SUMMARY

[0015] The disclosure relates to methods of using injectable botulinum toxin formulations for administration to an individual to achieve a therapeutic and/or cosmetic benefit having surprisingly high responder rate and long duration of effect.

[0015a] In one aspect, the present disclosure refers to a method of administering botulinum toxin to achieve an extended duration cosmetic effect in an individual, the method comprising:

administering by injection a treatment dose of a sterile injectable composition into an area of the individual in need thereof to achieve the cosmetic effect following treatment with the composition;

wherein the composition comprises a pharmaceutically acceptable diluent suitable for injection; and

a botulinum toxin component selected from the group consisting of a botulinum toxin, a botulinum toxin complex, or a reduced botulinum toxin complex; and

a positively charged carrier component having an amino acid sequence RKKRRQRRRG-$(K)_{15}$-GRKKRRQRRR (SEQ ID NO: 4);

wherein said positively charged carrier is present in said pharmaceutical composition in an amount of 0.2 µg to 0.4 µg per 1 U of said botulinum toxin component, wherein the cosmetic effect is a reduction in the appearance of glabellar lines.

[0015b] In another aspect, the present disclosure refers to a pharmaceutical composition in a sterile injectable formulation for use in reducing wrinkles, lines, or furrows in an individual in need thereof, said composition comprising:

a 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 having an amino acid sequence RKKRRQRRRG-$(K)_{15}$-GRKKRRQRRR (SEQ ID NO: 4); and

a pharmaceutically acceptable diluent for injection;

wherein said positively charged carrier is present in said pharmaceutical composition in an amount of 0.2 µg to 0.4 µg per 1 U of said botulinum toxin component.

[0015c] In another aspect, the present disclosure refers to a method of reducing wrinkles, lines, or furrows in an individual in need thereof, the method comprising:

administering to the individual by injection to one or more muscles or facial structures associated with the glabellar lines of the individual a composition comprising:

a pharmaceutically acceptable diluent for injection;

a botulinum toxin component selected from the group consisting of a botulinum toxin, a botulinum toxin complex, or a reduced botulinum toxin complex; and

a positively charged carrier component having an amino acid sequence RKKRRQRRRG-$(K)_{15}$-GRKKRRQRRR (SEQ ID NO: 4);

wherein the botulinum toxin component is administered to the individual in a treatment dose amount of 10 U to 100 U per injection treatment;

wherein said positively charged carrier is present in said pharmaceutical composition in an amount of 0.2 µg to 0.4 µg per 1 U of said botulinum toxin component,

wherein the injection of the composition provides a single treatment dose having at least a 28-week duration of effect in reducing wrinkles, lines, or furrows of the individual.

[0015d] In yet another aspect, the present disclosure refers to a pharmaceutical composition in a sterile injectable formulation for use in administering botulinum toxin to achieve an extended duration therapeutic or cosmetic effect in an individual,

said composition comprising a pharmaceutically acceptable diluent suitable for injection

a botulinum toxin component in a treatment dose of 10 U to 100 U, wherein said botulinum toxin component is selected from the group consisting of a botulinum toxin complex, a reduced botulinum toxin complex, or a botulinum toxin; and

a positively charged carrier component having an amino acid sequence RKKRRQRRRG-$(K)_{15}$-GRKKRRQRRR (SEQ ID NO: 4);

wherein the positively charged carrier is non-covalently associated with the botulinum toxin component and wherein said positively charged carrier is present in said pharmaceutical composition in an amount of 0.2 µg to 0.45 µg per 1 U of said botulinum toxin component.

[0015e] In yet another aspect, the present disclosure refers to a use of a pharmaceutical composition in the manufacture of a medicament for achieving an extended duration therapeutic effect in an individual, wherein the therapeutic effect is reduction of a symptom

associated with a disorder selected from the group consisting of hemifacial spasm, adult onset spasmodic torticollis, anal fissure, blepharospasm, cerebral palsy, cervical dystonia, plantar fasciitis, migraine headaches, strabismus, temporomandibular joint disorder, neurologic pain, overactive bladder, rhinitis, sinusitis, acne, dystonia, dystonic contractions, hyperhidrosis, and hypersecretion of a gland controlled by the cholinergic nervous system, wherein the pharmaceutical composition comprises a pharmaceutically acceptable diluent suitable for injection; a botulinum toxin component in a treatment dose of 10 U to 100 U, wherein said botulinum toxin component is selected from the group consisting of a botulinum toxin complex, a reduced botulinum toxin complex, or a botulinum toxin; and a positively charged carrier component having an amino acid sequence RKKRRQRRRG-$(K)_{15}$-GRKKRRQRRR (SEQ ID NO: 4), wherein the positively charged carrier is non-covalently associated with the botulinum toxin component and wherein said positively charged carrier is present in said pharmaceutical composition in an amount of 0.2 µg to 0.4 µg per 1 U of said botulinum toxin component.

[0016] In one example, this disclosure provides injectable compositions comprising botulinum toxin non-covalently associated with a positively charged carrier. 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 toxin 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 formulations.

[0017] In preferred examples, the botulinum toxin component comprises serotype A botulinum toxin having a molecular weight of 150 kDa. The botulinum toxin component may be selected from a botulinum toxin complex (including the 150 kD neurotoxin with accessory proteins found in native complexes produced by *C. botulinum*), a reduced botulinum toxin complex (including the 150 kD neurotoxin with some, but not all, of the native accessory proteins), and the 150 kD botulinum toxin molecule itself without accessory (non-toxin) proteins.

[0018] In preferred examples, certain non-native molecules (*i.e.*, molecules not found in botulinum toxin complexes obtained from *Clostridium botulinum* bacteria) are added to botulinum toxin, botulinum toxin complexes, and in particular reduced botulinum toxin complexes (as

defined herein), to improve toxin diffusion through tissues. The non-native molecules associate hh 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.

We claim:

1. A method of administering botulinum toxin to achieve an extended duration cosmetic effect in an individual, the method comprising:

administering by injection a treatment dose of a sterile injectable composition into an area of the individual in need thereof to achieve the cosmetic effect following treatment with the composition;

wherein the composition comprises a pharmaceutically acceptable diluent suitable for injection; and

a botulinum toxin component selected from the group consisting of a botulinum toxin, a botulinum toxin complex, or a reduced botulinum toxin complex; and

a positively charged carrier component having an amino acid sequence RKKRRQRRRG-$(K)_{15}$-GRKKRRQRRR (SEQ ID NO: 4) ;

wherein said positively charged carrier is present in said pharmaceutical composition in an amount of 0.2 µg to 0.4 µg per 1 U of said botulinum toxin component, wherein the cosmetic effect is a reduction in the appearance of glabellar lines.

2. A pharmaceutical composition in a sterile injectable formulation for use in reducing wrinkles, lines, or furrows in an individual in need thereof, said composition comprising:

a 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 having an amino acid sequence RKKRRQRRRG-$(K)_{15}$-GRKKRRQRRR (SEQ ID NO: 4); and

a pharmaceutically acceptable diluent for injection;

wherein said positively charged carrier is present in said pharmaceutical composition in an amount of 0.2 µg to 0.4 µg per 1 U of said botulinum toxin component.

3. The method according to claim 1 or the composition according to claim 2, wherein the pharmaceutical composition comprises a botulinum toxin component at a dose between 10 U to 100 U.

4. The method according to claim 1 or 3 or the composition according to claim 2 or 3, wherein the treatment dose of the composition administered by injection to the individual achieves the extended duration cosmetic effect having at least a 28-week duration of effect.

5. The method or composition according to claim 4, wherein the composition achieves an extended duration of effect for at least 32 weeks.

6. The method or composition according to claim 5, wherein the composition comprises botulinum toxin of serotype A.

7. The method or composition according to claim 6, wherein the composition comprises botulinum toxin of serotype A having a molecular weight of 150 kDa without accessory proteins.

8. The method or composition according to any one of claims 1 to 7, wherein the composition does not locally diffuse from the site of injection following injection.

9. The method or composition according to any one of claims 1 to 8, wherein the treatment dose of botulinum toxin is 40 U per injection treatment.

10. The method or composition according to any one of claims 1 to 9, wherein said positively charged carrier is present in said pharmaceutical composition in an amount of 12 µg per 50 U of said botulinum toxin component.

11. The method or composition according to any one of claims 1 to 9, wherein the positively charged carrier is non-covalently associated with the botulinum toxin component and present in said pharmaceutical composition in an amount of 11.7 µg per 50 U.

12. The method or composition according to any one of claims 1 to 11, wherein said excipient comprises at least one component selected from the group consisting of L-Histidine, L-Histidine hydrochloride, polysorbate 20, and trehalose dihydrate.

13. The method or composition according to claim 12, wherein said excipient comprises trehalose dihydrate.

14. The method or composition according to any one of claims 1 to 13, wherein said method or use comprises a single administration of said pharmaceutical composition.

15. The composition according to any one of claims 2 to 14, wherein the reduction of wrinkles, lines, or furrows is a reduction in the severity of glabellar lines.

16. The method or composition according to claim 15, wherein the administration comprises at least one injection into one or more muscle selected from the group consisting of the right corrugator muscle, the left corrugator muscle, and the procerus muscle.

17. The method or composition according to claim 16, wherein about 8 U of said botulinum toxin component are injected into said the medial aspect of the right corrugator muscle, about 8 U of said botulinum toxin component are injected into said the lateral aspect of the right corrugator muscle; about 8 U of said botulinum toxin component are injected into said the medial aspect of the left corrugator muscle, about 8 U of said botulinum toxin component are injected into said the lateral aspect of the left corrugator muscle; and about 8 U of said botulinum toxin component are injected into a procerus muscle.

18. The method or composition according to any one of the above where the subject is at least 65 years of age.

19. A method of reducing wrinkles, lines, or furrows in an individual in need thereof, the method comprising:

administering to the individual by injection to one or more muscles or facial structures associated with the glabellar lines of the individual a composition comprising:

a pharmaceutically acceptable diluent for injection;

a botulinum toxin component selected from the group consisting of a botulinum toxin, a botulinum toxin complex, or a reduced botulinum toxin complex; and

a positively charged carrier component having an amino acid sequence RKKRRQRRRG-$(K)_{15}$-GRKKRRQRRR (SEQ ID NO: 4) ;

wherein the botulinum toxin component is administered to the individual in a treatment dose amount of 10 U to 100 U per injection treatment;

wherein said positively charged carrier is present in said pharmaceutical composition in an amount of 0.2 µg to 0.4 µg per 1 U of said botulinum toxin component,

wherein the injection of the composition provides a single treatment dose having at least a 28-week duration of effect in reducing wrinkles, lines, or furrows of the individual.

20. A pharmaceutical composition in a sterile injectable formulation for use in administering botulinum toxin to achieve an extended duration therapeutic or cosmetic effect in an individual,

said composition comprising a pharmaceutically acceptable diluent suitable for injection;

a botulinum toxin component in a treatment dose of 10 U to 100 U, wherein said botulinum toxin component is selected from the group consisting of a botulinum toxin complex, a reduced botulinum toxin complex, or a botulinum toxin; and

a positively charged carrier component having an amino acid sequence RKKRRQRRRG-$(K)_{15}$-GRKKRRQRRR (SEQ ID NO: 4);

wherein the positively charged carrier is non-covalently associated with the botulinum toxin component and wherein said positively charged carrier is present in said pharmaceutical composition in an amount of 0.2 µg to 0.4 µg per 1 U of said botulinum toxin component.

21. The method according to claim 19 or the composition according to claim 20, wherein the pharmaceutical composition comprises a botulinum toxin component at a dose between 10 U to 100 U.

22. The method according to claim 19 or 21 or the composition according to claim 20 or 21, wherein the treatment dose of the composition administered by injection to the individual achieves the extended duration cosmetic effect having at least a 28-week duration of effect.

23. Use of a pharmaceutical composition in the manufacture of a medicament for achieving an extended duration therapeutic effect in an individual, wherein the therapeutic effect is reduction of a symptom associated with a disorder selected from the group consisting of hemifacial spasm, adult onset spasmodic torticollis, anal fissure, blepharospasm, cerebral palsy, cervical dystonia, plantar fasciitis, migraine headaches, strabismus, temporomandibular joint disorder, neurologic pain, overactive bladder, rhinitis, sinusitis, acne, dystonia, dystonic contractions, hyperhidrosis, and hypersecretion of a gland controlled by the cholinergic nervous system, wherein the pharmaceutical composition comprises a pharmaceutically acceptable diluent suitable for injection; a botulinum toxin component in a treatment dose of 10 U to 100 U, wherein said botulinum toxin component is selected from the group consisting of a botulinum toxin complex, a reduced botulinum toxin complex, or a botulinum toxin; and a positively charged carrier component having an amino acid sequence RKKRRQRRRG-$(K)_{15}$-GRKKRRQRRR (SEQ ID NO: 4), wherein the positively charged carrier is non-covalently associated with the botulinum toxin component and wherein said positively charged carrier is present in said pharmaceutical composition in an amount of 0.2 µg to 0.4 µg per 1 U of said botulinum toxin component.

24. The use according to claim 23, wherein the pharmaceutical composition comprises a botulinum toxin component at a dose between 10 U to 100 U.

25. The use according to claim 23 or 24, wherein the treatment dose of the composition administered by injection to the individual achieves the extended duration therapeutic effect having at least a 28-week duration of effect.