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, which takes effect over a period of 4–7 days or longer after injection, the response to the treatment with botulinum toxin typically wears off after a 12 week period, often as early as at 10 weeks, requiring the person suffering from cervical dystonia to be injected again. Therefore, a durable, longer acting treatment requiring fewer neurological interventions would be desirable.

SUMMARY

[0013] In one of its examples, the disclosure relates to a method for producing a biologic effect in the treatment of cervical dystonia by injecting an effective amount, preferably a therapeutically effective amount, of the compositions of this disclosure to a subject or patient in need of such treatment.

[0014] In another of its examples, this disclosure provides a method of treating cervical dystonia in an individual in need thereof, the method comprising administering to the individual an injection of a composition comprising: a botulinum toxin, a botulinum toxin complex, or a reduced botulinum toxin complex and a positively charged carrier comprising a positively charged polylysine backbone having covalently attached thereto one or more positively charged efficiency groups having an amino acid sequence of (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; and a pharmaceutically acceptable diluent for injection; wherein the botulinum toxin is administered to the individual in an amount from about 100 U to about 450 U; wherein the positively charged carrier is non-covalently associated with the botulinum toxin, botulinum toxin complex, or reduced botulinum toxin complex component; and wherein injection of the composition provides a treatment having at least about a six month to about a 10 month duration of effect in reducing

the symptoms of cervical dystonia, thereby extending treatment interval duration for the individual.

[0015] In another of its examples, this disclosure provides injectable compositions comprising botulinum toxin non-covalently associated with a positively charged carrier molecule used to treat cervical dystonia. In preferred examples, the compositions of the disclosure possess one or more advantages over conventional commercial botulinum toxin formulations, such as BOTOX® or MYOBLOC®. For instance, in certain examples, the compositions may exhibit one or more advantages over conventional injectable botulinum formulations, including reduced antigenicity, a reduced tendency to undergo diffusion into surrounding tissue following injection, increased duration of clinical efficacy or enhanced potency relative to conventional botulinum toxin formulations, faster onset of clinical efficacy, and/or improved stability.

[0016] A further example of this disclosure is the recognition that certain non-native molecules (i.e., molecules not found in botulinum toxin complexes obtained from *Clostridium botulinum* bacteria) can be added to botulinum toxin, botulinum toxin complexes, and in particular reduced botulinum toxin complexes (as defined herein), to improve toxin diffusion through tissues in the treatment of cervical dystonia. The non-native molecules associate non-covalently with the toxin and act as penetration enhancers that improve the ability of the toxin to reach target structures after injection. Furthermore, the non-native molecules may increase the stability of the toxin prior to and after injection. By way of example, the penetration enhancers may be positively charged carriers, such as cationic peptides, which have no inherent botulinum-toxin-like activity and which also contain one or more protein transduction domains as described herein.

[0017] Another example of this disclosure is to provide a composition comprising botulinum toxin, a botulinum toxin complex (or a reduced protein botulinum toxin complex including just the 150 kD neurotoxin itself, or the neurotoxin with some, but not all, of the native complex proteins) and a positively charged carrier for use in a method of treatment for cervical dystonia.

[0017a] In one aspect, the present disclosure refers to a pharmaceutical composition in a sterile injectable formulation for use in treating cervical dystonia in an individual in need thereof, said composition comprising:

a botulinum toxin component in a dose of 100 U to 450 U, said botulinum toxin component selected from the group consisting of a botulinum toxin complex, a reduced botulinum toxin complex, or a botulinum toxin,

a positively charged carrier component comprising a positively charged polylysine backbone having covalently attached thereto one or more positively charged efficiency groups having an amino acid sequence of $(gly)_p$-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; and

a pharmaceutically acceptable diluent for injection;

wherein the positively charged carrier is non-covalently associated with the botulinum toxin component; and

wherein said dose of the composition provides a single treatment having at least about a 16 week duration of effect in reducing the symptoms of cervical dystonia in the individual, thereby extending treatment interval duration for the individual.

[0017b] In one aspect, the present disclosure refers to use of a botulinum toxin component for the manufacture of an injectable composition for the treatment of cervical dystonia in an individual in need thereof, said composition comprising:

the botulinum toxin component in a dose of 100 U to 450 U, said botulinum toxin component selected from the group consisting of a botulinum toxin complex, a reduced botulinum toxin complex, or a botulinum toxin,

a positively charged carrier component comprising a positively charged polylysine backbone having covalently attached thereto one or more positively charged efficiency groups having an amino acid sequence of $(gly)_p$-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; and

a pharmaceutically acceptable diluent for injection;

wherein the positively charged carrier is non-covalently associated with the botulinum toxin component; and

wherein said dose of the composition provides a single treatment having at least about a 16 week duration of effect in reducing the symptoms of cervical dystonia in the individual, thereby extending treatment interval duration for the individual.

[0018] In another example, the disclosure provides effective doses and amounts of the compositions of this disclosure in the treatment of cervical dystonia that afford a long-lasting, sustained efficacy e.g., a response rate of long duration, following administration by injection to

a subject or patient in need of treatment. Such doses and amounts are preferably therapeutically effective doses and amounts that produce or result in a desired therapeutic effect in a subject to whom the doses and amounts are administered. In particular examples, a single treatment of a subject or patient with a composition of the disclosure comprising a botulinum toxin, such as botulinum toxin A, and a positively charged carrier, as described herein, in therapeutically effective dose amounts of about 100 U to about 450 U per subject, afforded a response rate of in the reduction of cervical dystonia symptoms for at least 16 weeks, at least 20 weeks, at least 24 weeks, or about 6 to 10 months, or even longer. Moreover, the compositions of the disclosure provide an attribute of reduced diffusion or spread from the injection site following injection, thereby localizing the toxin and its effect where desired and decreasing nonspecific or unwanted effects of the toxin at sites or locations distant from the site of injection for treatment.

[0019] The duration of effect provided by compositions of the disclosure, e.g., RT002 as well as by the described treatment methods and uses, affords significant advantages compared to the art. By way of example, subjects undergoing treatment with compositions containing botulinum toxin consider that duration of effect following treatment is of high importance to them. Such a long, sustained duration of effect, which is achieved by even a single treatment with an effective dose and treatment regime of a product of the disclosure, for example, RT002, permits fewer injections per treatment course for a subject, which is extremely important for the subject. A prolonged duration effect from a single treatment with a product which has clear efficacy and safety, as provided by the inventive compositions and methods described herein, offer less discomfort, less cost and more convenience to subjects undergoing a course of treatment. Furthermore, a product that affords significant and sustained effects, which are maintained for at least a 16 or 24 week period, or for at least a 6-month period, or for greater than a 6-month period, following the single injectable treatment of the product to a subject, provides a solution to an unmet need in the art for both practitioners and patients alike. Thus, the compositions and methods of the disclosure provide a solution to the problem of too frequent treatments and improve patients' overall well-being. Such prolonged duration of action provides for fewer treatments over an entire treatment course.

[0020] In another example, the disclosure provides a method of administering botulinum toxin to achieve an extended duration therapeutic effect in an individual suffering from cervical dystonia, in which the method comprises administering by injection a dose of a sterile injectable

composition into an area of the individual in need of treatment to achieve the therapeutic effect following a first treatment with the composition; wherein the composition comprises a botulinum toxin, a botulinum toxin complex, or a reduced botulinum toxin complex component and a positively charged carrier component comprising a positively charged polylysine backbone having covalently attached thereto one or more positively charged efficiency groups having an amino acid sequence of (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; wherein the botulinum toxin, botulinum toxin complex, or reduced botulinum toxin complex component is administered to the individual in a treatment dose of about 100 U to 450 U; or more specifically, from about 100 U to 200 U or from about 200 U to 300 U or from about 300 U to 450 U, wherein the positively charged carrier is non-covalently associated with the botulinum toxin, botulinum toxin complex, or reduced botulinum toxin complex component; and a pharmaceutically acceptable diluent suitable for injection; and wherein the first treatment dose of the composition administered by injection to the individual achieves the extended duration therapeutic effect having at least about a 6 month to about a 10 month duration of effect, optionally, before a second or subsequent treatment dose is administered.

[0021] In another example, the disclosure provides for the treatment of cervical dystonia, a sterile injectable composition comprising a botulinum toxin, a botulinum toxin complex, or a reduced botulinum toxin complex in a dosage amount selected from more than 100 U, from 100 U to 200 U, 200-300 U, or 300-450 U; and a positively charged carrier comprising a positively charged polylysine backbone having covalently attached thereto one or more positively charged efficiency groups having an amino acid sequence of (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; and a pharmaceutically acceptable diluent for injection; wherein the positively charged carrier is non-covalently associated with the botulinum toxin, botulinum toxin complex, or reduced botulinum toxin complex component; and wherein the composition provides a therapeutic effect which endures for at least 20 to 24 weeks, or for at least 6 months, or greater than 6 months, e.g., about 6 months to about 10 months, following a treatment of an individual with an effective dose of the injectable composition.

[0022] In some examples of these above methods and composition, the composition comprises botulinum toxin of serotype A, preferably a serotype A botulinum toxin having a molecular weight of 150 kDa. In an example, the positively charged carrier has the amino acid sequence RKKRRQRRRG-$(K)_{15}$-GRKKRRQRRR (SEQ ID NO: 4). In an example, the botulinum toxin is present in the composition in a dosage amount from more than 100 U, 100-200 U, or 200-300 U or 300-450 U. In an example, the botulinum toxin is present in the composition in a dosage amount selected from the group consisting of 100 U, 200U, 300U and 450U. In an example, the composition reduces the symptoms of cervical dystonia in an individual who has undergone a single treatment by injection of the composition. In certain examples, the duration of the treatment effect comprises greater than 6 months; greater than 7 months; greater than 8 months; greater than 9 months; or at least 6 months through 10 months.

[0023] In another of its examples, the disclosure provides a method of treating an individual suffering from cervical dystonia who is in need of treatment with injectable botulinum toxin, in which the method of treatment comprises a treatment course having multiple treatment intervals with prolonged duration of effect and duration time between each treatment interval, the treatment course comprising: administering by injection an initial treatment dose of a sterile injectable composition into an area of the individual in need of treatment to achieve a therapeutic effect following the initial treatment with the composition; wherein the composition comprises a botulinum toxin, a botulinum toxin complex, or a reduced botulinum toxin complex component and a positively charged carrier component comprising a positively charged polylysine backbone having covalently attached thereto one or more positively charged efficiency groups having an amino acid sequence of (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; and a pharmaceutically acceptable diluent suitable for injection; wherein the botulinum toxin, botulinum toxin complex, or reduced botulinum toxin complex component is administered to the individual in a treatment dose of from more than 100 U, 100-200 U, 200-300 U, or 300-450 U; wherein the positively charged carrier is non-covalently associated with the botulinum toxin, botulinum toxin complex, or reduced botulinum toxin complex component; wherein the initial treatment dose of the composition administered by injection to the individual provides a therapeutic duration of effect lasting through at least about 10 months; and administering

subsequent treatment doses of the composition by injection to the individual at treatment intervals comprising a duration of greater than or equal to 3 months to at least about 10 months following the initial treatment dose and between each subsequent treatment dose.

[0024] In examples of the above-described treatment method, the therapeutic effect is treatment of the symptoms of cervical dystonia. In an example, the composition comprises botulinum toxin of serotype A, preferably, botulinum toxin of serotype A having a molecular weight of 150 kDa. In an example, the positively charged carrier is a positively charged peptide having the amino acid sequence RKKRRQRRRG-(K)15-GRKKRRQRRR (SEQ ID NO: 4). In an example, the composition does not locally diffuse from the site of injection following injection. In specific examples, the botulinum toxin is administered to the individual in an amount of more than 100 U, 100-200 U, 200-300 U, or 300-450 U. In certain examples, the duration of the treatment interval comprises greater than 3 months; greater than 4 months; greater than 5 months; greater than 6 months; greater than 7 months; greater than 8 months; greater than 9 months; or at least 6 months through 10 months.

DESCRIPTION OF THE FIGURES

[0025] **Figure 1** shows the Cervical Dystonia Phase 2 Study Design as described in the Example herein.

[0026] **Figure 2** shows the Demographics by Cohort in the Study as described in the Example herein.

[0027] **Figure 3** shows Number (%) of Subjects with Treatment-Related AE's by Cohort By Preferred Term in the Study as described in the Example herein.

[0028] **Figure 4** shows Primary Endpoint by Cohort - Reduction in TWSTRS-Total Score at Week 4 in the Study as described in the Example herein.

[0029] **Figure 5** shows Secondary Endpoint by Cohort - Change from Baseline in TWSTRS-Total Score over Time in the Study as described in the Example herein.

[0030] **Figure 6** shows Secondary Endpoint by Cohort - Duration of Response Defined by Subject Reaching Target-TWSTRS Score (of subjects with improvement at Week 4. Withdrawals due to need for retreatment are considered as events) in the Study as described in the Example herein.

Claims:

1. A pharmaceutical composition in a sterile injectable formulation for use in treating cervical dystonia in an individual in need thereof, said composition comprising:

a botulinum toxin component in a dose of 100 U to 450 U, said botulinum toxin component selected from the group consisting of a botulinum toxin complex, a reduced botulinum toxin complex, or a botulinum toxin,

a positively charged carrier component comprising a positively charged polylysine backbone having covalently attached thereto one or more positively charged efficiency groups having an amino acid sequence of $(gly)_p$-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; and

a pharmaceutically acceptable diluent for injection;

wherein the positively charged carrier is non-covalently associated with the botulinum toxin component; and

wherein said dose of the composition provides a single treatment having at least about a 16 week duration of effect in reducing the symptoms of cervical dystonia in the individual, thereby extending treatment interval duration for the individual.

2. The pharmaceutical composition for use according to claim 1, wherein the composition achieves the extended duration therapeutic effect for at least about 8 months.

3. The pharmaceutical composition for use according to claim 2, wherein the composition comprises botulinum toxin of serotype A.

4. The pharmaceutical composition for use according to claim 2, wherein the composition comprises botulinum toxin of serotype A having a molecular weight of 150 kDa.

5. The pharmaceutical composition for use according to any one of claims 1 to 4, wherein the positively charged polylysine backbone has covalently attached thereto one or more positively charged efficiency groups having the amino acid sequence $(gly)_p$-RGRDDRRQRRR-$(gly)_q$ (SEQ ID NO: 1), wherein the subscripts p and q are each independently an integer of from 0 to 20.

6. The pharmaceutical composition for use according to any one of claims 1 to 4, wherein the positively charged polylysine backbone has covalently attached thereto one or more positively charged efficiency groups having the amino acid sequence $(gly)_p$-YGRKKRRQRRR-$(gly)_q$ (SEQ ID NO: 2), wherein the subscripts p and q are each independently an integer of from 0 to 20.

7. The pharmaceutical composition for use according to any one of claims 1 to 4, wherein the positively charged polylysine backbone has covalently attached thereto one or more positively charged efficiency groups having the amino acid sequence $(gly)_p$-RKKRRQRRR-$(gly)_q$ (SEQ ID NO: 3), wherein the subscripts p and q are each independently an integer of from 0 to 20.

8. The pharmaceutical composition for use according to any one of claims 1 to 7, wherein (i) the subscripts p and q are each independently an integer of from 0 to 8; or (ii) are each independently an integer of from 2 to 5.

9. The pharmaceutical composition for use according to any one of claims 1 to 8, wherein the one or more positively charged efficiency groups are attached to either end, or both ends, of the positively charged polylysine backbone of the positively charged carrier.

10. The pharmaceutical composition for use according to claim 9, wherein the positively charged carrier has the amino acid sequence RKKRRQRRRG-$(K)_{15}$-GRKKRRQRRR (SEQ ID NO: 4).

11. The pharmaceutical composition for use according to any one of claims 1 to 10, wherein the composition does not locally diffuse from the site of injection following injection.

12. The pharmaceutical composition for use according to any one of claims 1 to 11, wherein the treatment dose of botulinum toxin is administered to the individual in an amount of about 100 U to 200U.

13. The pharmaceutical composition for use according to any one of claims 1 to 11, wherein the treatment dose of botulinum toxin is administered to the individual in an amount of about 200 U to 300 U.

14. The pharmaceutical composition for use according to any one of claims 1 to 11, wherein the treatment dose of botulinum toxin is administered to the individual in an amount of 300 U to 450 U.

15. The pharmaceutical composition for use according to any one of claims 1 to 14, wherein the duration of effect comprises greater than 20 weeks.

16. The pharmaceutical composition for use according to any one of claims 1 to 14, wherein the duration of effect comprises greater than 24 weeks.

17. The pharmaceutical composition for use according to any one of claims 1 to 14, wherein the duration of effect comprises greater than 5 months.

18. The pharmaceutical composition for use according to any one of claims 1 to 14, wherein the duration of effect comprises greater than 6 months.

19. The pharmaceutical composition for use according to any one of claims 1 to 14, wherein the duration of effect comprises greater than 7 months.

20. The pharmaceutical composition for use according to any one of claims 1 to 14, wherein the duration of effect comprises greater than 8 months.

21. The pharmaceutical composition for use according to any one of claims 1 to 14, wherein the duration of effect comprises greater than 9 months.

22. The pharmaceutical composition for use according to any one of claims 1 to 14, wherein the duration of effect comprises at least 6 months through 10 months.

23. Use of a botulinum toxin component for the manufacture of an injectable composition for the treatment of cervical dystonia in an individual in need thereof, said composition comprising:

the botulinum toxin component in a dose of 100 U to 450 U, said botulinum toxin component selected from the group consisting of a botulinum toxin complex, a reduced botulinum toxin complex, or a botulinum toxin,

a positively charged carrier component comprising a positively charged polylysine backbone having covalently attached thereto one or more positively charged efficiency groups having an amino acid sequence of $(gly)_p$-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; and

a pharmaceutically acceptable diluent for injection;

wherein the positively charged carrier is non-covalently associated with the botulinum toxin component; and

wherein said dose of the composition provides a single treatment having at least about a 16 week duration of effect in reducing the symptoms of cervical dystonia in the individual, thereby extending treatment interval duration for the individual.