[0011] Botulinum toxin type A is said to be the most lethal natural biological agent known to man. Spores of *C. botulinum* are found in soil and can grow in improperly sterilized and sealed food containers. Ingestion of the bacteria can cause botulism, which can be fatal. At the same time, the muscle-paralyzing effects of botulinum toxin have been used for therapeutic effects. Controlled administration of botulinum toxin has been used to provide muscle paralysis to treat conditions, for example, neuromuscular disorders characterized by hyperactive skeletal muscles. Conditions that have been treated with botulinum toxin include hemifacial spasm, adult onset spasmodic torticollis, anal fissure, blepharospasm, cerebral palsy, cervical dystonia, migraine headaches, strabismus, temperomandibular joint disorder, and various types of muscle cramping and spasms. More recently the muscle-paralyzing effects of botulinum toxin have been taken advantage of in therapeutic and cosmetic facial applications such as treatment of wrinkles, frown lines, and other results of spasms or contractions of facial muscles.

[0012] Topical application of botulinum toxin would provide for a safer and more desirable treatment alternative due to the painless nature of application, the larger treatment surface area that can be covered, the ability to formulate a pure toxin with higher specific activity, the reduced training necessary for applying the botulinum therapeutic, the smaller doses that would be necessary to produce the desired effect, and the lack of a requirement for large wells of toxin to reach a therapeutic clinical result. An effective means for transdermal delivery of botulinum toxin, as well as an effective means for administering botulinum toxin to treat or prevent a number of conditions that does not require injection is thus highly desirable.

SUMMARY ~~OF THE INVENTION~~

[0013] This disclosure~~invention~~ relates to new compositions comprising a botulinum toxin, more specifically to such compositions that enable the transport or delivery of a botulinum toxin through the skin or epithelium (also referred to as "transdermal delivery"), and that therefore may be used as topical applications for providing a botulinum toxin to a subject, for various therapeutic, aesthetic and/or cosmetic purposes, as described herein.

[0014] Disclosed is~~One aspect of this invention is to provide~~ a composition containing a botulinum toxin and a carrier. The carrier has a polymeric backbone with attached

positively charged branching groups. The association between the carrier and the botulinum toxin is non-covalent.

[0014a] In one aspect, there is provided a composition comprising a botulinum toxin and a positively charged carrier comprising a positively charged polymeric backbone having attached positively charged efficiency groups, wherein the positively charged carrier and the botulinum toxin non-covalently and directly associate.

[0015] Also disclosed is~~another aspect of this invention is to provide~~ a kit for administration of a botulinum toxin to a subject. The kit includes a botulinum toxin present in an effective amount for transdermal delivery thereof, and a carrier that has a polymeric backbone with attached positively charged branching groups. The association between the carrier and the botulinum toxin is non-covalent.

[0015a] In another aspect, there is provided a kit for administration of a botulinum toxin to a subject, the kit comprising a botulinum toxin, an effective amount for transdermal delivery thereof of a positively charged carrier comprising a positively charged polymeric backbone having attached positively charged efficiency groups, wherein the positively charged carrier and the botulinum toxin directly and non-covalently associate upon administration, and instructions for administering the botulinum toxin.

[0016] Also disclosed is~~Yet another aspect of this invention is to provide~~ a kit for administration of a botulinum toxin to a subject. The kit includes a device for delivering the botulinum toxin to the skin and a composition containing a carrier having a polymeric backbone with attached positively charged branching groups selected from – $(gly)_{n1}$-$(arg)_{n2}$, HIV-TAT and fragments thereof, and Antennapedia PTD, in which the subscript n1 is an integer of from 0 to about 20, and the subscript n2 is independently an odd integer of from about 5 to about 25.

[0017] This disclosure~~invention~~ also provides a method of administering a botulinum toxin to a subject involving topically applying to the skin or epithelium of the subject the botulinum toxin in conjunction with an effective amount of a carrier. The carrier has a polymeric backbone with attached positively charged branching groups, and associates non-covalently with the botulinum toxin.

[0018] The disclosure~~In one aspect, this invention~~ relates to a composition comprising a botulinum toxin (as defined herein) and a carrier comprising a positively charged "backbone" having positively charged branching or "efficiency" groups, as described herein. Most preferably the positively charged carrier is a long-chain positively charged polypeptide or a positively charged nonpeptidyl polymer, for example, a polyalkyleneimine. The disclosure~~invention~~ further relates to a method for producing a biologic effect such as muscle paralysis, reducing hypersecretion or sweating, treating neurologic pain or migraine headache, reducing muscle spasms, preventing or reducing acne, or reducing or enhancing an immune response, by topically applying an effective amount of such a composition, preferably to the skin, of a subject or patient in need of such treatment. The disclosure~~invention~~ also relates to a method for producing an aesthetic or cosmetic effect, for example by topical application of botulinum toxin to the face instead of by injection into facial muscles.

[0019] This disclosure~~invention~~ also provides kits for preparing or formulating a composition that comprises the carrier and the botulinum toxin, as well as such additional items that are needed to produce a usable formulation, or a premix that may in turn be used to produce such a formulation. Alternatively the kit comprises means for separately but in conjunction administering the botulinum toxin and the carrier to a subject.

BRIEF DESCRIPTION OF THE DRAWINGS

[0020] **Figure 1** represents the results of an experiment demonstrating efficiency of transdermal delivery of botulinum toxin using a composition of the invention comprising a peptide backbone.

[0021] **Figure 2** is a photograph depicting the state of the hind limbs of a mouse in which the area of one limb was treated with a composition of the invention and the area of the other was treated with another botulinum toxin-containing composition that did not contain a carrier according to the invention.

[0022] **Figure 3** is a photograph depicting wrinkles on subject's forehead before and after treatment with "Essentia Botox lotion" topically.

[0023] **Figure 4** shows a Mikrosil cast of the forehead (A) after topical treatment of wrinkles with "Essentia Botox Lotion", and (b) before treatment. These Mikrosil casts, which are useful because they minimize artifacts that can result from photographing the actual subject, clearly show that the untreated side has deeper wrinkles.

[0024] **Figure 5** shows photographs depicting Minor's starch/iodine test performed on subject's forehead five days after application of "Essentia Botox Lotion" (A) and a control lotion (B). The control lotion contained a positively charged polylysine backbone with a molecular weight of about 21,000 and with TAT branching groups. These pictures were taken two minutes after application.

[0025] **Figure 6** is the same as Figure 5, but except that they were taken after four minutes had elapsed. .

[0026] **Figure 7** shows the dose area used in the axillary hyperhidrosis studies. Note that the dose area extends one centimeter beyond the area of the skin covered by axillary hair.

CLAIMS

WHAT IS CLAIMED IS:

1. A formulation comprising:

a botulinum toxin,

a positively charged backbone, and

and at least one member selected from the group consisting of a partitioning agent, oligo-bridge, and polyanion bridge,

wherein the botulinum toxin is non-covalently complexed with the positively charged backbone.

2. The formulation according to claim 1, wherein the positively charged backbone further comprises branching groups.

3. The formulation according to claims 1 or 2, wherein the positively charged backbone has a molecular weight less than 21,000.

4. The formulation according to claim 3, wherein the positively charged backbone is polylysine or PEI.

5. The formulation of claim 2, wherein the branching groups are either protected oligoarginine or TAT domains.

6. The formulation according to claim 1, further comprising a viscosity modifying agent.

7. The formulation according to claim 6, wherein the viscosity-modifying agent is either hydroxypropylcellulose or Cetaphil.

8. A kit for transdermal delivery of botulinum toxin, comprising the formulation according to claim 1.

9. Use of

a botulinum toxin,

a positively charged backbone, and

and at least one member selected from the group consisting of a partitioning agent, oligo-bridge, and polyanion bridge,

wherein the botulinum toxin is non-covalently complexed with the positively charged backbone, in the manufacture of a formulation for treating wrinkles

wherein said formulation is to be applied to an area of skin of a patient, and wherein an occlusion agent is to be optionally applied afterwards.

10. Use of

a botulinum toxin,

a positively charged backbone, and

and at least one member selected from the group consisting of a partitioning agent, oligo-bridge, and polyanion bridge,

wherein the botulinum toxin is non-covalently complexed with the positively charged backbone, in the manufacture of a formulation for treating hyperhidrosis

wherein said formulation is to be applied to an area of skin of a patient, and wherein an occlusion agent is to be optionally applied afterwards.

11. The use according to claim 9, wherein the area of skin is selected from the group consisting of the face, forehead, neck, hands and feet.

12. The use according to claim 10, wherein the area of skin is selected from the axillae, forehead, back, chest, palms, dorsal portion of the hand, instep, or plantar portion of the foot.

Claims

1. A composition comprising a botulinum toxin and a positively charged carrier comprising a positively charged polymeric backbone having attached positively charged efficiency groups, wherein the positively charged carrier and the botulinum toxin non-covalently and directly associate.

2. The composition according to claim 1, wherein the botulinum toxin comprises a recombinant botulinum toxin.

3. The composition according to claim 1, wherein the botulinum toxin comprises a modified botulinum toxin.

4. The composition according to claim 1, wherein the botulinum toxin is selected from botulinum toxin serotypes A, B, C, D, E, F and G.

5. The composition according to claim 4, wherein the botulinum toxin is botulinum toxin A.

6. The composition according to claim 4, wherein the botulinum toxin is botulinum toxin B.

7. The composition according to claim 4, wherein the botulinum toxin is botulinum toxin C_1.

8. The composition according to claim 4, wherein the botulinum toxin is botulinum toxin D.

9. The composition according to claim 4, wherein the botulinum toxin is botulinum toxin E.

10. The composition according to claim 1, wherein the efficiency groups are positively charged HIV-TAT fragments that have the formula $(gly)_p$-RGRDDRRQRRR-$(gly)_q$ (SEQ ID NO: 2), $(gly)_p$-YGRKKRRQRR-$(gly)_q$ (SEQ ID NO: 3), or $(gly)_p$-RKKRRQRRR-$(gly)_q$ (SEQ ID NO: 4), wherein the subscripts p and q are each independently an integer of from 0 to 20.

11. The composition according to claim 1, wherein the positively charged efficiency groups comprise at least about 0.05% by weight of the total carrier weight.

12. The composition according to claim 1, wherein the positively charged efficiency groups comprise from about 0.5% to about 45% by weight of the total carrier weight.

13. The composition according to claim 1, wherein the positively charged efficiency groups comprise from about 0.1% to about 30% by weight of the total carrier weight.

14. The composition according to claim 1, wherein the backbone comprises a positively charged polypeptide.

15. The composition according to claim 14, wherein the backbone comprises a positively charged polylysine.

16. The composition according to claim 15, wherein the polylysine has a molecular weight of from about 10,000 to 1,500,000.

17. The composition according to claim 15, wherein the polylysine has a molecular weight of from about 25,000 to about 1,200,000.

18. The composition according to claim 15, wherein the polylysine has a molecular weight of from about 100,000 to about 1,000,000.

19. The composition according to claim 1, wherein the backbone comprises a positively charged nonpeptidyl carrier.

20. The composition according to claim 19, wherein the backbone comprises a positively charged polyalkyleneimine.

21. The composition according to claim 20, wherein the polyalkyleneimine is a polyethyleneimine.

22. The composition according to claim 21, wherein the polyethyleneimine has a molecular weight of from about 10,000 to about 2,500,000.

23. The composition according to claim 21, wherein the polyethyleneimine has a molecular weight of from about 100,000 to about 1,800,000.

24. The composition according to claim 21, wherein the polyethyleneimine has a molecular weight of from about 500,000 to about 1,400,000.

25. The composition according to claim 1 having a pH of from about 4.5 to about 6.3.

26. The composition according to claim 1, wherein the composition is stable when stored at room temperature or under refrigerated conditions.

27. The composition according to claim 1, wherein the composition is formulated to have a controlled release.

28. The composition according to claim 1, wherein the composition is a liquid.

29. The composition according to claim 1, wherein the composition is a gel.

30. The composition according to claim 1, wherein the composition is a cream, lotion or ointment.

31. The composition according to claim 1 further comprising saline.

32. The composition according to claim 1 further comprising saline and a pH buffer system.

33. A kit for administration of a botulinum toxin to a subject, the kit comprising a botulinum toxin, an effective amount for transdermal delivery thereof of a positively charged carrier comprising a positively charged polymeric backbone having attached positively charged efficiency groups, wherein the positively charged carrier and the botulinum toxin directly and non-covalently associate upon administration, and instructions for administering the botulinum toxin.

34. The kit according to claim 33 further comprising a custom applicator.

35. The kit according to claim 34, wherein the custom applicator is designed for use by a health care professional.

36. The kit according to claim 34, wherein the custom applicator is designed for self-administration by a subject.

37. The kit according to claim 33 comprising a pre-formulated composition comprising the botulinum toxin and the carrier.

38. The kit according to claim 33, wherein the botulinum toxin and the carrier are separately formulated for combining prior to administration.

39. The kit according to claim 33, wherein the botulinum toxin is contained in a device for administering the botulinum toxin to a subject via the skin.

40. The kit according to claim 39, wherein the device is a skin patch.

41. The composition according to claim 4, wherein the botulinum toxin is botulinum toxin F.

42. The composition according to claim 4, wherein the botulinum toxin is botulinum toxin G.

43. The composition according to claim 15, wherein the polylysine has a molecular weight less than 75,000.

44. The composition according to claim 43, wherein the polylysine has a molecular weight less than 30,000.

45. The composition according to claim 44, wherein the polylysine has a molecular weight less than 25,000.

46. The composition according to claim 11, wherein the polymeric backbone comprises polylysine.

47. The composition according to claim 46, wherein the polylysine has a molecular weight less than 75,000.

48. The composition according to claim 47, wherein the polylysine has a molecular weight less than 30,000.

49. The composition according to claim 48, wherein the polylysine has a molecular weight less than 25,000.

50. The composition according to claim 10, wherein at least one of the efficiency groups has the formula $(gly)_p$-RGRDDRRQRRR-$(gly)_q$ (SEQ ID NO: 2) and the subscripts p and q are independently an integer in the range of 0 to 8.

51. The composition according to claim 50, wherein the polymeric backbone comprises polylysine.

52. The composition according to claim 51, wherein the polylysine has a molecular weight less than 75,000.

53. The composition according to claim 52, wherein the polylysine has a molecular weight less than 30,000.

54. The composition according to claim 53, wherein the polylysine has a molecular weight less than 25,000.

55. The composition according to any one of claims 51-54, wherein the botulinum toxin comprises a type A, B, C, D, E, F, or G botulinum neurotoxin.

56. The composition according to claim 55, wherein the botulinum toxin is a type A botulinum neurotoxin with a molecular weight of about 150,000.

57. The composition according to claim 10, wherein at least one of the efficiency groups has the formula $(gly)_p$-YGRKKRRQRR-$(gly)_q$ (SEQ ID NO: 3) and the subscripts p and q are independently an integer in the range of 0 to 8.

58. The composition according to claim 57, wherein the polymeric backbone comprises polylysine.

59. The composition according to claim 58, wherein the polylysine has a molecular weight less than 75,000.

60. The composition according to claim 59, wherein the polylysine has a molecular weight less than 30,000.

61. The composition according to claim 60, wherein the polylysine has a molecular weight less than 25,000.

62. The composition according to any one of claims 58-61, wherein the botulinum toxin comprises a type A, B, C, D, E, F, or G botulinum neurotoxin.

63. The composition according to claim 62, wherein the botulinum toxin is a type A botulinum neurotoxin with a molecular weight of about 150,000.

64. The composition according to claim 10, wherein at least one of the efficiency groups has the formula $(gly)_p$-RKKRRQRRR-$(gly)_q$ (SEQ ID NO: 4) and the subscripts p and q are independently an integer in the range of 0 to 8.

65. The composition according to claim 64, wherein the polymeric backbone comprises polylysine.

66. The composition according to claim 65, wherein the polylysine has a molecular weight less than 75,000.

67. The composition according to claim 66, wherein the polylysine has a molecular weight less than 30,000.

68. The composition according to claim 67, wherein the polylysine has a molecular weight less than 25,000.

69. The composition according to any one of claims 65-68, wherein the botulinum toxin comprises a type A, B, C, D, E, F, or G botulinum neurotoxin.

70. The composition according to claim 69, wherein the botulinum toxin is a type A botulinum neurotoxin with a molecular weight of about 150,000.

71. The composition according to claim 1, wherein the efficiency groups attached to the polymeric backbone are present in an amount sufficient to enhance transdermal delivery of botulinum toxin relative to transdermal delivery of botulinum toxin using the same polymeric backbone but without attached efficiency groups.

72. The kit according to claim 33, wherein the efficiency groups attached to the polymeric backbone are present in an amount sufficient to enhance transdermal delivery of botulinum toxin relative to transdermal delivery of botulinum toxin using the same polymeric backbone but without attached efficiency groups.

73. The composition according to claim 1, wherein the botulinum toxin is in the form of a botulinum toxin complex.

74. The composition according to claim 50, wherein the subscripts p and q are independently an integer in the range of 2 to 5.

75. The composition according to claim 57, wherein the subscripts p and q are independently an integer in the range of 2 to 5.

76. The composition according to claim 64, wherein the subscripts p and q are independently an integer in the range of 2 to 5.