporated herein by reference in its entirety).

[0005] Since its introduction as a therapeutic agent, the pharmaceutical measurement of the denervating or biologic activity of botulinum toxin has been the LD₅₀ 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 LD₅₀ 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 LD₅₀ units to treat torticollis whereas another preparation of botulinum toxin Type A (BOTOX®) requires only 100-300 LD₅₀ units. Similarly, the LD₅₀ 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

¹Units (U) are LD₅₀ units determined using 20-30 g Swiss-Webster mice, as described herein.

A. Complications Associated with Conventional Botulinum-Toxin Formulations.

[0006] 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

96

135

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.

5 **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%

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

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

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

20 [0007] 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 peripharyngeal 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 LD₅₀ units, that is, the greater the LD₅₀ units used, the greater the diffusion from a point injection. From literature summary from the 1980's and early 1990's, dysphagia is more common with use of DYSPORT® than BOTOX® at effective doses. Recently, from studies done at European centers, the differences in dysphagia rates have been confirmed (Ranoux et al. (2002) Respective potencies of DYSPORT® and BOTOX®: a double blind, randomized, crossover study in cervical dystonia. 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.

45 [0008] 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.

50 [0009] 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.

44

136

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

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

[0010] 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 hemidiaphragm 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. (Biglalk 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 LD₅₀ bioassay afforded by the albumin concentration increase. (Biglalk 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 LD₅₀ bioassay without reference to mechanism of action in tissues or pharmacologic-pharmacokinetic importance, that is, *in vivo* albumin binding, enhanced sequestration, and improvement in therapeutic effects. The same authors further observed in a rat-diaphragm preparation, that the addition of albumin to the BOTOX® preparation could not substantially increase regional denervative effects and did not advocate any changes in formulation. The findings of these researchers concluded that there was an effect of the albumin concentration on the LD₅₀ measurements however, there work did not demonstrate any increased potency of BOTOX® on regional denervation or that DYSPORT® could be enhance to give any greater denervation potency over BOTOX®. There work was limited by the *in vitro* nature of their experiments, that is, using a con blood perfused animal dissection of a motor nerve (phrenic nerve) and diaphragm muscle, which fails to accounts for dilutions and tissue fluid flow capable of washing injected toxin away from targeted tissue prior to binding with the nerve axon terminal receptors. The real time application requires an *in vivo* analysis of the effects of albumin on regional denervation as outlined in the following experiments. Their work did identify reasons for differences in LD₅₀ as measured by the mouse lethality assay. The conclusion were no improvement 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).

[0011] 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

[0012] The present invention provides a composition according to claim 1 comprising botulinum toxin and human serum albumin for use in treating various neuromuscular diseases and localized denervation.

[0013] 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.

[0014] 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 LD₅₀ units per nanogram botulinum toxin.

[0015] Each composition of the present invention, in addition to comprising a botulinum toxin and a human serum albumin, 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 human serum albumin. In another embodiment, the botulinum toxin

468

is covalently bound to the sequestration agent.

[0016] Furthermore, the albumin of the present compositions is preferably recombinantly produced. The albumin is present in an amount between 13,000 and 550,000 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.

[0017] 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.

[0018] 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.

[0019] 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.

[0020] 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.

[0021] 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.

[0022] 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.

[0023] 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.

[0024] 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.

[0025] 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.

[0026] 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.

[0027] The present invention provides a method of treating a hyperthyroidism 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.

[0028] 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.

[0029] 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

[0030] The present invention describes composition and a use thereof 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 human serum albumin". Enhanced sequestration using higher concentration of albumin 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 human serum albumin as 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 human serum albumin is an existing excipient at significantly higher concentrations than previously used such. The human serum albumin 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.

[0031] 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.

[0032] 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 in studies with blinded trials.

[0033] 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.

[0034] 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.

[0035] 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.

[0036] As used herein, "sequestration agent" means an agent that enhances localization and/or retention of the botulinum toxin to the site of administration.

[0037] As used herein, "subject" means a mammal.

B. Albumin.

[0038] 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, digitoxin, phenytoin, oxacillin, benzylpenicillin, lasix, indomethacin, diazepam, and quindine among others. Peptides and proteins also are known to bind human serum albumin. Peptide hormones such as gastrin, corticotropin, melatonin are also known to bind human serum albumin.

[0039] Several binding sites have been identified and binding has been thought to be noncovalent. 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.

[0040] 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 is much slower than removal of smaller molecular species. The relevance to Botulinum toxin pharmaceuticals relate to the role both in maintaining biologic activity by promoting nerve contact and preventing wash out from free neurotoxin release at injection points. DYSPORT®, with its lower albumin concentration, offers less sequestration for the neurotoxin complex, and subsequently, after injected, diffusion away from the targeted anatomic area are results. The clinical effect is a greater regional diffusion of the chemodenervation, which results in increased complications (ptosis, Dysphagia see Table 2). In order to compensate for this biologic behavior, the clinicians in practice or studies have had to give four to five times 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 doses 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).

[0041] 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 LD₅₀ 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).

[0042] 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.

[0043] 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 WydaseTM. 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.

[0044] 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.

[0045] 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 is 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.

Example 1: Treatment of benign essential blepharospasm.

[0046] 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 injection sites that are specific for benign essential blepharospasm. The high-albumin preparation was produced by adding 12,250 µg/cc (0.004 LD₅₀/µg albumin). Administration of 60 LD₅₀ units of the high-albumin preparation achieved excellent results when the administration of the conventional BOTOX® formulation had failed. Three months after the initial administration of the high-albumin botulinum toxin preparation, 40 LD₅₀ units of a high-albumin preparation comprising 25,000 µg albumin per 100 LD₅₀ units (0.002

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EP 1 594 523 B1

LD₅₀/μg albumin) were administered and produced greater than 80% relief of the clinical symptoms of blepharospasm.

Example 2: Treatment of blepharospasm.

5 [0047] 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.

[0048] Albumin was added to a conventional BOTOX® preparation to produce a high-albumin preparation of botulinum toxin with a concentration of 50,250 μg albumin/cc (0.001 LD₅₀/μg albumin). Injection of 50 units the high-albumin preparation produced a greater than 50% reduction of symptoms.

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Example 3: Treatment of blepharospasm.

[0049] 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.

15 [0050] Human serum albumin and 0.5 cc Healon® (hyaluronate) were both added to a 100 LD₅₀ units of Botulinum toxin type A (BOTOX®). The high-albumin preparation produced contained 25,500 μg albumin per 100 LD₅₀ units (0.005 LD₅₀/μg albumin). Administration of 60 LD₅₀ units reduced the clinically-observed involuntary-eyelid contractions,

Reference Example 4: Treatment of essential blepharospasm.

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[0051] The subject was a 66-year-old female with essential blepharospasm. Repeated treatment with BOTOX® (type A), using a range between 40 to 300 LD₅₀ units, produced no therapeutic benefit Botulinum toxin type B (MYOBLOC®) was administered at a dose of 10,000 LD₅₀ units within the periocular region and also failed to produce any relief. Bilateral-facial neurectomy also failed to produce any substantial relief of symptoms. Additional surgical procedures to remove muscles necessary for eyelid closure were similarly ineffective.

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[0052] Human serum albumin was added to a 100 LD₅₀ units of botulinum toxin type A (BOTOX®). The high-albumin preparation produced contained 12,750 μg albumin per 100 LD₅₀ units (0.00196 LD₅₀/μg albumin). Administration of 50 LD₅₀ units produced substantial relief of symptoms for a period of three to four months, when other formulations and surgical approaches had failed.

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Example 5: Treatment of severe chronic blepharospasm.

[0053] 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,

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[0054] The patient received an a high-albumin formulation of botulinum toxin that was produced by mixing 25,000 μg human serum albumin 100 LD₅₀ units of BOTOX®. The high-albumin preparation contained 12,750 μg albumin per cc (0.004 LD₅₀/μg albumin). Using the high-albumin preparation, 60-70 LD₅₀ units were administered with excellent clinical results and no evidence of ptosis after the therapy. The enhanced sequestration of much higher concentrations of botulinum toxin depressed the spread of the neurotoxin into the muscles within the eye socket

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Example 6: Treatment of essential blepharospasm.

45 [0055] 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.

[0056] A high-albumin botulinum toxin composition (20,000 μg albumin per cc; 0.0025 LD₅₀ BOTOX®/μg albumin) was administered to the subject with a resultant clinical improvement of the blepharospasm and no diffusion-related side effects (ptosis).

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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	12,500	0.0040	0.2	5	10
	25,000	0.0020			
2	50,250	0.0001	0.2	5	10
3	10,200	0.0050	0.2	5	10
4	25,000	0.0020	0.2	5	10
5	12,500	0.0040	0.2	5	10
6	20,000	0.0025	0.2	5	10
LD ₅₀ /mcg albumin/cc for BOTOX®, DYSPORT®, MYOBLOC® given for direct comparison					

Example 7: Preparation of a high-albumin composition of botulinum toxin.

[0057] 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 LD₅₀. The increased albumin binds to botulinum toxin and enhances sequestration of the injected neurotoxin providing for better saturation of neurotoxin receptors and improved clinical effects

Example 8: Preparation of a high-albumin composition of botulinum toxin further comprising hyaluronate.

[0058] 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 LD₅₀ units. Additionally, another sequestration agent, which further enhances sequestration, is added to keep the botulinum neurotoxin from diffusing away from the injections site. Such a sequestration agent includes but is not limited to a diluted solution of sodium hyaluronate. The increased albumin non-covalently binds to botulinum toxin and an enhances the sequestration of the neurotoxin providing better saturation of neurotoxin receptors and, consequently, an improved clinical effect.

Example 9: Preparation of a high-albumin composition of botulinum toxin further comprising collagen.

[0059] After quantifying the denervating effect of a botulinum neurotoxin by dilution of a purified botulinum toxin, albumin is mixed with the lyophilized botulinum neurotoxin in a quantity sufficient to exceed 500 μg albumin per 100 LD₅₀ units. Additionally, another physical agent, which further enhances sequestration, is added to keep botulinum neurotoxin from diffusing away from the injections field. Such an agent would be a diluted mixture of animal or human collagen. The increased albumin non-covalently binds to botulinum toxin and an enhances to the sequestration of the neurotoxin proving better saturation of neurotoxin receptors and improved clinical effect.

Example 10: Preparation of a high-albumin composition of botulium toxin comprising a recombinantly-produced botulinum toxin-albumin fusion protein.

[0060] Botulinum toxin is produced as a fusion protein with albumin thereby producing an albumin molecule that is covalently linked to a botulinum toxin. The fusion protein is tested using the mouse LD₅₀ bioassay to determine the effective amount The region 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.

Claims

1. A pharmaceutical composition comprising an effective amount of a botulinum toxin and a human serum albumin, wherein the human serum albumin is present in an amount between 13,000 and 550,000 μg human serum albumin

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1217
142

EP 1 594 523 B1

per 100 LD₅₀ units botulinum toxin.

2. The pharmaceutical composition of claim 1, wherein the human serum albumin is present in an amount between 13,000 and 50,500 µg human serum albumin per 100 LD₅₀ units botulinum toxin.
3. The pharmaceutical composition of claim 1, wherein the human serum albumin is present in an amount between 50,500 and 505,000 µg human serum albumin per 100 LD₅₀ units botulinum toxin.
4. The pharmaceutical composition of claim 1, wherein the human serum albumin is present in an amount between 50,500 and 90,500 µg human serum albumin per 100 LD₅₀ units botulinum toxin.
5. A pharmaceutical composition of any one of claim 1-4, wherein the composition is lyophilized.
6. A composition of any of the previous claims 1-5, wherein the botulinum toxin is selected from the group consisting of: botulinum toxin types A, B, C, D, E, F and G.
7. A composition of any of the previous claims 1-5, wherein the botulinum toxin is botulinum toxin type A from Hall strain *Clostridium botulinum*.
8. A composition of any of the previous claims 1-5, wherein the botulinum toxin comprises fractionated-light-chain botulinum toxin.
9. A composition of any of the previous claims 1-5, wherein the botulinum toxin consists essentially of fractionated light chain botulinum toxin.
10. A composition of any of the previous claims 1-5, wherein the botulinum toxin consists essentially of a mixture of hybrid and chain-translocated forms of botulinum toxin.
11. A composition of any of the previous claims 1-5, wherein the botulinum toxin consists essentially of chimeric forms of botulinum toxin.
12. A composition of any of the previous claims 1-5, wherein the betulinum toxin has a specific activity greater than or equal to 20 LD₅₀ units per nanogram botulinum toxin.
13. A composition of any of the previous claims 1-5, further comprising a pharmaceutically acceptable carrier.
14. A composition of any of the previous claims 1-5, wherein the botulinum toxin is covalently bound to the human serum albumin.
15. A composition of any of the previous claims 1-5, further comprising zinc or a zinc salt.
16. A composition of any of the previous claims 1-5, wherein the human serum albumin is formulated as a solid albumin particle.
17. A composition of any of the previous claims 1-5, wherein the human serum albumin is recombinantly produced.
18. A composition of any of the previous claims 1-5, further comprising one or more additional sequestration agents.
19. The use of an effective amount of a pharmaceutical composition according to any of the previous claims 1-18 for the manufacture of a drug for the treatment of localized denervation.
20. The use of claim 19, wherein the subject suffers from a neuromuscular disease associated with increased muscle tone or involuntary movement.
21. The use of claim 20, wherein the neuromuscular disease associated with increased muscle tone with involuntary movement is blepharospasm or torticollis.
22. The use of claim 21, wherein the neuromuscular disease associated with increased muscle tone with involuntary

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11
143

EP 1 594 523 B1

movement is blepharospasm.

23. The use of claim 22, wherein the effective amount is between 10-200 LD₅₀ units.
- 5 24. The use of claim 21, wherein the neuromuscular disease associated with increased muscle tone with involuntary movement is torticollis.
25. The use of claim 24, wherein the effective amount is between 10 and 3000 LD₅₀ units.
- 10 26. The use of an effective amount of a pharmaceutical composition according to any of the previous claims 1-18 for the manufacture of a drug for the treatment of a condition selected from the group consisting of facial wrinkles, rhytides and cosmetic alteration of lip and brow.
27. The use of claim 26, wherein the effective amount is between 2.5 and 400 LD₅₀ units.
- 15 28. The use of an effective amount of a pharmaceutical composition according to any of the previous claims 1-18 for the manufacture of a drug for the treatment of human headache disorders.
29. The use of claim 28, wherein the effective amount is between 5 and 1,000 LD₅₀ units.
- 20 30. The use of an effective amount of a pharmaceutical composition according to any of the previous claims 1-18 for the manufacture of a drug for the treatment of human migraine headache disorders.
31. The use of claim 30, wherein the effective amount is between 5 and 1,000 LD₅₀ units.
- 25 32. The use of an effective amount of a pharmaceutical composition according to any of the previous claims 1-18 for the manufacture of a drug for the treatment of human inflammatory conditions.
33. The use of claim 32, wherein the effective amount is between 5 and 4,000 LD₅₀ units.
- 30 34. The use of an effective amount of a pharmaceutical composition according to any of the previous claims 1-18 for the manufacture of a drug for the treatment of myopathic or neuropathic pain.
35. The use of claim 34, wherein the effective amount is between 5 and 4,000 LD₅₀ units.
- 35 36. The use of an effective amount of a pharmaceutical composition according to any of the previous claims 1-18 for the manufacture of a drug for the treatment of back pain or arthritic pain.
37. The use of claim 36, wherein the effective amount is between 5 and 4,000 LD₅₀ units.
- 40 38. The use of an effective amount of a pharmaceutical composition according to any of the previous claims 1-18 for the manufacture of a drug for the treatment of gastrointestinal spasm and strictures.
39. The use of claim 38, wherein the effective amount is between 5 and 4,000 LD₅₀ units.
- 45 40. The use of an effective amount of a pharmaceutical composition according to any of the previous claims 1-18 for the manufacture of a drug for the treatment of hyperhidrosis syndrome.
41. The use of claim 40, wherein the effective amount is between 5 and 4,000 LD₅₀ units.
- 50 42. A method for preparing a pharmaceutical composition comprising the steps of lyophilizing a composition comprising a botulinum toxin and optionally human serum albumin and optionally a stabilizing sugar and adding a quantity of human serum albumin to the lyophilized botulinum toxin composition in an amount sufficient to exceed 500 µg human serum albumin per 100 LD₅₀ units botulinum toxin.
- 55 43. The method of claim 42, wherein the lyophilized botulinum toxin composition comprises 500 µg human serum albumin per 100 LD₅₀ units botulinum toxin.

118
149

EP 1 594 523 B1

- 5 44. The method of claim 42, wherein human serum albumin is added to the lyophilized botulinum toxin composition in an amount sufficient to achieve between 5,500 and 13,000 μg human serum albumin per 100 LD₅₀ units botulinum toxin, between 13,000 and 50,500 μg human serum albumin per 100 LD₅₀ units botulinum toxin, between 50,500 and 505,000 μg human serum albumin per 100 LD₅₀ units botulinum toxin, or between 50,500 and 90,500 μg human serum albumin per 100 LD₅₀ units botulinum toxin.
- 10 45. The method of claim 42, wherein human serum albumin is added to the lyophilized botulinum toxin composition in an amount sufficient to achieve between 5,500 and 13,000 μg human serum albumin per 100 LD₅₀ units botulinum toxin.
- 15 46. The method of claim 42, wherein human serum albumin is added to the lyophilized botulinum toxin composition in an amount sufficient to achieve between 13,000 and 50,500 μg human serum albumin per 100 LD₅₀ units botulinum toxin.
- 20 47. The method of claim 42, wherein human serum albumin is added to the lyophilized botulinum toxin composition in an amount sufficient to achieve between 50,500 and 505,000 μg human serum albumin per 100 LD₅₀ units botulinum toxin.
- 25 48. The method of claim 42, wherein human serum albumin is added to the lyophilized botulinum toxin composition in an amount sufficient to achieve between 50,500 and 90,500 μg human serum albumin per 100 LD₅₀ units botulinum toxin.

Patentansprüche

- 25 1. Pharmazeutische Zusammensetzung, die eine wirksame Menge eines Botulinumtoxins und ein Human-Serumalbumin umfaßt, wobei das Human-Serumalbumin in einer Menge von 13.000 bis 550.000 μg Human-Serumalbumin pro 100 LD₅₀-Einheiten Botulinumtoxin vorliegt.
- 30 2. Pharmazeutische Zusammensetzung nach Anspruch 1, wobei das Human-Serumalbumin in einer Menge von 13.000 bis 50.500 μg Human-Serumalbumin pro 100 LD₅₀-Einheiten Botulinumtoxin vorliegt.
- 35 3. Pharmazeutische Zusammensetzung nach Anspruch 1, wobei das Human-Serumalbumin in einer Menge von 50.500 bis 505.000 μg Human-Serumalbumin pro 100 LD₅₀-Einheiten Botulinumtoxin vorliegt.
- 40 4. Pharmazeutische Zusammensetzung nach Anspruch 1, wobei das Human-Serumalbumin in einer Menge von 50.500 bis 90.500 μg Human-Serumalbumin pro 100 LD₅₀-Einheiten Botulinumtoxin vorliegt.
- 45 5. Pharmazeutische Zusammensetzung nach einem der Ansprüche 1 bis 4, wobei die Zusammensetzung gefriergetrocknet ist.
- 50 6. Zusammensetzung nach einem der vorstehenden Ansprüche 1 bis 5, wobei das Botulinumtoxin aus der Gruppe ausgewählt ist, die aus den Botulinumtoxin-Typen A, B, C, D, E, F und G besteht.
- 55 7. Zusammensetzung nach einem der vorstehenden Ansprüche 1 bis 5, wobei das Botulinumtoxin Botulinumtoxin Typ A vom Stamm Clostridium botulinum nach Hall ist.
8. Zusammensetzung nach einem der vorstehenden Ansprüche 1 bis 5, wobei das Botulinumtoxin Botulinumtoxin mit abgebauten leichten Ketten umfaßt.
9. Zusammensetzung nach einem der vorstehenden Ansprüche 1 bis 5, wobei das Botulinumtoxin im wesentlichen aus Botulinumtoxin mit abgebauten leichten Ketten besteht.
10. Zusammensetzung nach einem der vorstehenden Ansprüche 1 bis 5, wobei das Botulinumtoxin im wesentlichen aus einem Gemisch von Hybrid- und Kettentranslokations-Formen von Botulinumtoxin besteht.
11. Zusammensetzung nach einem der vorstehenden Ansprüche 1 bis 5, wobei das Botulinumtoxin im wesentlichen aus chimären Formen von Botulinumtoxin besteht.

56

145

EP 1 594 523 B1

12. Zusammensetzung nach einem der vorstehenden Ansprüche 1 bis 5, wobei das Botulinumtoxin eine spezifische Aktivität von größer als oder gleich 20 LD₅₀-Einheiten pro Nanogramm Botulinumtoxin aufweist.
- 5 13. Zusammensetzung nach einem der vorstehenden Ansprüche 1 bis 5, die ferner einen pharmazeutisch akzeptablen Träger umfaßt.
14. Zusammensetzung nach einem der vorstehenden Ansprüche 1 bis 5, wobei das Botulinumtoxin kovalent an Human-Serumalbumin gebunden ist.
- 10 15. Zusammensetzung nach einem der vorstehenden Ansprüche 1 bis 5, die ferner Zink oder ein Zinksalz umfaßt.
16. Zusammensetzung nach einem der vorstehenden Ansprüche 1 bis 5, wobei das Human-Serumalbumin als ein festes Albuminpartikel formuliert ist.
- 15 17. Zusammensetzung nach einem der vorstehenden Ansprüche 1 bis 5, wobei das Human-Serumalbumin rekombinant erzeugt worden ist.
18. Zusammensetzung nach einem der vorstehenden Ansprüche 1 bis 5, die ferner ein oder mehrere zusätzliche Sequestrationsmittel umfaßt.
- 20 19. Verwendung einer wirksamen Menge einer pharmazeutischen Zusammensetzung nach einem der vorstehenden Ansprüche 1 bis 18 für die Herstellung eines Medikaments zur Behandlung einer örtlichen Denervierung.
- 25 20. Verwendung nach Anspruch 19, wobei der Patient an einer neuromuskulären Erkrankung leidet, die mit einem verstärkten Muskeltonus oder einer unwillkürlichen Bewegung verbunden ist.
21. Verwendung nach Anspruch 20, wobei die neuromuskuläre Erkrankung, die mit einem verstärkten Muskeltonus mit einer unwillkürlichen Bewegung verbunden ist, Blepharospasmus oder Tortikollis ist.
- 30 22. Verwendung nach Anspruch 21, wobei die neuromuskuläre Erkrankung, die mit einem verstärkten Muskeltonus mit unwillkürlicher Bewegung verbunden ist, Blepharospasmus ist.
23. Verwendung nach Anspruch 22, wobei die wirksame Menge zwischen 10 und 200 LD₅₀-Einheiten liegt.
- 35 24. Verwendung nach Anspruch 21, wobei die neuromuskuläre Erkrankung, die mit einem verstärkten Muskeltonus mit unwillkürlicher Bewegung verbunden ist, Tortikollis ist.
25. Verwendung nach Anspruch 24, wobei wirksame Menge zwischen 10 und 3.000 LD₅₀-Einheiten liegt.
- 40 26. Verwendung einer wirksamen Menge einer pharmazeutischen Zusammensetzung nach einem der vorstehenden Ansprüche 1 bis 18 für die Herstellung eines Medikamentes zur Behandlung eines Zustandes, der aus der Gruppe ausgewählt ist, die aus Gesichtsfalten, Runzeln und einer kosmetischen Veränderung von Lippen und Brauen besteht.
- 45 27. Verwendung nach Anspruch 26, wobei die wirksame Menge zwischen 2,5 und 400 LD₅₀-Einheiten liegt.
28. Verwendung einer wirksamen Menge einer pharmazeutischen Zusammensetzung nach einem der vorstehenden Ansprüche 1 bis 18 für die Herstellung eines Medikamentes zur Behandlung von Störungen in Form von Kopfschmerzen beim Menschen.
- 50 29. Verwendung nach Anspruch 28, wobei die wirksame Menge zwischen 5 und 1.000 LD₅₀-Einheiten liegt.
30. Verwendung einer wirksamen Menge einer pharmazeutischen Zusammensetzung nach einem der vorstehenden Ansprüche 1 bis 18 für die Herstellung eines Medikamentes zur Behandlung von Störungen in Form von Migränekopfschmerzen beim Menschen.
- 55 31. Verwendung nach Anspruch 30, wobei die wirksame Menge zwischen 5 und 1.000 LD₅₀-Einheiten liegt.

57

146

EP 1 594 523 B1

32. Verwendung einer wirksamen Menge einer pharmazeutischen Zusammensetzung nach einem der vorstehenden Ansprüche 1 bis 18 für die Herstellung eines Medikamentes zur Behandlung von entzündlichen Zuständen beim Menschen.
- 5 33. Verwendung nach Anspruch 32, wobei die wirksame Menge zwischen 5 und 4.000 LD₅₀-Einheiten liegt.
34. Verwendung einer wirksamen Menge einer pharmazeutischen Zusammensetzung nach einem der vorstehenden Ansprüche 1 bis 18 für die Herstellung eines Medikamentes zur Behandlung von myopathischen oder neuropathischen Schmerzen.
- 10 35. Verwendung nach Anspruch 34, wobei die wirksame Menge zwischen 5 und 4.000 LD₅₀-Einheiten liegt.
36. Verwendung einer wirksamen Menge einer pharmazeutischen Zusammensetzung nach einem der vorstehenden Ansprüche 1 bis 18 für die Herstellung eines Medikamentes zur Behandlung von Rückenschmerzen oder arthritischen Schmerzen.
- 15 37. Verwendung nach Anspruch 36, wobei die wirksame Menge zwischen 5 und 4.000 LD₅₀-Einheiten liegt.
38. Verwendung einer wirksamen Menge einer pharmazeutischen Zusammensetzung nach einem der vorstehenden Ansprüche 1 bis 18 für die Herstellung eines Medikamentes zur Behandlung von gastrointestinalem Spasma und Strikturen.
- 20 39. Verwendung nach Anspruch 38, wobei die wirksame Menge zwischen 5 und 4.000 LD₅₀-Einheiten liegt.
40. Verwendung einer wirksamen Menge einer pharmazeutischen Zusammensetzung nach einem der vorstehenden Ansprüche 1 bis 18 für die Herstellung eines Medikamentes zur Behandlung der Hyperhyrise-Syndroms.
- 25 41. Verwendung nach Anspruch 40, wobei die wirksame Menge zwischen 5 und 4.000 LD₅₀-Einheiten liegt.
- 30 42. Verfahren zur Herstellung einer pharmazeutischen Zusammensetzung, umfassend die Schritte des Gefriertrocknens einer Zusammensetzung, die ein Botulinumtoxin und gegebenenfalls Human-Serumalbumin und gegebenenfalls einen stabilisierenden Zucker umfaßt, und des Zugabens einer Menge von Human-Serumalbumin zu der gefriergetrockneten Botulinumtoxin-Zusammensetzung in einer ausreichenden Menge, so daß 500 µg Human-Serumalbumin pro 100 LD₅₀-Einheiten Botulinumtoxin überschritten werden.
- 35 43. Verfahren nach Anspruch 42, wobei die gefriergetrocknete Botulinumtoxin-Zusammensetzung 500 µg Human-Serumalbumin pro 100 LD₅₀-Einheiten Botulinumtoxin umfaßt.
- 40 44. Verfahren nach Anspruch 42, wobei das Human-Serumalbumin der gefriergetrockneten Botulinumtoxin-Zusammensetzung in einer ausreichenden Menge zugesetzt wird, so daß 5.500 bis 13.000 µg Human-Serumalbumin pro 100 LD₅₀-Einheiten Botulinumtoxin, 13.000 bis 50.500 µg Human-Serumalbumin pro 100 LD₅₀-Einheiten Botulinumtoxin, 50.500 bis 505.000 µg Human-Serumalbumin pro 100 LD₅₀-Einheiten Botulinumtoxin oder 50.500 bis 90.500 µg Human-Serumalbumin pro 100 LD₅₀-Einheiten Botulinumtoxin erreicht werden.
- 45 45. Verfahren nach Anspruch 42, wobei das Human-Serumalbumin der gefriergetrockneten Botulinumtoxin-Zusammensetzung in einer ausreichenden Menge zugesetzt wird, so daß 5.500 bis 13.000 µg Human-Serumalbumin pro 100 LD₅₀-Einheiten Botulinumtoxin erreicht werden.
- 50 46. Verfahren nach Anspruch 42, wobei das Human-Serumalbumin der gefriergetrockneten Botulinumtoxin-Zusammensetzung in einer ausreichenden Menge zugesetzt wird, so daß 13.000 bis 50.500 µg Human-Serumalbumin pro 100 LD₅₀-Einheiten Botulinumtoxin erreicht werden.
- 55 47. Verfahren nach Anspruch 42, wobei das Human-Serumalbumin der gefriergetrockneten Botulinumtoxin-Zusammensetzung in einer ausreichenden Menge zugesetzt wird, so daß 50.500 bis 505.000 µg Human-Serumalbumin pro 100 LD₅₀-Einheiten Botulinumtoxin erreicht werden.
48. Verfahren nach Anspruch 42, wobei das Human-Serumalbumin der gefriergetrockneten Botulinumtoxin-Zusammensetzung in einer ausreichenden Menge zugesetzt wird, so daß 50.500 bis 90.500 µg Human-Serumalbumin

58

147

EP 1 594 523 B1

pro 100 LD₅₀-Einheiten Botulinumtoxin erreicht werden.

Revendications

- 5 1. Composition pharmaceutique comprenant une quantité efficace d'une toxine botulinique et d'une albumine sérique humaine, dans laquelle l'albumine sérique humaine est présente en une quantité comprise entre 13 000 µg et 550 000 µg d'albumine sérique humaine pour 100 unités DL₅₀ de toxine botulinique.
- 10 2. Composition pharmaceutique selon la revendication 1, dans laquelle l'albumine sérique humaine est présente en une quantité comprise entre 13 000 µg et 50 500 µg d'albumine sérique humaine pour 100 unités DL₅₀ de toxine botulinique.
- 15 3. Composition pharmaceutique selon la revendication 1, dans laquelle l'albumine sérique humaine est présente en une quantité comprise entre 50 500 µg et 505 000 µg d'albumine sérique humaine pour 100 unités DL₅₀ de toxine botulinique.
- 20 4. Composition pharmaceutique selon la revendication 1, dans laquelle l'albumine sérique humaine est présente en une quantité comprise entre 50 500 µg et 90 500 µg d'albumine sérique humaine pour 100 unités DL₅₀ de toxine botulinique.
5. Composition pharmaceutique selon l'une quelconque des revendications 1 à 4, dans laquelle la composition est lyophilisée.
- 25 6. Composition selon l'une quelconque des revendications précédentes 1 à 5, dans laquelle la toxine botulinique est sélectionnée parmi le groupe consistant en : toxine botulinique de type A, B, C, D, E, F et G.
7. Composition selon l'une quelconque des revendications précédentes 1 à 5, dans laquelle la toxine botulinique est une toxine botulinique de type A provenant de la souche Hall de *Clostridium botulinum*.
- 30 8. Composition selon l'une quelconque des revendications précédentes 1 à 5, dans laquelle la toxine botulinique comprend de la toxine botulinique à chaîne légère fractionnée.
- 35 9. Composition selon l'une quelconque des revendications précédentes 1 à 5, dans laquelle la toxine botulinique comprend essentiellement de la toxine botulinique à chaîne légère fractionnée.
10. Composition selon l'une quelconque des revendications précédentes 1 à 5, dans laquelle la toxine botulinique comprend essentiellement un mélange de formes hybrides et à chaîne déplacée de toxine botulinique.
- 40 11. Composition selon l'une quelconque des revendications précédentes 1 à 5, dans laquelle la toxine botulinique comprend essentiellement des formes chimères de toxine botulinique.
12. Composition selon l'une quelconque des revendications précédentes 1 à 5, dans laquelle la toxine botulinique a une activité spécifique supérieure ou égale à 20 unités DL₅₀ par nanogramme de toxine botulinique.
- 45 13. Composition selon l'une quelconque des revendications précédentes 1 à 5, comprenant en outre un excipient pharmaceutiquement acceptable.
14. Composition selon l'une quelconque des revendications précédentes 1 à 5, dans laquelle la toxine botulinique est liée de manière covalente à l'albumine sérique humaine.
- 50 15. Composition selon l'une quelconque des revendications précédentes 1 à 5, comprenant en outre du zinc ou un sel de zinc.
- 55 16. Composition selon l'une quelconque des revendications précédentes 1 à 5, dans laquelle l'albumine sérique humaine est formulée en tant qu'une particule d'albumine solide.
17. Composition selon l'une quelconque des revendications précédentes 1 à 5, dans laquelle l'albumine sérique humaine

59

148

EP 1 594 523 B1

est produite de manière recombinante.

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18. Composition selon l'une quelconque des revendications précédentes 1 à 5, comprenant en outre un ou plusieurs agents séquestrants additionnels.
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19. Utilisation d'une quantité efficace d'une composition pharmaceutique selon l'une quelconque des revendications précédentes 1 à 18 pour la fabrication d'un médicament pour le traitement de la dénerivation localisée.
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20. Utilisation selon la revendication 19, dans laquelle le sujet souffre d'une maladie neuromusculaire associée à une augmentation de la tonicité musculaire ou de mouvement involontaire.
21. Utilisation selon la revendication 20, dans laquelle la maladie neuromusculaire associée à une augmentation de la tonicité musculaire avec mouvement involontaire est le blépharospasme ou le torticolis.
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22. Utilisation selon la revendication 21, dans laquelle la maladie neuromusculaire associée à une augmentation de la tonicité musculaire avec mouvement involontaire est le blépharospasme.
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23. Utilisation selon la revendication 22, dans laquelle la quantité efficace est comprise entre 10 unités DL₅₀ et 200 unités DL₅₀.
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24. Utilisation selon la revendication 21, dans laquelle la maladie neuromusculaire associée à une augmentation de la tonicité musculaire avec mouvement involontaire est le torticolis.
- 30
25. Utilisation selon la revendication 24, dans laquelle la quantité efficace est comprise entre 10 unités DL₅₀ et 3000 unités DL₅₀.
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26. Utilisation d'une quantité efficace d'une composition pharmaceutique selon l'une quelconque des revendications précédentes 1 à 18 pour la fabrication d'un médicament pour le traitement d'une pathologie choisie parmi le groupe consistant en rides faciales, rhytides et altération cosmétique de la lèvre et du front.
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27. Utilisation selon la revendication 26, dans laquelle la quantité efficace est comprise entre 2,5 unités DL₅₀ et 400 unités DL₅₀.
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28. Utilisation d'une quantité efficace d'une composition pharmaceutique selon l'une quelconque des revendications précédentes 1 à 18 pour la fabrication d'un médicament pour le traitement de troubles de maux de tête humains.
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29. Utilisation selon la revendication 28, dans laquelle la quantité efficace est comprise entre 5 unités DL₅₀ et 1000 unités DL₅₀.
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30. Utilisation d'une quantité efficace d'une composition pharmaceutique selon l'une quelconque des revendications précédentes 1 à 18 pour la fabrication d'un médicament pour le traitement de troubles migraineux humains.
31. Utilisation selon la revendication 30, dans laquelle la quantité efficace est comprise entre 5 unités DL₅₀ et 1000 unités DL₅₀.
32. Utilisation d'une quantité efficace d'une composition pharmaceutique selon l'une quelconque des revendications précédentes 1 à 18 pour la fabrication d'un médicament pour le traitement de pathologies inflammatoires humaines.
33. Utilisation selon la revendication 32, dans laquelle la quantité efficace est comprise entre 5 unités DL₅₀ et 4000 unités DL₅₀.
34. Utilisation d'une quantité efficace d'une composition pharmaceutique selon l'une quelconque des revendications précédentes 1 à 18 pour la fabrication d'un médicament pour le traitement de douleurs myopathiques ou neuropathiques.
35. Utilisation selon la revendication 34, dans laquelle la quantité efficace est comprise entre 5 unités DL₅₀ et 4000 unités DL₅₀.

60

149

EP 1 594 523 B1

36. Utilisation d'une quantité efficace d'une composition pharmaceutique selon l'une quelconque des revendications précédentes 1 à 18 pour la fabrication d'un médicament pour le traitement des douleurs du dos ou des douleurs arthritiques.
- 5 37. Utilisation selon la revendication 36, dans laquelle la quantité efficace est comprise entre 5 unités DL₅₀ et 4000 unités DL₅₀.
38. Utilisation d'une quantité efficace d'une composition pharmaceutique selon l'une quelconque des revendications précédentes 1 à 18 pour la fabrication d'un médicament pour le traitement de spasmes gastro-intestinaux et de strictures.
- 10 39. Utilisation selon la revendication 38, dans laquelle la quantité efficace est comprise entre 5 unités DL₅₀ et 4000 unités DL₅₀.
- 15 40. Utilisation d'une quantité efficace d'une composition pharmaceutique selon l'une quelconque des revendications précédentes 1 à 18 pour la fabrication d'un médicament pour le traitement du syndrome d'hyperhydrose.
41. Utilisation selon la revendication 40, dans laquelle la quantité efficace est comprise entre 5 unités DL₅₀ et 4000 unités DL₅₀.
- 20 42. Procédé de préparation d'une composition pharmaceutique comprenant les étapes de lyophilisation d'une composition comprenant une toxine botulinique et éventuellement de l'albumine sérique humaine et éventuellement un sucre stabilisant et d'addition d'une quantité d'albumine sérique humaine à la composition de toxine botulinique lyophilisée en une quantité suffisante pour dépasser 500 µg d'albumine sérique humaine par 100 unités DL₅₀ de toxine botulinique.
- 25 43. Procédé selon la revendication 42, dans lequel la composition de toxine botulinique lyophilisée comprend 500 µg d'albumine sérique humaine pour 100 unités DL₅₀ de toxine botulinique.
- 30 44. Procédé selon la revendication 42, dans lequel l'albumine sérique humaine est ajoutée à la composition de toxine botulinique lyophilisée en une quantité suffisante pour obtenir entre 5 500 µg et 13 000 µg d'albumine sérique humaine pour 100 unités DL₅₀ de toxine botulinique, entre 13 000 µg et 50 500 µg d'albumine sérique humaine pour 100 unités DL₅₀ de toxine botulinique, entre 50 500 µg et 505 000 µg d'albumine sérique humaine pour 100 unités DL₅₀ de toxine botulinique, ou entre 50 500 µg et 90 500 µg d'albumine sérique humaine pour 100 unités DL₅₀ de toxine botulinique.
- 35 45. Procédé selon la revendication 42, dans lequel l'albumine sérique humaine est ajoutée à la composition de toxine botulinique lyophilisée en une quantité suffisante pour obtenir entre 5 500 µg et 13 000 µg d'albumine sérique humaine pour 100 unités DL₅₀ de toxine botulinique.
- 40 46. Procédé selon la revendication 42, dans lequel l'albumine sérique humaine est ajoutée à la composition de toxine botulinique lyophilisée en une quantité suffisante pour obtenir entre 13 000 µg et 50 500 µg d'albumine sérique humaine pour 100 unités DL₅₀ de toxine botulinique.
- 45 47. Procédé selon la revendication 42, dans lequel l'albumine sérique humaine est ajoutée à la composition de toxine botulinique lyophilisée en une quantité suffisante pour obtenir entre 50 500 µg et 505 000 µg d'albumine sérique humaine pour 100 unités DL₅₀ de toxine botulinique.
- 50 48. Procédé selon la revendication 42, dans lequel l'albumine sérique humaine est ajoutée à la composition de toxine botulinique lyophilisée en une quantité suffisante pour obtenir entre 50 500 µg et 90 500 µg d'albumine sérique humaine pour 100 unités DL₅₀ de toxine botulinique.
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61

150

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62



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(12) **United States Patent**
Borodic

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(45) **Date of Patent:** **Feb. 17, 2009**

(54) **PHARMACEUTICAL BOTULINUM TOXIN COMPOSITIONS**

(75) Inventor: **Gary Borodic**, Canton, MA (US)

(73) Assignee: **Botulinum Toxin Research Associates**, Quincy, MA (US)

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(52) **U.S. Cl.** **424/239.1**; 514/12; 424/184.1; 424/234.1; 424/247.1

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See application file for complete search history.

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Primary Examiner Mark Navarro
Assistant Examiner Ginny Portner
(74) **Attorney, Agent, or Firm**—Milbank, Tweed, Hadly & McLoey LLP

(57) **ABSTRACT**

Botulinum toxin, a well know systemic poison, produces favorable therapeutic effect by virtue of regionally attaching to nerves within the myoneural junction and possibly other tissues in a target region of a particular tissue. The present invention provides compositions of *botulinum* toxin and a sequestration agent that increase sequestration and delivery of the *botulinum* toxin to neural and associated tissues, as compared with available formulations of *botulinum* toxins, and thereby produce a beneficial clinical effect. The sequestration agents of the present invention include proteins, lipids and carbohydrates. A preferred composition of the present invention comprises a *botulinum* toxin and an albumin. The present invention also provides methods of treating neuromuscular diseases and pain using the disclosed compositions and methods of making the disclosed compositions.

55 Claims, No Drawings

152

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64

1
PHARMACEUTICAL BOTULINUM TOXIN
COMPOSITIONS

This application claims benefit to U. S. Provisional Appli-
cation Ser. No. 60/435,901 that was filed on Dec. 20, 2002.

TECHNICAL FIELD OF THE INVENTION

This invention relates to improved pharmaceutical compo-
sitions comprising *botulinum* neurotoxin and a sequestration
agent. The invention further provides pharmaceutical compo-
sitions and methods for the treatment of a variety of neuro-
muscular diseases.

BACKGROUND OF THE INVENTION

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

Although a deadly toxin at higher concentrations and quan-
tities, *botulinum* toxin has been used as a valuable therapeutic
for the treatment of many neuromuscular diseases (e.g., dys-
tonia, hemifacial spasm, bruxism, spasticity, cerebral palsy,
torticollis), as well as sensory disorders and cutaneous disor-
ders (myofascial pain, migraine, tension headaches, neuropathy,
hyperhidrosis). Prior to this invention, the in vivo bind-
ing 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®, DYS-
SPORT®, 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 effective-
ness where other available *botulinum* toxin preparations have
failed.

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

2
wrinkling from depression of facial muscle tone. (Borodic
(1992) *botulinum* A toxin for (expressionistic) ptosis over-
correction 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 pharmaeu-
tical measurement of the denervating or biologic activity of
botulinum toxin has been the LD₅₀ unit using a 18-22 gram
Swiss-Webster mouse, quantitated statistically by injecting
cohorts of mice at different dilutions from the purified *botu-
linum* neurotoxin protein and its protein complexes. This
measurement has the advantage of simplicity of a clear end-
point determination (living or dead mouse), however the
LD₅₀ unit does not predict clinical behavior of various *botu-
linum* toxin formulations when compared in clinical studies.
For instance, one preparation of type B *botulinum* toxin
(MYOBLOC®) requires 5,000-15,000 LD₅₀ units to treat
torticollis whereas another preparation of *botulinum* toxin
Type A (BOTOX®) requires only 100-300 LD₅₀ units. Simi-
larly, the LD₅₀ unit has failed to distinguish differences in
therapeutic behavior of different sources of the same *botuli-
num* toxin immunotype. For instance, approximately 50-300
units of BOTOX® is required to treat blepharospasm and
cervical dystonia compared to 200-1200 units of DYS-
SPORT®, 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
DYS-SPORT®	200 U	600-1,200 U
MYOBLOC®	3,000-5,000 U	10,000-15,000 U

¹Units (U) are LD₅₀ 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 differ-
ences 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 *botu-
linum* 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; incorpo-
rated 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 injec-
tions 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®	DYSPOET®	MYOBLOC®
Ptosis ¹	<2%	12-15%	30-40%
Dysphagia	<2%	14-21%	10-17%

¹Nussgens et al. (1997) Comparison of two botulinum-toxin preparations in the treatment of essential blepharospasm. *Graefes Arch Clin Exp Ophthalmol* 235(4): 197-199.
²Phase 3 Studies 1998-1989 for Oculinum Meta-analysis of clinical studies on Dysphagia and Botulinum 1995 at NIH (Borodic).
³Lew 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 histologic model demonstrating a histochemical and morphologic diffusion gradient from point injections of *botulinum* toxin. (Borodic et al. (1991) *botulinum* toxin: Clinical and scientific aspects. *Ophthalmology Clinics of North America* 4: 491-503; incorporated herein by reference in its entirety). The gradient was further demonstrated to be dose dependent over single muscle strips and capable of crossing fascial planes. The diffusion model was further demonstrated on the facial wrinkling pattern of the human forehead. (Borodic et al (1992) *botulinum* toxin for spasmodic torticollis, multiple vs single point injections per muscle. *Head and Neck* 14: 33-37). Diffusion was thereafter used to explain the mechanism for dysphagia after surface injections of *botulinum* injection for the human neck and ptosis (drooping eyelid complication) after periorcular 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 stemomastoid muscle, targeted for treatment of torticollis, to peripharyngeal musculature which generates the force for effective swallowing. From both histologic models and clinical experience, diffusion appears to be directly related to the quantity of toxin given in I.D.₅₀ units, that is, the greater the I.D.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 DYSPOET® 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 DYSPOET® 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 DYSPOET® 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 Rhytids American Society of Ophthalmic Plastic and reconstructive Surgeons Annual Meeting, Anaheim, Calif. Nov. 14, 2003).

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 DYSPOET® 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 DYSPOET® is derived from British Microbiology Collection. Immunologic cross reactivity exists between the products as both products were derived from immunotype A strains. Despite similar immunotypes, the clinical responses between BOTOX® and DYSPOET® may be explained by the differences in the excipients used in each formulation. The difference in human serum albumin concentrations between BOTOX® and DYSPOET® are outlined in Table 3.

TABLE 3

Human Serum Albumin content of various pharmaceutical formulations of botulinum toxin.		
Formulation	Albumin ¹	I.D. ₅₀ µg albumin
BOTOX®	500 µg	0.2
DYSPOET®	125 µg	5.0

¹Albumin is represented in mg per 100 I.D.₅₀ units of botulinum toxin. Other differences exist including the presence of stabilizing sugars. Lactose is used in DYSPOET® and not used in BOTOX®.

The albumin discrepancy between BOTOX® and DYSPOET® is almost identical to the difference in dose requirements observed between BOTOX® and DYSPOET® in multiple clinical studies. The correlation between the albumin ratio/clinical potency ratio is further strengthened by changes in pharmacologic properties of DYSPOET® when albumin is added to the vials using a mouse hemidiaphragm animal model. Wohlfahrt et al. noted using this model that adding albumin to one vials of DYSPOET® brought biologic activity higher using the mouse hemi-diaphragm model. (Biglalke et al (2001) *Botulinum* A toxin: DYSPOET® 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 I.D.₅₀ bioassay afforded by the albumin concentration increase. (Biglalke et al (2001) *Botulinum* A toxin: DYSPOET® improvement of biological availability. *Exp. Neurol.* 168(1): 162-170). The authors explained the differences of albumin on the I.D.₅₀ bioassay without reference to mechanism of action in tissues or pharmacologic-pharmacokinetic importance, that is, in vivo albumin binding, enhanced sequestration, and improvement in therapeutic effects. The same authors further observed in a rat-diaphragm preparation, that the addition of albumin to the BOTOX® preparation could not substantially increase regional denervative effects and did not advocate any changes in formulation. The findings of these researchers concluded that there was an effect of the

albumin concentration on the LD₅₀ measurements however, there work did not demonstrate any increased potency of BOTOX® on regional denervation or that DYSPORT® could enhance to give any greater denervation potency over BOTOX®. Their 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 account for dilutions and tissue fluid flow capable of washing injected toxin away from targeted tissue prior to binding with the nerve axon terminal receptors. The real time application requires an in vivo analysis of the effects of albumin on regional denervation as outlined in the following experiments. Their work did identify reasons for differences in LD₅₀ as measured by the mouse lethality assay. The conclusion were no improvements in potency or effectiveness could be made over existing BOTOX® preparation and is directly contrary to the conclusion derived herein. (Hanover Germany International Botulinum Toxin Meeting 2002).

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

SUMMARY OF THE INVENTION

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

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

In another embodiment, the compositions of the present invention comprise a *botulinum* toxin that consists essentially of fractionated-light-chain *botulinum* toxin. In yet another embodiment, the *botulinum* toxin consists essentially of a mixture of hybrid and chain-translocated forms of *botulinum* toxin. In a further embodiment, the *botulinum* toxin consists essentially of chimeric forms of *botulinum* toxin. Although the present invention may utilize any *botulinum* toxin, *botulinum* toxin fragment that retains neurotoxic activity, *botu-*

num toxin chimeras and hybrids, chemically-modified *botulinum* toxin, and specific activities well known to those of ordinary skill in the art, in one embodiment the *botulinum* toxin is purified to a specific activity greater than or equal to 20 LD₅₀ units per nanogram *botulinum* toxin.

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

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

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

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

In another embodiment, the present invention provides a composition comprising *botulinum* toxin and a sequestration agent, wherein the sequestration agent is present in an amount between 550 and 900,500 µg sequestration agent per 100 LD₅₀ units *botulinum* toxin, wherein the albumin may be formulated as a solid albumin particle.

In one embodiment of the present invention, the compositions comprise a *botulinum* toxin and at least one sequestration agent. In a preferred embodiment, the compositions of the present invention 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 LD₅₀ units of a composition of the present invention, as described herein. In another embodiment, the present invention provides methods for producing denervation in a subject suffering from torticollis. Preferably, the effective amount of a composition of the present invention is between 10 and 3000 LD₅₀ units.

In another embodiment, the present invention provides a method of treating a condition selected from the group consisting of facial wrinkles, rhytides and cosmetic alteration of lip and brow, in a subject in need thereof, comprising administering an effective amount of a composition of the present invention, as disclosed herein. Preferably, the effective amount is between 2.5 and 400 LD₅₀ 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 LD₅₀ 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 LD₅₀ 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 LD₅₀ 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 LD₅₀ 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 LD₅₀ 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 LD₅₀ units.

The present invention provides a method of treating a hyperphysis syndrome in a subject in need thereof, comprising administering an effective amount of a composition of the present invention, as disclosed herein. Preferably, the effective amount is between 5 and 4,000 LD₅₀ units.

The present invention also provides a method of producing the compositions described herein. In one embodiment, the method comprises mixing a sequestration agent with *botulinum* toxin. In another embodiment, the method comprises freeze drying or flash drying a sequestration agent with *botulinum* toxin. Preferably, the *botulinum* toxin and the seques-

tration agent are in a weight to weight ratio which exceeds 100 µg sequestration agent to 1 ng of *botulinum* toxin.

DETAILED DESCRIPTION OF THE INVENTION

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

A. Definitions.

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

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

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

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

As used herein, the term "pharmaceutically acceptable carrier" means a chemical composition, compound, or solvent with which an active ingredient may be combined and which, following the combination, can be used to administer the active ingredient to a subject. As used herein, "pharmaceutically acceptable carrier" includes, but is not limited to, one or more of the following: excipients; surface active agents; dispersing agents; inert diluents; granulating and disintegrating

agents; binding agents; lubricating agents; preservatives; physiologically degradable compositions such as gelatin; aqueous vehicles and solvents; oily vehicles and solvents; suspending agents; dispersing or wetting agents; emulsifying agents; demulcents; buffers; salts; thickening agents; fillers; antioxidants; stabilizing agents; and pharmaceutically acceptable polymeric or hydrophobic materials and other ingredients known in the art and described, for example in Genaro, ed., 1985, Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, Pa., which is incorporated herein by reference.

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

As used herein, "subject" means a mammal.

B. Albumin.

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

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

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

MYOBLOC® is formulated at an acidic pH<6.0 which provides for increased stability and stability of the liquid

formulation at room temperature. Unfortunately, the acidic pH has an adverse side effect on the structure and probably tissue carrying properties of the human serum albumin in this biologic drug's formulation. At varying pH, the isomerization of albumin can be considerable as well as the tertiary configuration of the albumin protein and physical properties (see Peters (1996) *All about Albumin*, Academic Press, New York; incorporated herein by reference in its entirety). Alterations in physical properties (via changes in binding of *botulinum* toxin and dynamics of *botulinum* toxin molecular release in tissues) can be used to explain some of the considerable differences in dose requirements comparing BOTOX® and MYOBLOC® in clinical practice. With higher pH, type B formulation, similar histologic effects can be seen with equivalent LD₅₀ units (see Borodic et al. (1993) *Botulinum B Toxin as an Alternative to Botulinum A Toxin*, A Histologic study, *Ophthalmic Plastic and Reconstructive Surgery* 9(3): 182-190). Although other proteins (e.g. gelatin, lactalbumin, lysozyme), lipids and carbohydrates may serve as effective sequestration agents, albumin, including encapsulated albumin and solid microspheres is the preferred protein sequestration agent, in part, because of its low immunogenicity. Other proteins, polysaccharides, lipids, polymers, gels and hydrogels that are potentially suitable as sequestration agents are disclosed in U.S. Pat. No.: 4,861,627, which is incorporated herein by reference in its entirety. Methods of using and making protein microspheres, including albumin microspheres, are disclosed in U.S. Pat. Nos.: 6,620,617; 6,210,707; 6,100,306; and 5,069,936 which are each incorporated herein by reference in their entirety.

C. Sequestration.

The concept of sequestration has been used by the inventor to explain altered lidocaine toxicity when periocular injections are given in the absence of Wydase. (Iroll 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 is 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 Occulinum 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 (eg, BOTOX®, MYOBLOC®) currently in use have failed or given suboptimal results.

158

11

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

Producing compositions of *botulinum* toxin that require a lower effective amount to treat a particular condition is desired, because the administration of *botulinum* toxin has been associated with the development of immunologic resistance. Consequently, this complication requires increased dosing (higher LD₅₀ 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 LD₅₀ units/ng toxin and 900 µg human serum albumin to 100 LD₅₀ units of *botulinum* toxin (0.11 LD₅₀ 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 LD₅₀ units of *botulinum* type A toxin formulated with the increased amount of albumin to 1 LD₅₀ content.

TABLE 5

Reduction in effective amount of botulinum toxin using high-albumin botulinum toxin compositions.			
Open-Label 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 LD₅₀ 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 LD₅₀ 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 LD₅₀ units to achieve acceptable therapeutic results (reduction in effective amount of toxin) and was associated with limited diffusion into the orbit which frequently results in ptosis. The decreased incidence of this complication indicated sequestration of the effects of *botulinum* toxin was enhanced by the higher albumin content.

12

EXAMPLES

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

Example 1

Treatment of Blepharospasm

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

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

Example 2

Treatment of Hemifacial Spasm

The subject is a 62-year-old male with a history of bilateral hemifacial spasm. *Botulinum*-toxin therapy using BOTOX® had been ineffective. The spasms impaired his day to day ability to function. Decompression of a facial nerve was attempted surgically on two separate occasions. Both surgeries proved ineffective in attaining acceptable relief of involuntary facial spasms and produced deafness in one ear. The albumin content of the BOTOX® was increased by adding human serum albumin sufficient to achieve a concentration of 5,250 µg/cc (0.00952 LD₅₀/µg albumin). Administration of 30 LD₅₀ units of the high-albumin preparation proved highly effective and substantially relieved the clinical symptoms.

Example 3

Treatment of Hemifacial Spasm

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

70

159

13

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

Example 4

Treatment of Benign Essential Blepharospasm

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

Example 5

Treatment of Blepharospasm

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

Albumin was added to a conventional BOTOX® preparation to produce a high-albumin preparation of *botulinum* toxin with a concentration of 50,250 µg albumin/cc (0.001 LD₅₀/µg albumin). Injection of 50 units of 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 LD₅₀ units of *botulinum* toxin type A (BOTOX®). The high-albumin preparation produced contained 25,500 µg albumin per 100 LD₅₀ units (0.005 LD₅₀/µg

14

albumin). Administration of 60 LD₅₀ units reduced the clinically-observed involuntary-eyelid contractions.

Example 7

Treatment of Essential Blepharospasm

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

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

Example 8

Treatment of Severe Chronic Blepharospasm

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

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

Example 9

Treatment of Essential Blepharospasm

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

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

77

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

LD₅₀/mcg albumin/cc for BOTOX ®, DYSPORT ®, MYOBLOC ® given for direct comparison

Example 10

Preparation of a High-Albumin Composition of *Botulinum* Toxin

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

Example 11

Preparation of a High-Albumin Composition of *Botulinum* Toxin Further Comprising Hyaluronate

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

Example 12

Preparation of a High-Albumin Composition of *Botulinum* Toxin Further Comprising Collagen

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

and an enhances to the sequestration of the neurotoxin providing 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 LD₅₀ bioassay to determine the effective amount. The regional denervation rabbit ptosis bioassay and mouse hindlimb bioassay may be used to confirm the effective amount of a composition comprising the fusion protein. A clinical-dose-escalation study would be further used to confirm and refine effective amount.

Example 14

Testing PURTOX™ and Other Forms of *Botulinum* Toxin

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

I claim:

1. A composition comprising a therapeutically effective amount of a *botulinum* toxin, and a human serum albumin, wherein the human serum albumin is present in an amount between 13,000 and 550,000 µg human serum albumin per 100 LD₅₀ units *botulinum* toxin.

2. 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.

17

3. The composition of claim 2, wherein the *botulinum* toxin is *botulinum* toxin type A from Hall strain *Clostridium botulinum*.

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

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

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

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

8. The composition of claim 1, wherein the *botulinum* toxin has a specific activity greater than or equal to 20 LD₅₀ units per nanogram *botulinum* toxin.

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

10. The composition of claim 1, wherein the *botulinum* toxin is noncovalently bound to the human serum albumin.

11. The composition of claim 1, wherein the *botulinum* toxin is covalently bound to the human serum albumin.

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

13. The composition of claim 1, wherein the human serum albumin is present in an amount between 13,000 and 50,500 µg human serum albumin per 100 LD₅₀ units *botulinum* toxin.

14. The composition of claim 1, wherein the human serum albumin is present in an amount between 50,500 and 505,000 µg human serum albumin per 100 LD₅₀ units *botulinum* toxin.

15. The composition of claim 1, wherein the human serum albumin is formulated as encapsulated microspheres in an amount between 50,500 and 90,500 µg human serum albumin per 100 LD₅₀ units *botulinum* toxin.

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

17. The composition of claim 1, wherein the human serum albumin is recombinantly produced.

18. The composition of claim 1 further comprising one or more additional sequestration agents.

19. A pharmaceutical composition comprising a therapeutically effective amount of a *botulinum* toxin, a human serum albumin and a pharmaceutically acceptable carrier, wherein the human serum albumin is present in an amount between 13,000 and 550,000 µg human serum albumin per 100 LD₅₀ units *botulinum* toxin.

20. The pharmaceutical composition of claim 19, wherein the human serum albumin is present in an amount between 13,000 and 50,500 µg human serum albumin per 100 LD₅₀ units *botulinum* toxin.

21. The pharmaceutical composition of claim 19, wherein the human serum albumin is present in an amount between 50,500 and 505,000 µg human serum albumin per 100 LD₅₀ units *botulinum* toxin.

22. The pharmaceutical composition of claim 19, wherein the human serum albumin is present in an amount between 50,500 and 90,500 µg human serum albumin per 100 LD₅₀ units *botulinum* toxin.

23. A composition comprising a therapeutically effective amount of a *botulinum* toxin and a human serum albumin, wherein the human serum albumin is present in an amount between 13,000 and 50,500, between 50,500 and 505,000, or between 50,500 and 90,500, µg human serum albumin per 100 LD₅₀ units *botulinum* toxin.

24. A lyophilized pharmaceutical composition comprising a therapeutically effective amount of a *botulinum* toxin, a

18

human serum albumin and a pharmaceutically acceptable carrier, wherein the human serum albumin is present in an amount between 13,000 and 550,000 µg human serum albumin per 100 LD₅₀ units *botulinum* toxin.

25. The lyophilized pharmaceutical composition of claim 24, wherein the human serum albumin is present in an amount between 13,000 and 50,500 µg human serum albumin per 100 LD₅₀ units *botulinum* toxin.

26. The lyophilized pharmaceutical composition of claim 24, wherein the human serum albumin is present in an amount between 50,500 and 505,000 µg human serum albumin per 100 LD₅₀ units *botulinum* toxin.

27. The lyophilized pharmaceutical composition of claim 24, wherein the human serum albumin is present in an amount between 50,500 and 90,500 µg human serum albumin per 100 LD₅₀ units *botulinum* toxin.

28. A lyophilized composition comprising a therapeutically effective amount of a *botulinum* toxin and a human serum albumin, wherein the human serum albumin is present in an amount between 13,000 and 50,500, between 50,500 and 505,000, or between 50,500 and 90,500 µg human serum albumin per 100 LD₅₀ units *botulinum* toxin.

29. A method of localized denervation in a subject in need thereof, comprising administering a therapeutically effective amount of the composition of claim 1 to the subject to produce localized denervation.

30. The method of claim 29, wherein the subject suffers from a neuromuscular disease associated with increased muscle tone or involuntary movement.

31. The method of claim 30, wherein the neuromuscular disease associated with increased muscle tone with involuntary movement is blepharospasm or torticollis.

32. The method of claim 31, wherein the neuromuscular disease associated with increased muscle tone with involuntary movement is blepharospasm.

33. The method of claim 32, wherein the effective amount is between 10-200 LD₅₀ units.

34. The method of claim 31, wherein the neuromuscular disease associated with increased muscle tone with involuntary movement is torticollis.

35. The method of claim 34, wherein the effective amount is between 10 and 3000 LD₅₀ units.

36. 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 a therapeutically effective amount of the composition of claim 1 to the subject in order to alleviate at least one symptom of said condition.

37. The method of claim 36, wherein the effective amount is between 2.5 and 400 LD₅₀ units.

38. A method of treating human headache disorders in a subject in need thereof, comprising administering a therapeutically effective amount of the composition of claim 1 to the subject in order to alleviate at least one symptom of said human headache disorder.

39. The method of claim 38, wherein the effective amount is between 5 and 1,000 LD₅₀ units.

40. A method of treating human migraine headache disorders in a subject in need thereof, comprising administering a therapeutically effective amount of the composition of claim 1 to the subject in order to alleviate at least one symptom of said human migraine headache disorders.

41. The method of claim 40, wherein the effective amount is between 5 and 1,000 LD₅₀ units.

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

162

19

the subject in order to alleviate at least one symptom of said human inflammatory conditions.

43. The method of claim 42, wherein the effective amount is between 5 and 4,000 LD₅₀ units.

44. A method of treating myopathic or neuropathic pain in a subject in need thereof, comprising administering a therapeutically effective amount of the composition of claim 1 to the subject in order to alleviate at least one symptom of said myopathic or neuropathic pain.

45. The method of claim 44, wherein the effective amount is between 5 and 4,000 LD₅₀ units.

46. A method of treating back pain or arthritic pain in a subject in need thereof, comprising administering a therapeutically effective amount of the composition of claim 1 to the subject in order to alleviate at least one symptom of said back pain or arthritic pain.

47. The method of claim 46, wherein the effective amount is between 5 and 4,000 LD₅₀ units.

48. A method of treating gastrointestinal spasm and strictures in a subject in need thereof, comprising administering a therapeutically effective amount of the composition of claim 1 to the subject in order to alleviate at least one symptom of said gastrointestinal spasm and strictures.

49. The method of claim 48, wherein the effective amount is between 5 and 4,000 LD₅₀ units.

50. A method of treating a hyperhydrosis syndrome in a subject in need thereof, comprising administering a therapeu-

20

tically effective amount of the composition of claim 1 to the subject in order to alleviate at least one symptom of said hyperhydrosis syndrome.

51. The method of claim 50, wherein the effective amount is between 5 and 4,000 LD₅₀ units.

52. A method of producing the composition of claim 1, comprising mixing human serum albumin and *botulinum* toxin in an amount between 13,000 and 550,000 µg human serum albumin per 100 LD₅₀ units *botulinum* toxin.

53. A method of producing the composition of claim 1, comprising freeze drying or flash drying a mixture of a *botulinum* toxin and human serum albumin in an amount between 13,000 and 550,000 µg human serum albumin per 100 LD₅₀ units *botulinum* toxin.

54. A method of producing the composition of claim 1, comprising lyophilizing a mixture of a *botulinum* toxin and human serum albumin in an amount between 13,000 and 550,000 µg human serum albumin per 100 LD₅₀ units *botulinum* toxin.

55. A composition comprising a therapeutically effective amount of a *botulinum* toxin and a human serum albumin, wherein the human serum albumin is present in an amount between about 13,000 and 25,000 µg human serum albumin per 100 LD₅₀ units *botulinum* toxin.

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74