

SERIAL TREATMENT WITH BOTULINUM TOXIN FOR CERVICAL DYSTONIA**CROSS REFERENCE TO RELATED APPLICATIONS**

[0001] This International application is based upon and claims benefit of priority to US Provisional Application No. 63/583,215, filed on September 15, 2023, and US Provisional Application No. 63/583,579, filed on September 18, 2023, both of which are hereby incorporated by reference in their entireties.

REFERENCE TO A SEQUENCE LISTING

[0002] The Sequence Listing submitted herewith as a xml file named "13720.0036P1_Sequence_Listing.xml," created on September 16, 2024, and having a size of 17 Kilo bytes is hereby incorporated by reference pursuant to 37 C.F.R. § 1.52(e)(5).

FIELD OF THE INVENTION

[0003] This invention relates to methods to treat cervical dystonia with injectable compositions comprising botulinum toxin that may be administered to a subject suffering from such malady. The methods described herein involve a series of treatment sessions with a botulinum toxin composition that can be used to achieve for the patient maintenance of a peak treatment effect or within a narrow range thereof over the course of a plurality of treatment sessions.

BACKGROUND OF THE INVENTION

[0004] Cervical dystonia is an extremely painful, chronic neurological movement disorder where the neck and shoulder muscles contract involuntarily and contort, causing abnormal movements and awkward posture of the head and neck such as the head to twist or turn to the left or right (torticollis), upwards (retrocollis), downwards (antecollis) or sideways (laterocollis). The movements may be sustained (tonic), jerky (clonic), or a combination. Cervical dystonia (also referred to as neck dystonia or spasmodic torticollis) affects a person's ability to control muscle activity. Cervical dystonia may be primary (meaning that it is the only apparent neurological disorder, with or without a family history) or may be brought about by secondary causes (such as physical trauma) and is often attributed to nervous system damage caused by a stroke, disease or trauma. A rare disorder that can occur at any age, even during infancy, cervical dystonia most often occurs in middle-aged individuals, and is more prevalent in women than men. Those with a family

history of cervical dystonia or some other type of dystonia are at higher risk of developing the disorder.

[0005] Cervical dystonia is the third most common movement disorder following essential tremor and Parkinson's disease. An estimated 3 in every 10,000 people are known to suffer from cervical dystonia. The number of cases reported in North America alone is approximately 300,000.

[0006] Symptoms generally begin gradually and then reach a plateau where the symptoms don't get substantially worse. Unfortunately, there is no cure for cervical dystonia and the condition greatly impacts an individual's quality of life. In some cases, the disorder resolves without treatment, but sustained remissions are fairly uncommon.

[0007] Treatments for cervical dystonia include oral medications, botulinum toxin injections, surgery, and complementary therapies. The most commonly prescribed treatment for cervical dystonia is the use of botulinum toxin, typically type A (although Type B has also been used), which can reduce its signs and symptoms. Botulinum toxin can help block the communication between the nerve and the muscle and may alleviate abnormal movements and postures. The number of injections is typically based on the severity of the dystonia. Doctors injecting the toxin may select the muscles to be injected by observing abnormal postures or movements and feeling for the muscle spasm or by using an electromyography machine to measure muscle activity. Each muscle affected by dystonia typically has to be injected separately. As such, based on the diffusion characteristics of currently available toxin formulations, there is a limit to the total quantity of toxin that can be injected into the body at one time. While the treatment for cervical dystonia involves regular neurological intervention, insurance typically does not cover retreatment within 12 weeks of the prior dose. The response to the treatment with botulinum toxin typically wears off after a 12 week period, often as early as at 10 weeks. In addition, it can take over a period of 4-6 weeks after injection for the full effect of a treatment to be felt. Thus, patients are often in significant discomfort during the time leading up to retreatment and for a period of time thereafter. There is a need for a practical treatment regimen that reduces the symptom "roller-coaster" that a patient experiences.

SUMMARY OF THE INVENTION

[0008] The present disclosure is directed to a method of treating cervical dystonia in a subject, the method comprising administering a treatment regimen to said subject wherein the

treatment regimen comprises: administering a plurality of botulinum toxin treatments to treat cervical dystonia separated by an interval, wherein the interval between each of said botulinum treatments is at least 12 weeks; and wherein each treatment comprises injecting a therapeutically effective amount of botulinum toxin into one or more muscles contributing to cervical dystonia symptoms of said subject; and wherein a treatment administered subsequent to a prior treatment is administered when remaining efficacy of the prior treatment is greater than 40% of its peak efficacy.

[0009] A method of treating cervical dystonia (CD) in a subject comprising: administering a treatment to a subject that is prior to a subsequent treatment, wherein the treatment comprises injecting a therapeutically effective amount of botulinum toxin in a pharma composition to a plurality of muscles to treat torticollis only and administering the subsequent treatment to the subject, wherein the subsequent treatment comprises injecting a therapeutically effective amount of the botulinum toxin composition to a plurality of muscles to treat laterocollis, antecollis, or retrocollis and optionally torticollis.

DESCRIPTION OF THE FIGURES

[0010] **Figure 1** depicts injectable muscles as defined by the Study Design described in the Example Section.

[0011] **Figures 2 to 4** each show a treatment effect of patients with three or four serial treatments with a botulinum toxin composition described herein.

DETAILED DESCRIPTION OF THE INVENTION

[0012] This invention relates to treatment regimens wherein the timing of a retreatment and the dose amount is such that there is a significant amount of remaining efficacy from the prior treatment at the time of retreatment, wherein the time of retreatment is 12 or more weeks from the prior treatment. In embodiments, the remaining efficacy is greater than 40% of peak treatment effect or more specifically, greater than 50%, 60% of peak treatment effect, or more specifically, 70% of peak treatment effect. In other words, a significant amount of effect remains in the patient at the time of retreatment even though the retreatment time is beyond 12 weeks.

[0013] “Peak treatment effect” or “peak efficacy” is when the botulinum toxin treatment is having its maximum effect on the patient. Typically, this is achieved around 4-7 weeks after

injection and can be sustained for multiple weeks. To objectively quantify the patient's state of symptoms, TWSTRS or other suitable scales known to a person skilled in the art can be used. In an embodiment, TWSTRS score of patient within the time period of 4 weeks to 7 weeks post treatment can be used to determine the state of a subject's symptoms at "peak efficacy".

[0014] To quantify the percent of remaining treatment effect, which would be at a particular time of interest after 6-7 weeks post-treatment, the patient's symptomatic state at peak efficacy, at baseline, and at the particular post-treatment time of interest can be used. A patient's baseline is the state of symptoms without any treatment effect, e.g., when there is no toxin effect on the muscles. The percent of remaining treatment effect at time X is essentially the ratio of the difference between the symptomatic state at X and the baseline to difference between the symptomatic state at peak effect and the baseline. The percent of treatment effect loss at time X is essentially the ratio of the difference between the symptomatic state at X and the peak effect to difference between the symptomatic state at peak effect and the baseline. At retreatment, a patient is preferably within the peak effect of the treatment but near its end or has at least 60% of peak efficacy, or more preferably, at least 70, 80, 90, or 100% of peak efficacy.

[0015] It is understood that dose can impact what the symptomatic state is at "peak efficacy". In other words, at peak efficacy, TWSTRS scores can be lower for a patient when the amount of toxin administered is higher. In accordance with the present disclosure, the dose amount and the interval of time between treatments can be adjusted to achieve a symptomatic state that does not significantly deviate from the symptomatic state at peak efficacy and that symptomatic state at peak efficacy can be maximized without significant adverse events to the patient.

Treatment Regimens for Treating Cervical Dystonia

[0016] In accordance with the present disclosure, a treatment regimen to treat cervical dystonia comprises administering a plurality of botulinum toxin treatments separated by an interval. Each treatment comprises injecting a therapeutically effective amount of botulinum toxin into one or more muscles contributing to cervical dystonia symptoms of said subject. The interval between each of said botulinum treatments must be at least 12 weeks. In embodiments, the interval between treatments is approximately the time from treatment to about 3 to 21 days before efficacy begins to wane for a treatment. In this manner, as the prior treatment effect begins to wane, the retreatment effect starts its rise and the subject's symptom state remains near the state at peak

efficacy. The amount of an interval can vary between subjects depending on a particular patient's response to toxin but is usually between 12 to 36 weeks (e.g., 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, or 36 weeks).

[0017] In embodiments, a treatment that is administered subsequent to a prior treatment is administered when the remaining efficacy of the prior treatment is greater than 20% or more preferably greater than 40% of its peak efficacy. Preferably, the subsequent treatment can be administered when remaining efficacy of the prior treatment is greater than 50% of its peak efficacy or greater than 60% of its peak efficacy or greater than 70% of its peak efficacy or greater than 80% of its peak efficacy or greater than 90% of its peak efficacy or before peak efficacy begins to wane. In embodiments, the subsequent treatment can be administered when remaining efficacy of the prior treatment is 40% to 90% or 50% to 90% or 60% to 90% or 70% to 90% of its peak efficacy.

[0018] The dose can be titrated over time to determine the minimal amount of toxin that is needed to have the maximum treatment effect (e.g., lowest TWSTRS score possible) at peak efficacy. In embodiments, the therapeutically effective amount of the subsequent treatment can be reduced from that of the therapeutically effective amount of the prior treatment but because of the overlapping effect of the treatments a peak efficacy of the subsequent treatment is approximately equal to or is greater than the peak efficacy of the prior treatment. Conversely, the therapeutically effective amount of the subsequent treatment can be increased from that of the therapeutically effective amount of the prior treatment to assess if the subject's symptomatic state at peak efficacy can be improved. It may also be desired to begin with a high dose such as 300U for a prior treatment and reduce the amount of toxin after the prior treatment to determine whether there is a maintenance of peak treatment effect at the lower dose. Such strategy may shorten the interval of time to achieve maintenance of the peak treatment effect or maintain an effect within a narrow range thereof over the entire course of a plurality of treatment sessions. A narrow range of a peak treatment effect can be within 30%, 25%, 20%, or 15% of the peak treatment effect as compared to baseline measured by TWSTRS or another method of scaling cervical dystonia in a subject.

[0019] The muscles treated at different treatments can be varied, for example, when some muscles exhibit a longer duration of response to toxin than other muscles. In embodiment, the one or more muscles injected in a subsequent treatment can be different than the one or more muscles

of a prior treatment or can be a subgroup or an expanded group thereof. For example, splenius capitis, trapezius and levator scapulae on the same side and the sternocleidomastoid on the opposite side (or a sub-group thereof) may be treated in each treatment session but additional muscles can be treated in every other session.

[0020] It is contemplated that 2 to 6 treatment sessions will be needed before a subject is able to maintain its peak effect over the course of a plurality of treatment sessions. Thus, the method can further comprise administering a treatment following the subsequent treatment to the subject to treat CD, wherein the treatment following the subsequent treatment is administered when remaining efficacy of the subsequent treatment is greater than 20%, 30%, 40%, 50%, 60%, 70%, or 80%, 90% of its peak efficacy or at peak efficacy. In addition, the dosage can again be reduced or increased from the prior treatment, wherein the symptomatic state at peak efficacy of the subsequent treatment is the same as or improved from the state at the peak efficacy of the prior treatment. In addition, the one or more muscles injected in the treatment following the subsequent treatment can be varied from the prior treatment as described above.

[0021] Treatment regimens as described herein can lead to the subject's symptomatic state at peak efficacy improving with the first 2-6 session until a maximum treatment state at peak efficacy is achieved at which point the patient can receive a consistent dose, which may also be at a consistent interval. In an embodiment, the subsequent treatment is administered when remaining efficacy of the prior treatment is greater than 80% of its peak efficacy and wherein a peak efficacy of the subsequent treatment is approximately equal to or better than the peak efficacy of the prior treatment. In embodiments, following the subsequent treatment or the treatment following the subsequent treatment, repeated administrations maintain a peak treatment effect (also referred to as maximum treatment state) or maintain an effect within a narrow range of the peak treatment effect over the course of the repeated administrations. A narrow range of a peak treatment effect can be within 30%, 25%, 20%, or 15% of the peak treatment effect as compared to baseline measured by TWSTRS or another method of scaling cervical dystonia in a subject.

[0022] In another embodiment, a method of treating cervical dystonia (CD) in a subject can comprise a treatment regimen wherein the muscles targeted differ between two consecutive treatments and the muscle group alternates. In such embodiments, the method can comprise administering a therapeutically effective amount of botulinum toxin in a pharmaceutical

composition to a plurality of muscles to treat torticollis only and administering in a subsequent treatment to the subject a therapeutically effective amount of the botulinum toxin composition to a plurality of muscles to treat laterocollis, antecollis, or retrocollis and optionally torticollis. In embodiment, the subsequent treatment is to treat laterocollis only. In embodiments, the subsequent treatment is to treat laterocollis and torticollis. As explained before, the subsequent treatment is administered when remaining efficacy of the prior treatment is greater than 60%, 70, 80, or 90% or at 100% of its peak efficacy. The treatment interval can be at least 12 weeks after the administration of the prior treatment.

Botulinum Toxin Pharmaceutical Compositions

[0023] A composition that can be used with treatment regimens described herein are those with a duration of effect at least greater than 16 weeks, such as greater than 18, 20, 24, 28, 30 weeks, or more. Suitable composition may be used as injectable applications for providing a botulinum toxin to a subject with cervical dystonia as described herein. In embodiments, a composition in accordance with the present disclosure comprises a positively charged peptide carrier to which botulinum toxin non-covalently associates. A suitable composition can also have an improved safety profile, including fewer adverse events, over other compositions and methods of delivery of botulinum toxin so that shortened dosing intervals are well tolerated. In embodiments, suitable compositions are injectable and provide a duration of effect that lasts 16 weeks, 18 weeks, 20 weeks, or 24 weeks in subjects to whom such compositions, particularly those comprising botulinum toxin in amounts of 100U to 500 U, are administered by injection for the treatment of cervical dystonia.

[0024] The term “botulinum toxin” as used herein may refer to any of the known types of botulinum toxin (e.g., 150 kD botulinum toxin protein molecules associated with the different serotypes of *C. botulinum*), whether produced by the bacterium or by recombinant techniques, as well as any such types that may be subsequently discovered including newly discovered serotypes, and engineered variants or fusion proteins. Currently seven immunologically distinct botulinum neurotoxins have been characterized, namely botulinum neurotoxin serotypes A, B, C, D, E, F and G, each of which is distinguished by neutralization with type-specific antibodies. The botulinum toxin serotypes are commercially available, for example, from Sigma-Aldrich (St. Louis, MO) and from Metabionics, Inc. (Madison, WI), as well as from other sources. The different serotypes of

botulinum toxin vary in the animal species that they affect and in the severity and duration of the paralysis they evoke. At least two types of botulinum toxin, types A and B, are available commercially in formulations for treatment of certain conditions. Type A, for example, is contained in preparations of Allergan having the trademark BOTOX®, of Ipsen having the trademark DYSPORT®, and of Revance having the trademark DAXXIFY®, and type B is contained in preparations of Elan having the trademark MYOBLOC®.

[0025] The term “botulinum toxin” used in the compositions of this invention can alternatively refer to a botulinum toxin derivative, that is, a compound that has botulinum toxin activity but contains one or more chemical or functional alterations on any part or on any amino acid chain relative to naturally occurring or recombinant native botulinum toxins. For instance, the botulinum toxin may be a modified neurotoxin that is a neurotoxin which has at least one of its amino acids deleted, modified or replaced, as compared to a native form, or the modified neurotoxin can be a recombinantly produced neurotoxin or a derivative or fragment thereof. For instance, the botulinum toxin may be one that has been modified in a way that, for instance, enhances its properties or decreases undesirable side effects, but that still retains the desired botulinum toxin activity. Alternatively the botulinum toxin used in this invention may be a toxin prepared using recombinant or synthetic chemical techniques, e.g. a recombinant peptide, a fusion protein, or a hybrid neurotoxin, for example prepared from subunits or domains of different botulinum toxin serotypes (*See*, U.S. Patent No. 6,444,209, for instance). The botulinum toxin may also be a portion of the overall molecule that has been shown to possess the necessary botulinum toxin activity, and in such case may be used per se or as part of a combination or conjugate molecule, for instance a fusion protein. Alternatively, the botulinum toxin may be in the form of a botulinum toxin precursor, which may itself be non-toxic, for instance a non-toxic zinc protease that becomes toxic on proteolytic cleavage.

[0026] The term “botulinum toxin complex” or “toxin complex” as used herein refers to the approximately 150 kD botulinum toxin protein molecule (belonging to any one of botulinum toxin serotypes A-G), along with associated endogenous non-toxin proteins (i.e., hemagglutinin protein and non-toxin non-hemagglutinin protein produced by *Clostridium botulinum* bacteria). Note, however, that the botulinum toxin complex need not be derived from *Clostridium botulinum* bacteria as one unitary toxin complex. For example, botulinum toxin or modified botulinum toxin may be recombinantly prepared first and then subsequently combined with the non-toxin proteins.

Recombinant botulinum toxin can also be purchased (e.g., from List Biological Laboratories, Campbell, CA) and then combined with non-toxin proteins.

[0027] Suitable compositions can comprise botulinum toxin that is a “reduced botulinum toxin complex”, in which the botulinum toxin complexes have reduced amounts of endogenous non-toxin protein compared to the amounts naturally found in botulinum toxin complexes produced by *Clostridium botulinum* bacteria. In one embodiment, reduced botulinum toxin complexes are prepared using any conventional protein separation method to extract a fraction of the hemagglutinin protein or non-toxin non-hemagglutinin protein from botulinum toxin complexes derived from *Clostridium botulinum* bacteria. For example, reduced botulinum toxin complexes may be produced by dissociating botulinum toxin complexes through exposure to red blood cells at a pH of 7.3 (e.g., see EP 1514556 A1, hereby incorporated herein by reference). HPLC, dialysis, columns, centrifugation, and other methods for extracting proteins from proteins can be used. Alternatively, when the reduced botulinum toxin complexes are to be produced by combining synthetically produced botulinum toxin with non-toxin proteins, one may simply add less hemagglutinin or non-toxin, non-hemagglutinin protein to the mixture than what would be present for naturally occurring botulinum toxin complexes. Any of the non-toxin proteins (e.g., hemagglutinin protein or non-toxin non-hemagglutinin protein or both) in the reduced botulinum toxin complexes according to the invention may be reduced independently by any amount. In certain exemplary embodiments, one or more non-toxin proteins are reduced by at least about 0.5%, 1%, 3%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100% compared to the amounts normally found in botulinum toxin complexes. As noted above, *Clostridium botulinum* bacteria produce seven different serotypes of toxin and commercial preparations are manufactured with different relative amounts of non-toxin proteins (i.e. different amount of toxin complexes). For example, MYOBLOC™ has 5000 U of Botulinum toxin type B per ml with 0.05% human serum albumin, 0.01 M sodium succinate, and 0.1 M sodium chloride. DYSPORT™ has 500 U of botulinum toxin type A-hemagglutinin complex with 125 mcg albumin and 2.4 mg lactose. In certain embodiments, substantially all of the non-toxin protein (e.g., greater than 95%, 96%, 97%, 98% or 99% of the hemagglutinin protein and non-toxin non-hemagglutinin protein) that would normally be found in botulinum toxin complexes derived from *Clostridium botulinum* bacteria is removed from the botulinum toxin complex. Furthermore, although the amount endogenous non-toxin proteins may be reduced by the same amount in some cases, this invention also contemplates

reducing each of the endogenous non-toxin proteins by different amounts, as well as reducing at least one of the endogenous non-toxin proteins, but not the others. A form of botulinum toxin wherein substantially all of the non-toxin proteins have been removed is referred to as a 150 kD botulinum toxin.

[0028] A preferred composition suitable with the methods described herein is a botulinum toxin-containing composition that is stabilized a positively charged carrier (e.g., peptide). A positively charged carrier molecule having protein transduction domains or efficiency groups, as described herein, has been found suitable as a transport system for a botulinum toxin, enabling toxin to be injected with improved penetration to target structures such as muscles. The transport occurs without covalent modification of the botulinum toxin. Besides enhancing penetration of botulinum toxin, the positively charged carriers of the invention may stabilize the botulinum toxin against degradation. In such embodiments, the hemagglutinin protein and non-toxin, non-hemagglutinin protein that are normally present to stabilize the botulinum toxin may be reduced or omitted entirely. Similarly, albumin that is normally added during manufacturing to toxin formulations can be omitted.

[0029] By the use of the terms “positively charged” or “cationic” in connection with the term “carrier”, it is meant that the carrier has a positive charge under at least some solution-phase conditions, more preferably, under at least some physiologically compatible conditions. More specifically, “positively charged” and “cationic” as used herein, means that the group in question contains functionalities that are charged under all pH conditions, for instance, a quaternary amine, or contains a functionality which can acquire positive charge under certain solution-phase conditions, such as pH changes in the case of primary amines. More preferably, “positively charged” or “cationic” as used herein refers to those groups that have the behavior of associating with anions over physiologically compatible conditions. Polymers with a multiplicity of positively-charged moieties need not be homopolymers, as will be apparent to one skilled in the art. Other examples of positively charged moieties are well known in the prior art and can be employed readily, as will be apparent to those skilled in the art.

[0030] Generally, the positively-charged carrier (also referred to as a “positively charged backbone”) is typically a chain of atoms, either with groups in the chain carrying a positive charge at physiological pH, or with groups carrying a positive charge attached to side chains extending

from the backbone. In certain preferred embodiments, the positively charged backbone is a cationic peptide. As used herein, the term “peptide” refers to an amino acid sequence, but carries no connotation with respect to the number of amino acid residues within the amino acid sequence. Accordingly, the term “peptide” may also encompass polypeptides and proteins. In certain preferred embodiments, the positively charged backbone itself will not have a defined enzymatic or therapeutic biologic activity. In certain embodiments, the backbone is a linear hydrocarbon backbone which is, in some embodiments, interrupted by heteroatoms selected from nitrogen, oxygen, sulfur, silicon and phosphorus. The majority of backbone chain atoms are usually carbon. Additionally, the backbone will often be a polymer of repeating units (e.g., amino acids, poly(ethyleneoxy), poly(propyleneamine), polyalkyleneimine, and the like) but can be a heteropolymer. In one group of embodiments, the positively charged backbone is a polypropyleneamine wherein a number of the amine nitrogen atoms are present as ammonium groups (tetra-substituted) carrying a positive charge. In another embodiment, the positively charged backbone is a nonpeptidyl polymer, which may be a hetero- or homo-polymer such as a polyalkyleneimine, for example a polyethyleneimine or polypropyleneimine, having a molecular weight of from about 10,000 to about 2,500,000, preferably from about 100,000 to about 1,800,000, and most preferably from about 500,000 to about 1,400,000. In another group of embodiments, the backbone has attached a plurality of side-chain moieties that include positively charged groups (e.g., ammonium groups, pyridinium groups, phosphonium groups, sulfonium groups, guanidinium groups, or amidinium groups). The sidechain moieties in this group of embodiments can be placed at spacings along the backbone that are consistent in separations or variable. Additionally, the length of the sidechains can be similar or dissimilar. For example, in one group of embodiments, the sidechains can be linear or branched hydrocarbon chains having from one to twenty carbon atoms and terminating at the distal end (away from the backbone) in one of the above-noted positively charged groups. The association between the positively charged carrier and the botulinum toxin is by non-covalent interaction, non-limiting examples of which include ionic interactions, hydrogen bonding, van der Waals forces, or combinations thereof. Examples of positively charged backbones and efficiency groups for use of the invention are described in U.S. Patent Nos. 8,623,811 and 9,211,248, which are hereby incorporated by reference in their entirety.

[0031] In one group of embodiments, the positively charged backbone is a polypeptide having multiple positively charged sidechain groups (e.g., lysine, arginine, ornithine, homoarginine, and the like). Preferably, the polypeptide has a molecular weight from about 100 to about 1,500,000, more preferably from about 500 to about 1,200,000, most preferably from about 1000 to about 1,000,000. One of skill in the art will appreciate that when amino acids are used in this portion of the invention, the sidechains can have either the D- or L-form (R or S configuration) at the center of attachment. In certain preferred embodiments, the polypeptide has a molecular weight from about 500 to about 5000, more preferably from 1000 to about 4000, more preferably from 2000 to about 3000. In other preferred embodiments, the polypeptide comprises 10 to 20 amino acids, or 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acids, preferably polylysine.

[0032] Alternatively, the backbone may comprise amino acid analogs and/or synthetic amino acids. The backbone may also be an analog of a polypeptide such as a peptoid. See, for example, Kessler, *Angew. Chem. Int. Ed. Engl.* 32:543 (1993); Zuckermann et al. *Chemtracts-Macromol. Chem.* 4:80 (1992); and Simon et al. *Proc. Nat'l. Acad. Sci. USA* 89:9367 (1992). Briefly, a peptoid is a polyglycine in which the sidechain is attached to the backbone nitrogen atoms rather than the α -carbon atoms. As above, a portion of the sidechains will typically terminate in a positively charged group to provide a positively charged backbone component. Synthesis of peptoids is described in, for example, U.S. Patent No. 5,877,278, which is hereby incorporated by reference in its entirety. As the term is used herein, positively charged backbones that have a peptoid backbone construction are considered "non-peptide" as they are not composed of amino acids having naturally occurring sidechains at the alpha-carbon locations.

[0033] A variety of other backbones can be used employing, for example, steric or electronic mimics of polypeptides wherein the amide linkages of the peptide are replaced with surrogates such as ester linkages, thioamides (--CSNH--), reversed thioamide (--NHCS--), aminomethylene (--NHCH₂--), or the reversed methyleneamino (--CH₂NH--) groups, keto-methylene (--COCH₂--), groups, phosphinate (--PO₂RCH₂--), phosphoramidate and phosphoramidate ester (--PO₂RNH--), reverse peptide (--NHCO--), trans-alkene (--CR=CH--), fluoroalkene (--CF=CH--), dimethylene (--CH₂CH₂--), thioether (--CH₂S--), hydroxyethylene (--CH(OH)CH₂--), methyleneoxy (--CH₂O--), tetrazole (CN₄), sulfonamido (--SO₂NH--),

methylenesulfonamido (--CHRSO₂NH--), reversed sulfonamide (--NHSO₂--), and backbones with malonate and/or gem-diamino-alkyl subunits, for example, as reviewed by Fletcher et al. ((1998) Chem. Rev. 98:763) and detailed by references cited therein. Many of the foregoing substitutions result in approximately isosteric polymer backbones relative to backbones formed from α -amino acids.

[0034] In each of the backbones provided above, sidechain groups can be appended that carry a positively charged group. For example, the sulfonamide-linked backbones (--SO₂NH-- and --NHSO₂--) can have sidechain groups attached to the nitrogen atoms. Similarly, the hydroxyethylene (--CH(OH)CH₂--) linkage can bear a sidechain group attached to the hydroxy substituent. One of skill in the art can readily adapt the other linkage chemistries to provide positively charged sidechain groups using standard synthetic methods.

[0035] In one embodiment, the positively charged backbone is a polypeptide having protein transduction domains (also referred to as efficiency groups). As used herein, an efficiency group or protein transduction domain is any agent that has the effect of promoting the translocation of the positively charged backbone through a tissue or cell membrane. Non-limiting examples of protein transduction domains or efficiency groups include -(gly)_{n1}-(arg)_{n2} (SEQ ID NO: 5), HIV-TAT or fragments thereof, or the protein transduction domain (PTD) of Antennapedia, or a fragment thereof, in which the subscript n1 is an integer of from 0 to 20, more preferably 0 to 8, still more preferably 2 to 5, and the subscript n2 is independently an odd integer of from about 5 to about 25, more preferably about 7 to about 17, most preferably about 7 to about 13. In some embodiments, the HIV-TAT fragment does not contain the cysteine-rich region of the HIV-TAT molecule, in order to minimize the problems associated with disulfide aggregation. Preferably, the fragments of the HIV-TAT and Antennapedia protein transduction domains retain the protein transduction activity of the full protein. Still further preferred are those embodiments in which the HIV-TAT fragment has the amino acid sequence (gly)_p-RGRDDRRQRRR-(gly)_q (SEQ ID NO: 1), (gly)_p-YGRKKRRQRRR-(gly)_q (SEQ ID NO: 2) or (gly)_p-RKKRRQRRR-(gly)_q (SEQ ID NO: 3) wherein the subscripts p and q are each independently an integer of from 0 to 20, or wherein p and q are each independently the integer 1. In another embodiment, the fragment or efficiency group is attached to the backbone via either the C-terminus or the N-terminus of the fragment or amino acid sequence of the efficiency group. In certain preferred embodiments, p is one and q is zero or

p is zero and q is one. Preferred HIV-TAT fragments are those in which the subscripts p and q are each independently integers of from 0 to 8, more preferably 0 to 5. In another preferred embodiment the positively charged side chain or branching group is the Antennapedia (Antp) protein transduction domain (PTD), or a fragment thereof that retains activity. These are known in the art, for instance, from Console et al., *J. Biol. Chem.* 278:35109 (2003) and a non-limiting example of an Antennapedia PTD contemplated by this invention is the PTD having the amino acid sequence SGRQIKIWFQNRRMKWKKC (SEQ ID NO: 6). In other embodiments, the positively charged carrier is a positively charged peptide having the amino acid sequence RKKRRQRRR-G-(K)₁₅-G-RKKRRQRRR (SEQ ID NO: 4); or a positively charged peptide having the amino acid sequence YGRKKRRQRRR-G-(K)₁₅-G-YGRKKRRQRRR (SEQ ID NO: 7); or a positively charged peptide having the amino acid sequences RGRDDRRQRRR-G-(K)₁₅-G-RGRDDRRQRRR (SEQ ID NO: 8) for use in the compositions and methods of the invention.

[0036] Preferably the positively charged carrier includes side-chain positively charged protein transduction domains or positively charged efficiency groups in an amount of at least about 0.01%, as a percentage of the total carrier weight, preferably from about 0.01 to about 50 weight percent, more preferably from about 0.05 to about 45 weight percent, and most preferably from about 0.1 to about 30 weight %. For positively charged protein transduction domains having the formula -(gly)_{n1}-(arg)_{n2} (SEQ ID NO: 5), a preferred range is from about 0.1 to about 25%.

[0037] In another embodiment, the backbone portion is a polylysine and positively charged protein transduction domains are attached to the lysine sidechain amino groups or to the C- or N-termini. In some preferred embodiments, the polylysine may have a molecular weight that is at least 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, 5000, 5500, or 6000 D, and less than about 2,000,000, 1,000,000, 500,000, 250,000, 100,000, 75,000, 50,000, and 25,000 D. Within the range of 100 to 2,000,000 D, it is contemplated that the lower and/or upper range may be increased or decreased, respectively, by 100, with each resulting sub-range being a specifically contemplated embodiment of the invention. In some exemplary embodiments, the polylysine has a molecular weight from about 1,000 to about 1,500,000 D, from about 2,000 to about 800,000 D, or from about 3,000 to about 200,000 D. In other exemplary embodiments, the polylysine has molecular weight from about 100 to about 10,000 D, from about 500 to about 5,000 D, from about 1,000 to about 4,000 D, from about 1,500 to about 3,500 D or from about 2,000 to about 3,000 D. Preferred is a polylysine polypeptide having 10 to 20 lysines

(SEQ ID NO: 9), more preferably, 15 lysines. In some embodiments, the polylysine contemplated by this invention can be any of the commercially available (Sigma Chemical Company, St. Louis, Mo., USA) polylysines such as, for example, polylysine having MW>70,000, polylysine having MW of 70,000 to 150,000, polylysine having MW 150,000 to 300,000 and polylysine having MW>300,000. The selection of an appropriate polylysine will depend on the remaining components of the composition and will be sufficient to provide an overall net positive charge to the composition and provide a length that is preferably from one to four times the combined length of the negatively charged components. Preferred positively charged protein transduction domains or efficiency groups include, for example, -gly-gly-gly-arg-arg-arg-arg-arg-arg-arg (-Gly₃Arg₇ (SEQ ID NO: 10)) or HIV-TAT.

[0038] In another preferred embodiment the positively charged backbone is a polyalkyleneimine, non-limiting examples of which include polyethyleneimine, polypropyleneimine, and polybutyleneimine. In certain embodiments, the polyalkyleneimine has a molecular weight of at least 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, 5000, 5500, or 6000 D, and less than about 2,000,000, 1,000,000, 500,000, 250,000, 100,000, 75,000, 50,000, and 25,000 D. Within the range of 100 to 2,000,000 D, it is contemplated that the lower and/or upper range may be increased or decreased, respectively, by 100, with each resulting sub-range being a specifically contemplated embodiment of the invention.

[0039] In other embodiments of this invention, the carrier is a relatively short polylysine or polyethyleneimine (PEI) backbone (which may be linear or branched) and which has positively charged branching groups. Without wishing to be constrained by theory, it is believed that such carriers are useful for minimizing uncontrolled aggregation of the backbones and botulinum toxin in a therapeutic composition, which causes the transport efficiency to decrease dramatically. When the carrier is a relatively short linear polylysine or PEI backbone, the backbone will have a molecular weight of less than 75,000 D, more preferably less than 30,000 D, and most preferably, less than 25,000 D. When the carrier is a relatively short branched polylysine or PEI backbone, however, the backbone will have a molecular weight less than 60,000 D, more preferably less than 55,000 D, and most preferably less than 50,000 D.

[0040] In one particularly interesting embodiment, the non-native molecules are cationic peptides that have no inherent botulinum-toxin-like activity and that also contain one or more protein transduction domains as described herein. Without wishing to be bound by any particular scientific theory, it is believed that certain peptides can enhance tissue penetration of molecules associated in complex after injection, while enhancing stabilization of the botulinum toxin in skin and *in vitro*. It is believed that the enhanced tissue penetration afforded by certain peptides in particular affords reduced antigenicity, a better safety profile, enhanced potency, faster onset of clinical efficacy or longer duration of clinical efficacy compared to conventional commercial botulinum toxin complexes that are bound to exogenous albumin (e.g., BOTOX® or MYOBLOC®).

[0041] In preferred embodiments, the concentration of positively charged carriers in the compositions according to the invention is sufficient to enhance the delivery of the botulinum toxin to molecular targets such as, for example, motor nerve plates. Furthermore, without wishing to be bound by theory, it is believed that the penetration rate follows receptor-mediated kinetics, such that tissue penetration increases with increasing amounts of penetration-enhancing-molecules up to a saturation point, upon which the transport rate becomes constant. Thus, in a preferred embodiment, the amount of added penetration-enhancing-molecules is equal to the amount that maximizes penetration rate right before saturation. A useful concentration range for the positively charged carrier (or carrier peptide) in the injectable compositions of this invention is about 0.1 pg of carrier per Unit (U) of botulinum toxin (0.1 pg/U) to about 1.0 mg per Unit (mg/U) of the botulinum toxin as described herein. A useful concentration range for the positively charged carrier (or carrier peptide) in the topical compositions of the invention is about 1.0 pg/U to 0.5 mg/U of botulinum toxin (amount of carrier/U of botulinum toxin). In other embodiments, the positively charged carrier (or carrier peptide) is present in the injectable compositions of the invention in the range of, for example, 1 ng/U to 300 ng/U or 10 ng/U to 200 ng/U of botulinum toxin, or in the range of 1 ng/U to 1000 ng/U of botulinum toxin; or in the range of 0.1 ng/U to 10,000 ng/U of botulinum toxin. In some embodiments, the amount of positively charged carrier (or carrier peptide) to Units of botulinum toxin present in the compositions of the invention is, by way of nonlimiting example, 0.5, 1, 2, 5, 10, 20, 30, 40, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190,

200 ng of carrier per Unit of botulinum toxin (ng/U), or any amount therebetween. Preferably, the botulinum toxin is of serotype A, and particularly, the 150 kD form of serotype A botulinum toxin and the carrier peptide is that shown at SEQ ID NO: 4.

[0042] In general, methods and procedures for measuring the activity of botulinum toxin, i.e., units (U) of botulinum toxin activity, are known to and practiced by those having skill in the art. Briefly, median lethality assays (LD₅₀ assays) in mice are conventionally used to estimate the number of units of botulinum toxin with a high degree of precision. Doses of all commercially available botulinum toxins are expressed in terms of units of biologic activity. By way of example, one unit of botulinum toxin corresponds to the calculated median intraperitoneal lethal dose (LD₅₀) in female Swiss-Webster mice. See, Hoffman, R.O. et al., 1986, *Int. Ophthalmol. Clin.*, 26:241-50, as well as DePass, L.R., 1989, *Toxicol. Letters*, 49:159-170; and Pearce, L.B. et al., 1994, *Toxicol. Appl. Pharmacol.*, 128:69-77, which also describe lethality assays in the art. More particularly, a suitable method for determining botulinum toxin units for a botulinum toxin component of the compositions of the invention is as follows: Forty-eight (48) female CD-1 mice weighing 17-23 grams are randomly assigned to six doses of the test article (1.54, 1.31, 1.11, 0.95, 0.80, and 0.68 U/0.5 mL), eight (8) animals per dose group. The test article refers to the botulinum toxin preparation or sample being assayed or tested. The animals are housed eight per cage and are weighed within 24 hours of dosing with the test article. On the day of dosing, the test article is diluted to the appropriate concentrations in isotonic saline (0.9% NaCl). Each animal is administered 0.5 mL of diluted test article via intraperitoneal injection. After injection, mice are returned to the cage and fatalities are recorded daily for three days. Lethality is scored 72 hours post injection and the results are analyzed by probit or logistic analysis to derive the LD₅₀ value relative to a reference standard that is assessed using the same dosing regimen. By way of example, the reference standard is a specifically qualified and calibrated lot of the same composition of the invention that is used for comparison to derive relative potency of the test article. The determined LD₅₀ value is then corrected for the cumulative dilutions performed to assign a relative potency value for the neat (undiluted) test article.

[0043] Compositions of this invention are preferably in a form that permits injection into the skin or epithelium of subjects or patients. The term “in need” is meant to include both pharmaceutical or health-related needs (e.g., treating conditions involving undesirable dystonic contractions or muscle spasms). In preferred embodiments, the compositions are prepared by

mixing the botulinum toxin (either containing the associated non-toxin proteins or reduced associated non-toxin proteins) with the positively charged carrier, and usually with one or more additional pharmaceutically acceptable carriers or excipients. In their simplest form, they may contain an aqueous pharmaceutically acceptable diluent, such as buffered saline (e.g., phosphate buffered saline). However, the compositions may contain other ingredients typically found in injectable pharmaceutical or cosmeceutical compositions, including a dermatologically or pharmaceutically acceptable carrier, vehicle or medium that is compatible with the tissues to which it will be applied. The term “pharmaceutically acceptable,” as used herein, means that the compositions or components thereof so described are suitable for use in contact with these tissues or for use in patients in general without undue toxicity, incompatibility, instability, allergic response, and the like. As appropriate, compositions of the invention may comprise any ingredient conventionally used in the fields under consideration.

[0044] In terms of their form, compositions of this invention may include solutions, emulsions (including microemulsions), suspensions, gels, powders, or other typical solid or liquid compositions used for injection to muscle and other tissues where the compositions may be used. In preferred embodiments, the compositions of the invention are present in low-viscosity, sterile formulations suitable for injection with a syringe. As used herein, the terms compositions and formulations are essentially interchangeable when referring to the compositions and formulations according to the present invention. The compositions of the invention may be in the form of a lyophilized powder that is reconstituted using a pharmaceutically acceptable liquid diluent prior to injection. In certain embodiments, the lyophilized powder is reconstituted with a liquid diluent to form an injectable formulation with a viscosity of about 0.1 to about 2000 cP, more preferably about 0.2 to about 500 cP, even more preferably about 0.3 to about 50 cP, and even more preferably about 0.4 to about 2.0 cP. The compositions of the invention may contain, in addition to the botulinum toxin and positively charged carrier, other ingredients typically used in such products, such as antimicrobials, hydration agents, tissue bulking agents or tissue fillers, preservatives, emulsifiers, natural or synthetic oils, solvents, surfactants, detergents, gelling agents, antioxidants, fillers, thickeners, powders, viscosity-controlling agents and water, and optionally including anesthetics, anti-itch actives, botanical extracts, conditioning agents, minerals, polyphenols, silicones or derivatives thereof, vitamins, and phytomedicinals.

[0045] The injectable compositions according to this invention may be in the form of controlled-release or sustained-release compositions which comprise botulinum toxin and positively charged carrier encapsulated or otherwise contained within a material such that they are released within the tissue in a controlled manner over time. The composition comprising the botulinum toxin and positively charged carrier may be contained within matrixes, liposomes, vesicles, microcapsules, microspheres and the like, or within a solid particulate material, all of which is selected and/or constructed to provide release of the botulinum toxin over time. The botulinum toxin and the positively charged carrier may be encapsulated together (i.e., in the same capsule) or separately (i.e., in separate capsules).

[0046] In embodiments, compositions of the invention comprise compositions (or formulations) comprising a botulinum toxin as described herein, a positively charged carrier (or peptide) as described herein, a non-reducing disaccharide or a non-reducing trisaccharide, a non-ionic surfactant, and a physiologically compatible buffer, which is capable of maintaining a suitable pH, such as a pH in the range of pH 4.5 to pH 7.5, or pH 4.5 to pH 6.8, or pH 4.5 to pH 6.5. It is to be understood that a suitable pH also includes the upper and lower pH values in the range, e.g., a pH of 6.5 or a pH of 7.5. The concentration of the non-reducing sugar in the reconstituted composition is in the range of 10% through 40% (w/v) and the concentration of the non-ionic surfactant is in the range of 0.005% through 0.5% (w/v). The compositions can in powder form, preferably by lyophilization, to produce stabilized solid compositions, which may thereafter be reconstituted for use, for example, using sterile saline or other known physiologically and pharmaceutically acceptable diluents, excipients, or vehicles, especially those known for use in injectable formulations. Preferably, the dried, e.g., lyophilized, solid compositions are noncrystalline and amorphous solid compositions, and may be in the form of powders, for example. Also, preferably, the compositions of the invention do not include animal protein-derived products, such as albumin. Compositions that are suitable for the invention are also described in U.S. Patent No. 9,340,587 B2, the entire contents of which are incorporated herein by reference. In particular embodiments the compositions comprise botulinum toxin of serotype A. In other particular embodiments, the compositions comprise botulinum toxin of serotype A which has a molecular weight of 150 kDa.

[0047] In certain embodiments, the compositions of the invention contain a non-reducing sugar, which is preferably a disaccharide, non-limiting examples of which include trehalose,

including its anhydrous and hydrated forms, or sucrose, as well as combinations thereof. In some embodiments, the hydrated form of trehalose, trehalose-dihydrate, is preferable. In other embodiments, the compositions contain a trisaccharide, a non-limiting example of which is raffinose. In general, the concentration of the non-reducing sugar, preferably a disaccharide, e.g., sucrose, in the compositions of the invention are in the range of 10% to 40% (w/v), preferably 10% to 25% (w/v), more preferably 15% to 20% (w/v). In some preferred embodiments, the concentration of the non-reducing sugar, preferably a disaccharide, e.g., sucrose, is 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19% or 20% (w/v).

[0048] In general, the compositions of the invention may include any non-ionic surfactant that has the ability to stabilize botulinum toxin and that is suitable for pharmaceutical use. In some embodiments, the non-ionic surfactant is a polysorbate, such as, by way of nonlimiting example, polysorbate 20, polysorbate 40, polysorbate 60, and polysorbate 80. In other embodiments, the non-ionic surfactant is a sorbitan ester, non-limiting examples of which include SPAN[®] 20, SPAN[®] 60, SPAN[®] 65, and SPAN[®] 80. The non-ionic surfactants Triton[®] X-100 or NP-40 may also be used. In addition, a combination of the different non-ionic surfactants may be used. In certain preferred embodiments, the non-ionic surfactant is a polysorbate, a poloxamer and/or a sorbitan; polysorbates and sorbitans are particularly preferred. In embodiments, the non-ionic surfactant is present in the compositions of the invention in the range of 0.005% to 0.5%, or in the range of 0.01% to 0.2%, or in the range of 0.02% to 0.1% or in the range of 0.05 to 0.08%, inclusive of the upper and lower values. In addition, the compositions of the invention may contain a non-ionic surfactant in the amount of 0.01%, 0.02%, 0.03%, 0.04%, 0.05%, 0.06%, 0.07%, 0.08%, 0.09%, 0.10%, 0.11%, 0.12%, 0.13%, 0.14%, or 0.15%.

[0049] In general for the compositions of the invention, any physiologically compatible buffer capable of maintaining the pH in the above ranges is suitable for use. Non-limiting examples of such buffers include salts of citric acid, acetic acid, succinic acid, tartaric acid, maleic acid, and histidine. Non-limiting examples of suitable buffer concentrations include buffer concentrations in the range of 0.400% to 0.600%; 0.450% to 0.575%, or 0.500% to 0.565%. The compositions of the invention may also comprise a mixture of buffer salts, non-limiting examples of which include citrate/acetate, citrate/histidine, citrate/tartrate, histidine monohydrochloride, maleate/histidine, or succinate/histidine. Accordingly, a composition of the invention which provides a long duration effect after treatment by a single injection includes a botulinum toxin,

such as botulinum toxin A or botulinum toxin A of 150 kDa MW, as described herein, a positively charged carrier (or peptide) as described herein, a non-reducing disaccharide, such as sucrose, a non-ionic surfactant, such as polysorbate 20, polysorbate 40, polysorbate 60, polysorbate 80, or a sorbitan ester, and a physiologically compatible buffer, such as citric acid, acetic acid, succinic acid, tartaric acid, maleic acid, and histidine, which is capable of maintaining a suitable pH, such as a pH in the range of pH 4.5 to pH 6.5 or in the range of pH 4.5. to pH 7.5, in w/v amounts as described herein.

[0050] In an embodiment, a suitable composition to be used with described regimens comprises a botulinum toxin, e.g., a 150 kD toxin, a non-reducing disaccharide or a non-reducing trisaccharide, a non-ionic surfactant, a positively charged peptide carrier, and a physiologically compatible buffer for maintaining the pH between 4.5. and 7.5. The concentration of the non-reducing sugar in the composition is in the range of 10% through 40% (w/v) and the concentration of the non-ionic surfactant is in the range of 0.005% through 0.5% (w/v). The preferred composition comprises botulinum toxin, preferably botulinum toxin A, more preferably, of 150 kDa MW, a positively charged carrier (e.g., peptide) as described herein, a non-reducing disaccharide, such as sucrose, a non-ionic surfactant, such as polysorbate 20, polysorbate 40, polysorbate 60, polysorbate 80, or a sorbitan ester, and a physiologically compatible buffer, such as citric acid, acetic acid, succinic acid, tartaric acid, maleic acid, and histidine; and has a pH in the range of pH 4.5. to pH 7.5.

[0051] A particular composition of the invention is an albumin-free composition which comprises a botulinum toxin, preferably botulinum toxin of serotype A, or a botulinum toxin A having a molecular weight of 150 kDa; a positively charged carrier (e.g., peptide such as that of SEQ ID NO: 4) at a ratio with toxin as described above; a non-reducing disaccharide or a non-reducing trisaccharide, preferably a disaccharide, present in a range of 10% through 40% (w/v); a non-ionic surfactant, preferably, a polysorbate or sorbitan ester, present in the range of 0.005% through 0.5% (w/v); and a physiologically compatible buffer, such as citric acid, acetic acid, succinic acid, tartaric acid, maleic acid, or histidine, present in the range of 0.400% to 0.600%; 0.450% to 0.575%, or 0.500% to 0.565%, for maintaining the pH between 4.5. and 7.5. A preferred composition is DAXXIFY® manufactured by Revance.

Administration for a Treatment Session

[0052] Botulinum toxin formulations according to the invention can be delivered by injection (typically using a syringe) to muscles underlying the skin, or to glandular structures within the skin, in an effective amount to produce paralysis, produce relaxation, alleviate contractions, prevent or alleviate spasms, reduce glandular output, or other desired effects. Local delivery of the botulinum toxin in this manner could afford dosage reductions, reduce toxicity and allow more precise dosage optimization for desired effects relative to injectable or implantable materials.

[0053] The compositions of the invention are administered to deliver an effective amount, preferably a therapeutically effective amount, of the botulinum toxin. The term “effective amount” or “therapeutically effective amount” as used herein means an amount of a botulinum toxin as defined above that is sufficient to produce the desired muscular paralysis or other biological effect, but that implicitly is a safe amount, i.e., one that is low enough to avoid serious side effects.

[0054] The compositions of the invention may contain an appropriate effective amount of the botulinum toxin for application as a single-dose treatment, or may be more concentrated, either for dilution at the place of administration or for use in multiple applications and/or sequential applications over periods of time. Through the use of the positively charged carrier this invention, a botulinum toxin can be administered by injection to a subject for treating conditions such as cervical dystonia. The botulinum toxin is administered by injection to muscles or to other skin-associated or other target tissue structures.

[0055] Most preferably, the compositions are administered by or under the direction of a physician or other health care professional. They may be administered in a single treatment or in a series of treatments over time. In preferred embodiments, a composition according to the invention is injected at a location or locations where an effect associated with botulinum toxin is desired. In the treatment of cervical dystonia, the following Table 1 provides guidance as to the appropriate dosage of RT002 for injection where a dose is 125 U or 250 U, respectively:

Table 1: –Injectable Muscles and Pre-defined Injection Volume by Muscle	RT002 for Injection 125 U Group	RT002 for Injection 250U Group
Total volume of reconstituted study drug is 2.5mL		
Dilution	5 U/ 0.1 mL	10 U/ 0.1 mL

Injectable Muscles	Mandatory Injection Volume by Muscle (mL)	Dose Range (Units)	Dose Range (Units)
Sternocleidomastoid	0.2-0.5	10-25	20-50
Levator scapulae	0.2-0.6	10-30	20-60
Scalenus complex	0.2-0.3	10-15	20-30
Splenius capitis	0.2-1.0	10-50	20-100
Splenius cervicis	0.2-1.0	10-50	20-100
Trapezius	0.3-0.8	15-40	30-80
Longissimus	0.2-0.6	10-30	20-60

A total dose of 300 U, 350 U, 400 U, 450 U, or 500 U can be pro-rata adjusted from the above.

[0056] One or more muscles in the head, neck, and shoulder area are injected with the botulinum toxin composition. In some embodiments, the one or more muscular targets are selected from a group consisting of the sternocleidomastoid, levator scapulae, scalenus complex, splenius capitis, splenius cervicis, trapezius, and longissimus, the left and right side muscle or muscle complex each being an optional target. (see **FIG. 11**). In some embodiments, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or all muscular targets are selected from a group consisting of sternocleidomastoid, levator scapulae, scalenus complex, splenius capitis, splenius cervicis, trapezius, and longissimus, the left and right side muscle or muscle complex each being an optional target. In some embodiments, the splenius capitis, splenius cervicis, trapezius, and longissimus may receive unilateral or bilateral injections (i.e., injections in one or both sides of the body). In some embodiments, only unilateral injections are administered in the sternocleidomastoid, levator scapulae, and/or scalenus complex. In some embodiments, the sternocleidomastoid, levator scapulae, scalenus complex, splenius capitis, splenius cervicis, trapezius, and longissimus each receive at least one injection. In some embodiments, a muscular target can receive one or more injections, such as 1, 2, 3, 4, 5, etc. In some embodiments, the dosage range administered to each muscular target, by one or more injections, is provided in Table 1.

[0057] Because of its nature, the botulinum toxin preferably is administered at an amount, application rate, and frequency that will produce the desired result without producing any adverse or undesired results. In embodiments, a single treatment with an effective dose of the compositions of the invention affords an effect of long duration such that during a course of treatment for an indication treatable by botulinum toxin, or series of injections during a single multiple treatment session, with a concomitant effect that endures over extended periods of time, e.g., at least 12, 14,

16, 18, 20, 24, 26, 28, 32, 36, and 38 weeks; or 3 months, 4 months, 5 months, 6 months or greater than 6 months, namely, 6 months, 7 months, 8 months, 9 months, or longer, including 10 months. The longer duration of action provides for longer intervals or time periods between treatments where multiple treatments are used to maintain a treatment goal or effect. A therapeutically effective amount of toxin in a composition as described herein can be about 100 U to 500 U; or more specifically, from about 100U, 125U, 150U, 175U, 200U, 225U, 250U, 175U, 300U, 325U, 350U, 375U, 400U, 425U, 450U, or any amount therebetween of botulinum toxin. In embodiments, the composition is DAXXIFY.

[0058] This invention also contemplates the use of a variety of delivery devices for injecting botulinum toxin-containing compositions described herein across skin. Such devices may include, without limitation, a needle and syringe, or may involve more sophisticated devices capable of dispensing and monitoring the dispensing of the composition, and optionally monitoring the condition of the subject in one or more aspects (e.g., monitoring the reaction of the subject to the substances being dispensed).

[0059] In some embodiments, the compositions can be pre-formulated and/or pre-installed in a delivery device as such. This invention also contemplates embodiments wherein the compositions are provided in a kit that stores one or more components separately from the remaining components. For example, in certain embodiments, the invention provides for a kit that separately stores botulinum toxin and the positively charged carrier for combining at or prior to the time of application. The amount of positively charged carrier or the concentration ratio of these molecules to the botulinum toxin will depend on which carrier is chosen for use in the composition in question. The appropriate amount or ratio of carrier molecule in a given case can readily be determined, for example, by conducting one or more experiments such as those described below.

[0060] In some embodiments, the patient to be treated is 65 years of age, at least 65 years old, or over 65 years old. For example, the patient may be 65, 66, 68, 70, 75, 80 years, or older.

EXAMPLES

Example 1 – Formulation

[0061] An injectable botulinum toxin formulation (RT002) was prepared with ingredients according to Table 2 below. All inactive ingredients are listed in the US FDA inactive ingredients database, except for the novel excipient RTP004. RTP004 is a peptide with the following sequence: RKKRRQRRRG-(K)₁₅-GRKKRRQRRR (SEQ ID NO: 4). The botulinum toxin molecule is not covalently associated with R004. RT002 does not contain accessory proteins or animal-derived proteins such as albumin.

Table 2. Composition of RT002 for Injection

Ingredient	Quality Standard	Function	Quantity per vial (50 U/vial formulation)	Quantity per vial (100 U/vial formulation)
Botulinum toxin Type A, 150 kD	Internal	Active Ingredient	50 U (0.23 ng ^a)	100 U (0.50 ng ^d)
L-histidine	USP/Ph. Eur.	Buffer	0.14 mg	0.14 mg
L-histidine monohydrochloride	Ph. Eur.	Buffer	0.65 mg	0.65 mg
Trehalose dihydrate	NF/Ph. Eur.	Stabilizer	36 mg	36 mg
Polysorbate-20	NF/Ph. Eur.	Stabilizer	0.1 mg	0.1 mg
RPT004	Internal	Stabilizer	11.7 µg ^b	11.7 µg ^b
Water for Injection	USP	Processing Aid	N/A ^c	N/A ^c

^a, Theoretical amount of toxin required for 50U/vial drug product; ^b, Equivalent to 15µg RTP004-Acetate; ^c, Water added in manufacturing is removed during the lyophilization step, ^d, Theoretical amount of toxin required for 100U/vial drug product.

[0062] A dose escalation study using the RT002 formulation is described in the Example section of PCT Publication No. WO2018/213710 to Revance Therapeutics, Inc. This example and the entire specification of the '710 Publication is hereby incorporated by reference in its entirety.

Protocol for Examples 2 to 3

[0063] In an open-label study, subjects received DAXXIFY to treat their moderate-to-severe isolated cervical dystonia. Subjects with moderate-to-severe cervical dystonia were enrolled at multiple sites across the United States.

[0064] The recommended dose of DAXXIFY for the treatment of cervical dystonia ranged from 125 U to 250 U given intramuscularly as a divided dose among affected muscles. Subjects received an initial dose of either 125 U or 250 U of DAXXIFY. The assigned dose—either 125 U

or 250 U—depended on whether the subject was previously treated with another botulinum toxin, their past dose, response to treatment, duration of effect, and adverse event history. For example, subjects were assigned 125 U if they previously received a BOTOX or XEOMIN dose that was less than 190 U, or they were assigned 250 U if they previously received a BOTOX or XEOMIN dose that was equal to or greater than 190 U. FIG. 1 shows the muscles that would have been treated under the protocol.

[0065] The primacy efficacy endpoint of the study was an improvement in dystonia symptoms as measured by change (reduction) from the subject's Toronto Western Spasmodic Torticollis Rating Scale (TWSTRS)-Total score. TWSTRS is a validated composite scale that covers different features of the cervical dystonia condition. It has three parts. The first part of the scale is based on the physical findings and severity of dystonia, the second part rates the patient's perceived level of disability, and the third part rates pain associated with the condition.

[0066] Subjects presented with their baseline TWSTRS score before receiving an initial dose. After receiving an initial dose, subjects were retreated with a second dose and so on prior to or as their symptoms reemerged, which was in any case when the subject's efficacy was 40% or more of the initial dose's peak efficacy. Every two weeks during the study, the TWSTRS score for each subject was recorded. Throughout the study, subjects received a total of four treatments.

[0067] The study showed positive efficacy results and the DAXXIFY® botulinum toxin composition was generally safe and well-tolerated.

Example 2

[0068] In one example, a subject was treated in accordance with the above protocol. The subject presented with a baseline TWSTRS score of about 50 before receiving their first treatment. In response to the initial treatment, the patient's TWSTRS score decreased to 17 after 6 weeks, and to 15 after 12 weeks. The patient received their second treatment at 20 weeks from initial treatment. At this time, the patient had experienced a 59% loss of effect from the initial treatment (i.e., 41% remaining efficacy). The patient received their third treatment (18 weeks after the second treatment) and fourth treatment (22 weeks after the third treatment) with 63.0% and 23% efficacy remaining from the prior respective treatment. Each time, the subject was treated with 125 U of

DAXXIFY. The TWSTRS scores during the course of these serial treatments are shown in FIG. 1.

[0069] As can be appreciated from FIG. 2, the peak efficacy trended “higher” (i.e., a lower TWSTRS score) with repeat treatment and timing of the treatment at a time when prior treatment effect begins diminishing but is still measurable at least 4-6 weeks or more after the subsequent treatment. In particular, in FIG. 1, by the third treatment, the patient exhibited an improvement in peak efficacy, evidenced by a TWSTRS score of 10. The overlapping efficacy can cause a lowering of the peak effect over time at least for 3-4 treatment cycles.

[0070] Another benefit is that a patient will experience less symptom reemergence. For example, in FIG. 2, one can see that the patient’s TWSTRS score stayed below 30 soon after the second treatment. In other words, timing the dose of the third and fourth treatment during a period of significant efficacy from the prior treatment prevented the symptoms to reemerge to a significant degree. Indeed, the TWSTRS score exhibited a 60% change from before the first treatment to before the fourth treatment. Thus, the patient’s experience is improved with a serial treatment protocol that administers a subsequent treatment with Daxxify® and its long duration of effect at an early phase of when the prior treatment begins to wane.

Example 3

[0071] In another example, a subject was treated in accordance with the above protocol. The TWSTRS scores during the course of these serial treatments are shown in FIG. 3. In this case, the treatment interval was such that the subject was dosed near the peak efficacy, namely 77% remaining efficacy. The subject was retreated at 28 and 48 weeks, and received a dose of 125 U of DAXXIFY each time. The subject presented with a baseline TWSTRS score of 43 before the first treatment. The subject’s peak efficacy decreased to a TWSTRS score of 13. Before receiving a second treatment, the patient presented with only a 26% loss of effect from the first treatment, and a TWSTRS score of about 19. Thus, the patient had very little symptom reemergence, and the serial treatment protocol, wherein the patient was retreated when the patient was within 25% of the peak effect of the initial treatment, allowed for the patient to stay within 25% of the peak effect throughout the entire treatment cycles.

Example 4

[0072] In another example, a subject was treated. The protocol was the same as above except that the retreatment was closer to the return to baseline. The TWSTRS scores during the course of these serial treatments are shown in FIG. 4. The subject was retreated every 16 weeks, and received a dose of 250 U DAXXIFY each time. As can be appreciated from FIG. 4, the symptomatic state of the patient was not stable but was very close to baseline before retreatment. A treatment interval for this patient in accordance with the present disclosure would have been dosed every 12 weeks.

CLAIMS

We claim:

1. A method of treating cervical dystonia in a subject, the method comprising administering a treatment regimen to said subject wherein the treatment regimen comprises:

administering a plurality of botulinum toxin treatments to treat cervical dystonia separated by an interval, wherein the interval between each of said botulinum treatments is at least 12 weeks; and wherein each treatment comprises

injecting a therapeutically effective amount of botulinum toxin into one or more muscles contributing to cervical dystonia symptoms of said subject; and

wherein a treatment administered subsequent to a prior treatment is administered when remaining efficacy of the prior treatment is greater than 40% of its peak efficacy.
2. The method of claim 1 wherein the subsequent treatment is administered when remaining efficacy of the prior treatment is greater than 50% of its peak efficacy or greater than 60% of its peak efficacy or greater than 70% of its peak efficacy or greater than 80% of its peak efficacy or greater than 90% of its peak efficacy or at peak efficacy or between 50% and 80% of its peak efficacy.
3. The method of any one of claims 1 to 2, wherein the therapeutically effective amount administered for the subsequent treatment is less than the therapeutically effective amount administered for the prior treatment and wherein a peak efficacy of the subsequent treatment is approximately equal to or is greater than the peak efficacy of the prior treatment.
4. The method of any one of claims 1 to 3, wherein the one or more muscles injected in the subsequent treatment comprises one or more different muscles than or a subgroup of the one or more muscles injected in the prior treatment.
5. The method of any one of claims 1 to 4, wherein the subsequent treatment has a peak efficacy and wherein the peak efficacy of the subsequent treatment is increased compared to the peak efficacy of the prior treatment.

6. The method of any one of claims 1 to 5, further comprising administering a treatment following the subsequent treatment to the subject to treat CD, wherein the treatment following the subsequent treatment is administered when remaining efficacy of the subsequent treatment is greater than 20% or 40% of its peak efficacy.
7. The method of claim 6, wherein the therapeutically effective amount of the subsequent treatment administered is less than the therapeutically effective amount of the prior treatment administered and wherein a peak efficacy of the subsequent treatment is approximately equal to or is greater than the peak efficacy of the prior treatment.
8. The method of claim 6 or 7, wherein the one or more muscles injected in the treatment following the subsequent treatment comprises one or more different muscles than or a subgroup of the one or more muscles injected in the subsequent treatment.
9. The method of any one of claims 6 to 8, wherein the subsequent treatment and the treatment following the subsequent treatment has a peak efficacy and wherein the peak efficacy of the treatment following the subsequent treatment is increased compared to the peak efficacy of the prior treatment and/or the subsequent treatment.
10. The method of any one of claims 1 and 6-9, wherein the subsequent treatment is administered when remaining efficacy of the prior treatment is greater than 80% of its peak efficacy and wherein a peak efficacy of the subsequent treatment is approximately equal to the peak efficacy of the prior treatment.
11. The method of claim 10, further comprising administering treatments following the subsequent treatment wherein the subject maintains a treatment effect within a narrow range of the peak treatment effect as compared to a baseline of the subject.
12. A method of treating cervical dystonia (CD) in a subject comprising:

administering a treatment to a subject that is immediately prior to a subsequent treatment, wherein the treatment comprises injecting a therapeutically effective amount of a pharmaceutical composition comprising botulinum toxin to a plurality of muscles to treat torticollis only and

administering the subsequent treatment to the subject, wherein the subsequent treatment comprises injecting a therapeutically effective amount of the botulinum toxin composition to a plurality of muscles to treat laterocollis, antecollis, or retrocollis and optionally torticollis.

13. The method of claim 12, wherein the subsequent treatment is to treat laterocollis only.
14. The method of claim 12, wherein the subsequent treatment is to treat laterocollis and torticollis.
15. The method of any one of claims 12 to 14, wherein the subsequent treatment is administered when remaining efficacy of the prior treatment is greater than 40% of its peak efficacy and wherein the subsequent treatment is administered more than 12 weeks after the administration of the prior treatment.
16. The method of any one of claims 12 to 14, wherein the subsequent treatment is administered when remaining efficacy of the prior treatment is between 40% and 100% or 70% to 100% of its peak efficacy and wherein the subsequent treatment is administered more than 12 weeks after the administration of the prior treatment.
17. The method of any one of claims 12 to 16, further comprising repeating the administering of the treatment and the administering of the subsequent treatment
18. The method of any one of claims 1 to 17, wherein the botulinum toxin composition comprises a type A botulinum toxin.
19. The method of any one of claims 1 to 17, wherein the botulinum toxin composition comprises a 150 kD, Type A botulinum toxin.
20. The method of any one of claims 1 to 19, wherein the composition further comprises a positively charged carrier comprising a positively charged backbone and at least one efficiency group, wherein the positively charged efficiency groups are selected from an amino acid sequence selected from the group consisting of (gly)_p-RGRDDRRQRRR-(gly)_q (SEQ ID NO: 2), (gly)_p-YGRKKRRQRRR-(gly)_q (SEQ ID NO: 3), and (gly)_p-RKKRRQRRR-(gly)_q (SEQ ID NO: 4), wherein the subscripts p and q are each independently an integer of from 0 to 20 and mixtures thereof.

21. The method of claim 20, wherein the positively charged efficiency groups include the amino acid sequence RKKRRQRRR-(gly)_q-(K)_x-(gly)_p-RKKRRQRRR, wherein the subscript x is an integer from 5 to 50 or 10-30 or 10 to 20, wherein p and q are each an integer from 0 to 8.
22. The method of claim 20, wherein the positively charged efficiency groups has the amino acid sequence RKKRRQRRRG-(K)₁₅-GRKKRRQRRR.
23. The method of any one of claims 1 to 22, wherein the composition further comprises a non-reducing disaccharide and a polysorbate or wherein the composition further comprises polysorbate 20, histidine, and sucrose or trehalose and is formulated to have a pH greater than 7 upon reconstitution.
24. The method of any one of claims 1 to 23, wherein the subsequent treatment is administered between 13 and 24 weeks after the administration of the prior treatment.
25. The method of any one of claims 1 to 24, wherein the number of weeks between the subsequent treatment and the prior treatment is different than the number of weeks between the subsequent treatment and the treatment following the subsequent treatment.
26. A method of treating cervical dystonia in a subject, the method comprising administering a treatment regimen to said subject wherein the treatment regimen comprises:
 - administering a plurality of botulinum toxin treatments to treat cervical dystonia separated by an interval, wherein the interval between each of said botulinum treatments is at least 12 weeks; and wherein each treatment comprises
 - injecting a therapeutically effective amount of botulinum toxin into one or more muscles contributing to cervical dystonia symptoms of said subject; and
 - wherein a treatment administered subsequent to a prior treatment is administered when remaining efficacy of the prior treatment is greater than 20% of its peak efficacy.
27. A method of treating cervical dystonia in a subject in need thereof, the method comprising:

administering a plurality of botulinum toxin treatments to a subject,
wherein at least 12 weeks separate each treatment and
wherein the dose of and interval of time between each treatment is sufficient to prevent
symptom levels from returning to the level of symptoms prior to initiating botulinum
treatments.

28. The method according to claim 27, wherein subsequent administrations of botulinum toxin result in an increased reduction in the level of symptoms.
29. The method of claim 26, 27, or 38 and any one of claims 3 to 25.
30. A method of treating cervical dystonia in a subject, the method comprising administering a treatment regimen to said subject wherein the treatment regimen comprises:
- administering a plurality of botulinum toxin treatments to treat cervical dystonia separated by an interval, wherein the interval between each of said botulinum treatments is at least 12 weeks; and wherein each treatment comprises
- injecting a therapeutically effective amount of botulinum toxin into one or more muscles contributing to cervical dystonia symptoms of said subject; and
- wherein a treatment administered immediately subsequent to a prior treatment is administered during peak efficacy of the prior treatment and 1 to 21 days or 3 to 18 days or 3 to 14 days or 3 to 7 days before efficacy of the prior treatment begins to wane.
31. The method of claim 30 and any one of claims 3 to 5 and 18 to 24.