[0014] Typically, the botulinum toxin is administered to patients by carefully controlled injections of compositions containing botulinum toxin complex and albumin. However, there are several problems associated with this approach. Not only are the injections painful, but typically large subdermal wells of toxin are locally generated around the injection sites, in order to achieve the desired therapeutic or cosmetic effect. The botulinum toxin may migrate from these subdermal wells to cause unwanted paralysis in surrounding areas of the body. This problem is exacerbated when the area to be treated is large and many injections of toxin are required to treat the area. Moreover, because the injected toxin complexes contain non-toxin proteins and albumin that stabilize the botulinum toxin and increase the molecular weight of the toxin complex, the toxin complexes have a long half-life in the body and may cause an undesirable antigenic response in the patient. For example, some patients will, over time, develop an allergy to the albumin used as a stabilizer in current commercial formulations. Also, the toxin complexes may induce the immune system of the patient to form neutralizing antibodies, so that larger amounts of toxin are required in subsequent administrations to achieve the same effect. When this happens, subsequent injections must be carefully placed so that they do not release a large amount of toxin into the bloodstream of the patient, which could lead to fatal systemic poisoning, especially since the non-toxin proteins and albumin stabilize the botulinum toxin in blood.

[0015] In view of the drawbacks associated with current botulinum toxin formulations, it would be highly desirable to have an injectable botulinum toxin formulation that is efficacious and stable, but exhibits reduced antigenicity and a lower tendency to diffuse locally after injection. It would also be desirable to use such a botulinum toxin formulation for various therapeutic, aesthetic and/or cosmetic purposes.

SUMMARY

[0016] This disclosure provides injectable compositions comprising botulinum toxin non-covalently associated with a positively charged carrier molecule. In preferred embodiments, 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 embodiments, 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.

[0017] Disclosed 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. 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.

[0018] Disclosed is 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.

[0019] Disclosed is a method for producing a biologic effect by injecting an effective amount of the compositions of this disclosure to a subject or patient in need of such treatment. The biologic effect may include, for example, muscle paralysis, reduction of hypersecretion or sweating, treatment of neurologic pain or migraine headache, management of rhinitis or sinusitis, treatment of hyperactive bladder, reduction of muscle spasms, prevention or reduction of acne, reduction or enhancement of an immune response, reduction of wrinkles, or prevention or treatment of various other disorders.

[0020] Disclosed are kits for preparing formulations containing a botulinum toxin, a botulinum toxin complex, or a reduced protein botulinum toxin

complex and positively charged carrier, or a premix that may in turn be used to produce such a formulation. Also provided are kits that contain means for sequentially administering a botulinum toxin complex (or a reduced botulinum toxin complex including just the 150 KD neurotoxin itself or the neurotoxin with some native complex proteins) and a positively charged carrier.

[0020a] In one aspect, there is provided a method of administering botulinum toxin to achieve a cosmetic effect to an individual in need thereof, the method comprising

injecting an effective amount of a sterile injectable formulation comprising a botulinum toxin composition into the individual to achieve a cosmetic effect,

wherein the botulinum toxin composition comprises

a positively charged carrier comprising the amino acid sequence RKKRRQRRR G-$(K)_{15}$- GRKKRRQ RRR,

a botulinum toxin component present in an amount suitable for injection, the botulinum toxin component selected from the group consisting of a botulinum toxin complex, reduced botulinum toxin complex, and botulinum toxin, and

a pharmaceutically acceptable diluent suitable for injection,

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

[0020b] In another aspect, there is provided use of an effective amount of a botulinum toxin composition for the preparation of a sterile injectable formulation to achieve a therapeutic or cosmetic effect in an individual in need thereof,

wherein the botulinum toxin composition comprises

a positively charged carrier comprising the amino acid sequence RKKRRQ RRRG-$(K)_{15}$-GRKKRRQRRR,

a botulinum toxin component present in an amount suitable for injection, the botulinum toxin component selected from the group consisting of a botulinum toxin complex, reduced botulinum toxin complex, and botulinum toxin, and

a pharmaceutically acceptable diluent suitable for injection,

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

[0020c] In another aspect, there is provided a method of preparing a sterile injectable botulinum toxin formulation, the method comprising

providing an effective amount of a botulinum toxin component present in an amount suitable for injection, the botulin um toxin component selected from the group consisting of a botulinum toxin complex, a reduced botulinum toxin complex, and a botulinum toxin;

providing a positively charged carrier comprising the amino acid sequence RKKRRQ RRRG-$(K)_{15}$-GRKKRRQRRR ; and

combining the botulinum toxin component with the positively charged carrier and a pharmaceutically acceptable diluent to form the sterile injectable botulinum toxin formulation;

wherein the pharmaceutically acceptable diluent is suitable for use in injectable

pharmaceutical or cosmeceutical compositions; and

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

[0020d] In another aspect, there is provided use of an effective amount of a botulinum toxin composition for the preparation of a sterile injectable formulation for administration by injection to an individual, at an area affected by muscle spasm, to reduce or prevent said muscle spasm,
wherein the botulinum toxin composition comprises:

a positively charged carrier comprising the amino acid sequence RKKRRQRRRG-$(K)_{15}$-GRKKRRQRRR,

a botulinum toxin component present in an amount suitable for injection, the botulinum toxin component selected from the group consisting of a botulinum toxin complex, reduced botulinum toxin complex, and botulinum toxin, and

a pharmaceutically acceptable diluent suitable for injection;
wherein the positively charged carrier is non-covalently associated with the botulinum toxin component.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1: A bar graph showing the required time to return to the baseline DAS value (0.4) following repeated administration of either RT003 or BOTOX®

Figure 2: Figure 2A shows the hind leg of a mouse injected with a dark dye to indicate the portion of a mouse's gastrocnemius muscle that is affected by lateral-to-midline injection. Figure 2B shows the hind leg of a mouse injected with a dark dye to indicate the portion of a mouse's gastrocnemius muscle that is affected by midline injection.

Figure 3: Digital abduction scores measured as a function of time following injection of RT003, RTT150, or BOTOX® into either the lateral-to-midline or midline portion of the gastrocnemius muscle of a mouse.

DETAILED DESCRIPTION OF THE INVENTION

[0021] This invention relates to novel injectable compositions comprising botulinum toxin, a botulinum toxin complex, or a reduced botulinum toxin complex. In preferred embodiments, the compositions stabilize the toxin or enable the transport or delivery of toxin through tissues after injection such that the toxin has reduced antigenicity, a better safety profile, enhanced potency, faster onset of clinical efficacy and/or longer duration of clinical efficacy compared to conventional commercial botulinum toxin complexes that are bound to exogenous albumin (e.g., BOTOX® or MYOBLOC®). The compositions of the invention may be used as injectable applications for providing a botulinum toxin to a subject, for various therapeutic, aesthetic and/or cosmetic purposes, as described herein. The compositions of the invention also have an improved safety profile over other compositions and methods of delivery of botulinum toxin. In addition, these compositions can afford beneficial reductions in immune responses to the botulinum toxin.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A method of administering botulinum toxin to achieve a cosmetic effect to an individual in need thereof, the method comprising

injecting an effective amount of a sterile injectable formulation comprising a botulinum toxin composition into the individual to achieve a cosmetic effect,

wherein the botulinum toxin composition comprises

a positively charged carrier comprising the amino acid sequence RKKRRQRRR G-$(K)_{15}$-GRKKRRQ RRR,

a botulinum toxin component present in an amount suitable for injection, the botulinum toxin component selected from the group consisting of a botulinum toxin complex, reduced botulinum toxin complex, and botulinum toxin, and

a pharmaceutically acceptable diluent suitable for injection,

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

2. Use of an effective amount of a botulinum toxin composition for the preparation of a sterile injectable formulation to achieve a therapeutic or cosmetic effect in an individual in need thereof,

wherein the botulinum toxin composition comprises

a positively charged carrier comprising the amino acid sequence RKKRRQ RRRG-$(K)_{15}$-GRKKRRQRRR,

a botulinum toxin component present in an amount suitable for injection, the botulinum toxin component selected from the group consisting of a botulinum toxin complex, reduced botulinum toxin complex, and botulinum toxin, and

a pharmaceutically acceptable diluent suitable for injection,

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

3. The method according to claim 1 or use according to claim 2, wherein the botulinum toxin component is obtained from serotypes A, B, C, D, E, F, or G of C. *botulinum.*

4. The method or use according to any one of claims 1 to 3, wherein the positively charged carrier stabilizes botulinum toxin against degradation.

5. The method or use according to claim 4, wherein the positively charged carrier stabilizes botulinum toxin against degradation to retain potency after 6 months ageing at 4 °C.

6. The method or use according to any one of claims 1 to 5, wherein the positively charged carrier reduces local diffusion of the botulinum toxin following injection.

7. The method or use according to any one of claims 1 to 6, wherein antibody generation following injection of the formulation into the subject's body is reduced compared to antibody generation following injection of a formulation consisting of 100 U of type A botulinum toxin with accessory protein, 0.5 mg of human albumin, and 0.9 mg of sodium chloride.

8. The method or use according to any one of claims 1 to 7, wherein the cosmetic effect is selected from the group consisting of decreasing the appearance of fine lines and/or wrinkles, adjusting facial appearance in a desirable way, widening the eyes, lifting corners of the mouth, and smoothing lines that fan out from the upper lip.

9. The use according to any one of claims 2 to 7, wherein the therapeutic effect is reducing or enhancing immune response or reduction of a symptom associated with a disorder selected from the group consisting of muscle tension, muscle spasm, hemifacial spasm, adult onset spasmodic torticollis, anal fissure, blepharospasm, cerebral palsy, headaches, migraine headache, 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.

10. A method of preparing a sterile injectable botulinum toxin formulation, the method comprising

providing an effective amount of a botulinum toxin component present in an amount suitable for injection, the botulin um toxin component selected from the group consisting of a botulinum toxin complex, a reduced botulinum toxin complex, and a botulinum toxin;

providing a positively charged carrier comprising the amino acid sequence

RKKRRQ RRRG-$(K)_{15}$-GRKKRRQRRR ; and

combining the botulinum toxin component with the positively charged carrier and a pharmaceutically acceptable diluent to form the sterile injectable botulinum toxin formulation;

wherein the pharmaceutically acceptable diluent is suitable for use in injectable pharmaceutical or cosmeceutical compositions; and

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

11. The method of claim 10, further comprising the step of storing the injectable formulation in a syringe suitable for injecting the formulation.

12. The method according to claim 10 or 11, wherein the botulin um toxin component is obtained from serotype A, B, C, D, E, F, or G of *C. botuUnum.*

13. The method according to any one of claims 10 to 12, wherein the positively charged carrier stabilizes botulinum toxin against degradation.

14. The method according to claim 13, wherein the positively charged carrier stabilizes botulinum toxin against degradation to retain potency after 6 months ageing at 4 °C.

15. The method according to any one of claims 10 to 14, wherein the positively charged carrier reduces local diffusion of botulin um toxin following injection.

16. The method according to any one of claims 10 to 15, wherein antibody generation following injection of the formulation into the subject's body is reduced compared to antibody generation following injection of a formulation consisting of 100 U of type A botulinum toxin with accessory protein, 0.5 mg of human albumin, and 0.9 mg of sodium chloride.

17. The method or use according to any one of claims 1 to 9, wherein said botulinum toxin component is present in said formulation in an amount from 1 to 400 U per injection, or from 10 to 350 U per injection.

18. The method or use according to any one of claims 1 to 9, wherein said botulinum toxin component is present in said formulation in an amount from 30 to 250 U per injection, or from 50 to 200 U per injection.

19. The method or use according to any one of claims 1 to 9, wherein said botulinum toxin component is present in said formulation in an amount from 1 to 200 U per injection, or from 1 to 250 U per injection.

20. The method or use according to any one of claims 1 to 9, wherein said botulinum toxin component is present in said formulation in an amount from 10 to 200 U per injection, or from 10 to 250 U per injection.

21. The method or use according to any one of claims 1 to 9, wherein said botulinum toxin component is present in said formulation in an amount from 30 to 200 U per injection or from 50 to 250 U per injection.

22. The method or use according to any one of claims 1 to 9, wherein said formulation is administered to provide from about 1 U to about 20,000 U, or from about 1 U to about 10,000 U, of the botulinum toxin component per cm^2 of skin surface treated in a series of treatments over time.

23. The method or use according to any one of claims 1 to 22, wherein said botulinum toxin component is obtained from serotype A of *C. botulinum.*

24. The method according to claim 23, wherein the botulinum toxin component is present in said composition in the range of 10 to 200 U per injection.

25. The method according to claim 24, wherein the botulinum toxin component is present in said composition in the range of 30 to 200 U per injection.

26. Use of an effective amount of a botulinum toxin composition for the preparation of a sterile injectable formulation for administration by injection to an individual, at an area affected by muscle spasm, to reduce or prevent said muscle spasm,

wherein the botulinum toxin composition comprises:

a positively charged carrier comprising the amino acid sequence RKKRRQRRRG-$(K)_{15}$-GRKKRRQRRR,

a botulinum toxin component present in an amount suitable for injection, the botulinum toxin component selected from the group consisting of a botulinum toxin complex, reduced botulinum toxin complex, and botulinum toxin, and

a pharmaceutically acceptable diluent suitable for injection;

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

27. The use according to claim 26, wherein the botulinum toxin component is obtained from serotypes A, B, C, D, E, F, or G of C. *botulinum.*

28. The use according to claim 27, wherein the botulinum toxin component is a type A botulinum toxin component.

29. The use according to any one of claims 26 to 28, wherein the reduction or prevention of muscle spasm results from local muscle paralysis following injection of said composition and wherein said local muscle paralysis lasts longer than local muscle paralysis following injection of a formulation of the same dose of units ofbotulinum toxin consisting of type A botulinum toxin with accessory protein, 0.5 mg of human albumin, and 0.9 mg of sodium chloride.

30. The use according to claim 29, wherein said muscle paralysis lasts approximately twice as long as muscle paralysis following injection of a formulation of the same dose of units of botulinum toxin consisting of type A botulin um toxin with accessory protein, 0.5 mg of human albumin, and 0.9 mg of sodium chloride.

31. The use according to any one of claims 26 to 30, wherein the muscle spasm is associated with at least one condition selected from the group consisting of hemifacial spasm, adult onset spasmodic torticollis, anal fissure, blepharospasm, strabismus, temporomandibular joint

disorder, overactive bladder, facial muscle spasm, muscular cramp, muscular ache, dystonia, cervical dystonia, and dystonic contraction.

32. The use according to any one of claims 26 to 31, wherein the affected area is at least one area selected from the group consisting of the face, neck, shoulder, back, arm, elbow, hand, pelvis, leg, knee, and foot.

33. The use according to any one of claims 26 to 32, wherein said botulinum toxin component is present in said composition in an amount from 10 to 350 U per injection.

34. The use according to claim 33, wherein said botulinum toxin component is present in said composition in an amount from 30 to 250 U per injection.

35. The use according to claim 34, wherein said botulinum toxin component is present in said composition in an amount from 50 to 200 U per injection.

36. The use according to any one of claims 26 to 32, wherein said botulinum toxin component is present in said composition in an amount from 10 to 200 U per injection.

37. The use according to claim 36, wherein said botulinum toxin component is present in said composition in an amount from 30 to 200 U per injection.

38. The use according to any one of claims 26 to 32, wherein said botulinum toxin component is present in said composition in an amount of 10 U per injection.

39. The use according to any one of claims 26 to 32, wherein said botulinum toxin component is present in said composition in an amount of 30 U per injection.