COMPOSITIONS AND METHODS FOR TOPICAL APPLICATION AND TRANSDERMAL DELIVERY OF BOTULINUM TOXINS

[0001] This application claim the benefit of priority to U.S. Provisional Patent Application No. 60/550,015, filed on March 3, 2004. U.S. Provisional Patent Application No. 60/550,015 is hereby incorporated by reference in its entirety.

BACKGROUND OF THE INVENTION

[0002] Skin protects the body's organs from external environmental threats and acts as a thermostat to maintain body temperature. It consists of several different layers, each with specialized functions. The major layers include the epidermis, the dermis and the hypodermis. The epidermis is a stratifying layer of epithelial cells that overlies the dermis, which consists of connective tissue. Both the epidermis and the dermis are further supported by the hypodermis, an internal layer of adipose tissue.

[0003] The epidermis, the topmost layer of skin, is only 0.1 to 1.5 millimeters thick (Inlander, Skin, New York, NY: People's Medical Society, 1-7 (1998)). It consists of keratinocytes and is divided into several layers based on their state of differentiation. The epidermis can be further classified into the stratum corneum and the viable epidermis, which consists of the granular melphigian and basal cells. The stratum corneum is hygroscopic and requires at least 10% moisture by weight to maintain its flexibility and softness. The hygroscopicity is attributable in part to the water-holding capacity of keratin. When the horny layer loses its softness and flexibility it becomes rough and brittle, resulting in dry skin.

[0004] The dermis, which lies just beneath the epidermis, is 1.5 to 4 millimeters thick. It is the thickest of the three layers of the skin. In addition, the dermis is also home to most of the skin's structures, including sweat and oil glands (which secrete substances through openings in the skin called pores, or comedos), hair follicles, nerve endings, and blood and lymph vessels (Inlander, Skin, New York, NY: People's Medical Society, 1-7 (1998)). However, the main components of the dermis are collagen and elastin.

[0005] The hypodermis is the deepest layer of the skin. It acts both as an insulator for body heat conservation and as a shock absorber for organ protection (Inlander, Skin, New York, NY: People's Medical Society, 1-7 (1998)). In addition,

the hypodermis also stores fat for energy reserves. The pH of skin is normally between 5 and 6. This acidity is due to the presence of amphoteric amino acids, lactic acid, and fatty acids from the secretions of the sebaceous glands. The term "acid mantle" refers to the presence of the water-soluble substances on most regions of the skin. The buffering capacity of the skin is due in part to these secretions stored in the skin's horny layer.

[0006] Wrinkles, one of the telltale signs of aging, can be caused by biochemical, histological, and physiologic changes that accumulate from environmental damage (Benedetto, International Journal of Dermatology, 38:641-655 (1999)). In addition, there are other secondary factors that can cause characteristic folds, furrows, and creases of facial wrinkles (Stegman et al., The Skin of the Aging Face Cosmetic Dermatological Surgery, 2nd ed., St. Louis, MO: Mosby Year Book: 5-15 (1990)). These secondary factors include the constant pull of gravity, frequent and constant positional pressure on the skin (i.e., during sleep), and repeated facial movements caused by the contraction of facial muscles (Stegman et al., The Skin of the Aging Face Cosmetic Dermatological Surgery, 2nd ed., St. Louis, MO: Mosby Year Book: 5-15 (1990)). Different techniques have been utilized in order potentially to mollify some of the signs of aging. These techniques range from facial moisturizers containing alpha hydroxy acids and retinol to surgical procedures and injections of neurotoxins.

[0007] One of the principal functions of skin is to provide a barrier to the transportation of water and substances potentially harmful to normal homeostasis. The body would rapidly dehydrate without a tough, semi-permeable skin. The skin helps to prevent the entry of harmful substances into the body. Although most substances cannot penetrate the barrier, a number of strategies have been developed to selectively increase the permeability of skin with variable success.

[0008] Botulinum toxins (also known as botulin toxins or botulinum neurotoxins) are neurotoxins produced by the gram-positive bacteria *Clostridium botulinum*. They act to produce paralysis of muscles by preventing synaptic transmission or release of acetylcholine across the neuromuscular junction, and are thought to act in other ways as well. Their action essentially blocks signals that normally would cause muscle spasms or contractions, resulting in paralysis.

[0009] Botulinum toxin is classified into eight neurotoxins that are serologically related, but distinct. Of these, seven can cause paralysis, namely botulinum neurotoxin serotypes A, B, C, D, E, F and G. Each of these is distinguished by neutralization with type-specific antibodies. Nonetheless, the molecular weight of the botulinum toxin protein molecule, for all seven of these active botulinum toxin serotypes, is about 150 kD. As released by the bacterium, the botulinum toxins are complexes comprising the 150 kD botulinum toxin protein molecule in question along with associated non-toxin proteins. The botulinum toxin type A complex can be produced by Clostridia bacterium as 900 kD, 500 kD and 300 kD forms. Botulinum toxin types B and C are apparently produced as only a 700 kD or 500 kD complex. Botulinum toxin type D is produced as both 300 kD and 500 kD complexes. Botulinum toxin types E and F are produced as only approximately 300 kD complexes. The complexes (i.e. molecular weight greater than about 150 kD) are believed to contain a non-toxin hemaglutinin protein and a non-toxin and non-toxic nonhemaglutinin protein. These two non-toxin proteins (which along with the botulinum toxin molecule comprise the relevant neurotoxin complex) may act to provide stability against denaturation to the botulinum toxin molecule and protection against digestive acids when toxin is ingested. Additionally, it is possible that the larger (greater than about 150 kD molecular weight) botulinum toxin complexes may result in a slower rate of diffusion of the botulinum toxin away from a site of intramuscular injection of a botulinum toxin complex.

[0010] 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. For example, it has been determined that botulinum toxin type A is 500 times more potent, as measured by the rate of paralysis produced in the rat, than is botulinum toxin type B. Additionally, botulinum toxin type B has been determined to be non-toxic in primates at a dose of 480 U/kg, about 12 times the primate LD_{50} for type A. Due to the molecule size and molecular structure of botulinum toxin, it cannot cross stratum corneum and the multiple layers of the underlying skin architecture.

[0010a] Botulism, the characteristic symptom complex from systemic botulinum toxin exposure, has existed in Europe since antiquity. In 1895, Emile P. van Ermengem first isolated the anaerobic spore-forming bacillus from raw salted pork meat obtained from post-mortem tissue of victims who died of botulism in

Belgium. Van Ermengem found the disease to be caused by an extracellular toxin that was produced by what he called Bacillus botulinus (Van Ermengem, Z Hyyg Infektionskr, 26:1-56; Rev Infect (1897)). The name was changed in 1922 to Clostridium botulinum. The name Clostridium was used to reflect the anaerobic nature of the microorganism and also its morphologic characteristics (Carruthers and Carruthers, Can J Ophthalmol, 31:389-400 (1996)). In the 1920's, a crude form of Botulinum toxin type A was isolated after additional outbreaks of food poisoning. Dr. Herman Sommer at the University of California, San Francisco made the first attempts to purify the neurotoxin (Borodic et al., Ophthalmic Plast Recostr Surg, 7:54-60 (1991)). In 1946, Dr. Edward J. Schantz and his colleagues isolated the neurotoxin in crystalline form (Schantz et al., In: Jankovi J, Hallet M (Eds) Therapy with Botulinum Toxin, New York, NY: Marcel Dekker, 41-49 (1994)). By 1949, Burgen and his associates were able to demonstrate that the Botulinum toxin blocks impulses across the neuromuscular junction (Burgen et al., J Physiol, 109:10-24 (1949)). Allan B. Scott first used botulinum toxin A (BTX-A) in monkeys in 1973. Scott demonstrated reversible ocular muscle paralysis lasting 3 months (Lamanna, Science, 130:763-772 (1959)). Soon afterwards, BTX-A was reported to be a successful treatment in humans for strabismus, blepharospasm, and spasmodic torticollis (Baron et al., In: Baron EJ, Peterson LR, Finegold SM (Eds), Bailey & Scotts Diagnostic Microbiology, St. Louis, MO: Mosby Year Book, 504-523 (1994); Carruthers and Carruthers, Adv Dermatol, 12:325-348 (1997); Markowitz, In: Strickland GT (Eds) Hunters Tropical Medicine, 7th ed. Philadelphia: W.B. Saunders, 441-444 (1991)). In 1986, Jean and Alastair Carruthers, a husband and wife team consisting of an ocuplastic surgeon and a dermatologist, began to evolve the cosmetic use of BTX-A for treatment of movement-associated wrinkles in the glabella area (Schantz and Scott, In Lewis GE (Ed) Biomedical Aspects of Botulinum, New York: Academic Press, 143-150 (1981)). The Carruthers' use of BTX-A for the treatment of wrinkles led to their seminal publication of this approach in 1992 (Schantz and Scott, In Lewis GE (Ed) Biomedical Aspects of Botulinum, New York: Academic Press, 143-150 (1981)). By 1994, the same team reported experiences with other movement-associated wrinkles on the face (Scott, Ophthalmol, 87:1044-1049 (1980)). This in turn led to the birth of the era of cosmetic BTX-A treatment.

[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

[0013] This disclosure 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 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.

[0015] Also disclosed is 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.

[0016] Further disclosed is 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 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] In one embodiment, this disclosure 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 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 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.

[0018a] In one example, there is disclosed use of 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 botulinum toxin is not covalently modified and wherein the positively charged carrier and the botulinum toxin non-covalently and directly associate;

in the manufacture of a medicament for use in treatment of:

(a) symptoms associated with urological disorders;

(b) symptoms associated with subjective or clinical hyperhidrosis;

(c) symptoms associated with subjective or clinical dystonic contractions or dystonia;

(d) symptoms associated with muscle spasm;

(e) symptoms associated with migraine headache

(f) symptoms associated with acne;

(g) symptoms associated with mucous secretion;

(h) symptoms associated with obesity;

(i) symptoms associated with inflammation;

(j) symptoms associated with psoriasis;

(k) snoring;

(l) cutaneous symptoms associated with diabetes;

(m) wound healing;

(n) symptoms associated autonomic nerve dysfunction;

(o) symptoms associated with cerebral palsy;

(p) symptoms associated with Hashimoto's thyroiditis;

(q) symptoms associated with mammary gland disorders

(r) altering hair growth;

(s) symptoms associated with parathyroid disorders;

(t) symptoms associated with movement disorders;

(u) symptoms associated with parkinson's disease;

(v) symptoms associated with tremors;

(w) symptoms associated with epilepsy;

(x) symptoms associated with inner ear disorders;

(y) symptoms associated with cholinergic-controlled secretions;

(z) symptoms associated with neurophychiatric disorders;

(aa) symptoms associated with injured muscles;

(bb) symptoms associated with ear disorders;

(cc) symptoms associated with cancer;

(dd) symptoms associated with nerve entrapment disorders; or

(ee) symptoms associated with hypercalcemia.

[0018b] In one aspect, there is provided use of 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 botulinum toxin is not covalently modified, and wherein the positively charged carrier and the botulinum toxin non-covalently and directly associate;

in the manufacture of a medicament for treating, preventing, or reducing symptoms associated with subjective or clinical hyperhidrosis in a subject in need thereof, wherein the medicament is to be topically applied to the skin or epithelium of the subject.

[0018c] In one example, there is disclosed use of 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 botulinum toxin is not covalently modified, and wherein the positively charged carrier and the botulinum toxin non-covalently and directly associate;

in the manufacture of a medicament for treating, preventing, or reducing the severity of fine lines and/or wrinkles in a subject in need thereof, wherein the medicament is to be topically applied to the skin or epithelium of the subject.

[0018d] In one example, there is disclosed use of 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 botulinum toxin is not covalently modified, and wherein the positively charged carrier and the botulinum toxin non-covalently and directly associate;

in the manufacture of a medicament for treating urologic disorder in a subject in need thereof, wherein the medicament is to be topically applied to the skin or epithelium of the subject.

[0018e] In one example, there is disclosed use of 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 botulinum toxin is not covalently modified, and wherein the positively charged carrier and the botulinum toxin non-covalently and directly associate;

in the manufacture of a medicament for treating neurologic pain; migraine headache pain or other headache pain; or relieving muscle spasm-related pain in a subject in need thereof, wherein the medicament is to be topically applied to the skin or epithelium of the subject.

[0018f] In one example, there is disclosed use of 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 botulinum toxin is not covalently modified, and wherein the positively charged carrier and the botulinum toxin non-covalently and directly associate;

in the manufacture of a medicament for enhancing or reducing an immune response. In one aspect, the present invention refers to non-therapeutic use of 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 botulinum toxin is not covalently modified, and wherein the positively charged carrier and the botulinum toxin non-covalently and directly associate; for preventing, or reducing the severity of fine lines and/or wrinkles in a subject in need thereof, wherein the composition is to be topically applied to the skin or epithelium of the subject. In another aspect, the present invention refers to use of 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 botulinum toxin is not covalently modified, and wherein the positively charged carrier and the botulinum toxin non-covalently and directly associate; in the manufacture of a medicament for treating bladder dysfunction or spasm in a subject in need thereof, wherein the medicament is to be topically applied to the skin or epithelium of the subject. In yet another aspect, the present invention refers to use of 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 botulinum toxin is not covalently modified, and wherein the positively charged carrier and the botulinum toxin non-covalently and directly associate; in the manufacture of a medicament for treating neurologic pain; or preventing or reducing migraine headache pain or other headache pain; or relieving muscle spasm-related pain in a subject in need thereof, wherein the medicament is to be topically applied to the skin or epithelium of the affected area of the subject.

[0019] This disclosure 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.

[0027] **Figure 8a** represents the results of an experiment demonstrating efficiency of botulinum toxin therapeutically delivered across intact skin as a topical agent using a short peptidyl carrier for the treatment of axillary hyperhidrosis on human subjects. Graph depicts significant reduction in amount of sweat (mg per 5 minutes) measured gravimetrically 4 weeks after treatment with Botox plus a short peptidyl carrier or carrier alone. Results are 4 week values as ratio to baseline value for same group, with significance determined by Wilcoxon analysis with $P<0.05$. N=10 patients. **Figure 8b** represents the results of an experiment demonstrating efficiency of botulinum toxin therapeutically delivered across intact skin as a topical agent using a short peptidyl carrier for the treatment of axillary hyperhidrosis on human subjects. Graph depicts significant reduction in amount of sweat (mg per 5 minutes) measured gravimetrically 4 weeks after treatment with Botox plus a short peptidyl carrier or carrier alone. Results are treatment values as ratio to control value for both timepoints, with significance determined by Wilcoxon analysis with $P<0.05$. N=10 patients.

[0028] **Figure 9** shows photographs depicting Minor's starch/iodine test before and after treatement with "Essentia Botox lotion" topically for the treatment of axillary hyperhidrosis. Starch/iodine test at Baseline vs. 2 week is shown where right axilla was treated with "Essentia Botox lotion" (a and c) and left axilla was applied with the control (b and d) for subject #12. These photographs illustrate typical benefits observed after treatment with carrier+botox in starch iodine. Although some crossover is observed on the control side (consistent with 25% reduction in gravimetric data), significant reductions are afforded with treatment (consistent with 65% reduction in gravimetric data on treated side).

DETAILED DESCRIPTION OF THE INVENTION

[0029] This invention provides compositions and methods for delivery, particularly transdermal delivery, of a botulinum toxin by topical application of an appropriate formulation.

[0030] According to the present invention, a positively charged carrier molecule having efficiency groups, as described herein, has been found suitable as a transport system for a botulinum toxin, enabling that toxin to be administered

transdermally to muscles and/or other skin-associated structures. The transport occurs without covalent modification of the botulinum toxin.

[0031] By "positively charged" 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" 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" 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.

[0032] Generally, the positively-charged carrier (also referred to as a "positively charged backbone") is typically a linear 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. Preferably, the positively charged backbone itself will not have a defined enzymatic or therapeutic biologic activity. The linear backbone is a 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. In all aspects of the present invention, the association between the carrier and the biologically active agent is by non-covalent interaction, non-limiting examples of which include ionic interactions, hydrogen bonding, van der Waals forces, or combinations thereof.

[0033] 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 of from about 10,000 to about 1,500,000, more preferably from about 25,000 to about 1,200,000, most preferably from about 100,000 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. Alternatively, the backbone can 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 α-carbon locations.

[0034] 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 (-$NHCH_2$-) or the reversed methyleneamino (-CH_2NH-) groups, keto-methylene (-$COCH_2$-) groups, phosphinate (-PO_2RCH_2-), phosphonamidate and phosphonamidate ester (-PO_2RNH-), reverse peptide (-NHCO-), trans-alkene (-CR=CH-), fluoroalkene (-CF=CH-), dimethylene (-CH_2CH_2-), thioether (-CH_2S-), hydroxyethylene (-$CH(OH)CH_2$-), methyleneoxy (-CH_2O-), tetrazole (CN_4), sulfonamido (-SO_2NH-), methylenesulfonamido (-$CHRSO_2NH$-), reversed sulfonamide (-$NHSO_2$-), 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.

[0035] In each of the backbones provided above, sidechain groups can be appended that carry a positively charged group. For example, the sulfonamide-linked backbones (-SO_2NH- and –$NHSO_2$-) can have sidechain groups attached to the nitrogen atoms. Similarly, the hydroxyethylene (-$CH(OH)CH_2$-) 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.

[0036] In one embodiment, the positively charged backbone is a polypeptide having branching groups (also referred to as efficiency groups). As used herein, an efficiency group or branching group 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 branching or efficiency groups include -$(gly)_{n1}$-$(arg)_{n2}$, HIV-TAT or fragments thereof, or the protein transduction domain 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. Still further preferred are those embodiments in which the HIV-TAT fragment has the formula $(gly)_p$-RGRDDRRQRRR-$(gly)_q$, $(gly)_p$-YGRKKRRQRRR-$(gly)_q$ or $(gly)_p$-RKKRRQRRR-$(gly)_q$ wherein the subscripts p and q are each independently an integer of from 0 to 20 and the fragment is attached to the backbone via either the C-terminus or the N-terminus of the fragment. Preferred HIV-TAT fragments are those in which the subscripts p and q are

each independently integers of from 0 to 8, more preferably 2 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. Preferably the positively charged carrier includes side-chain positively charged branching groups in an amount of at least about 0.05 %, as a percentage of the total carrier weight, preferably from about 0.05 to about 45 weight %, and most preferably from about 0.1 to about 30 weight %. For positively charged branching groups having the formula $-(gly)_{n1}-(arg)_{n2}$, the most preferred amount is from about 0.1 to about 25 %.

[0037] In another embodiment, the backbone portion is a polylysine and positively charged branching groups are attached to the lysine sidechain amino groups. The polylysine may have a molecular weight of from about 10,000 to about 1,500,000, preferably from about 25,000 to about 1,200,000, and most preferably from about 100,000 to about 1,000,000. It can be any of the commercially available (Sigma Chemical Company, St. Louis, Missouri, 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 branching groups or efficiency groups include, for example, –gly-gly-gly-arg-arg-arg-arg-arg-arg-arg (-Gly_3Arg_7) or HIV-TAT. In another preferred embodiment the positively charged backbone is a long chain polyalkyleneimine such as a polyethyleneimine, for example, one having a molecular weight of about 1,000,000.

[0038] The positively charged backbones or carrier molecules comprising polypeptides or polyalkyleneimines, having the branching groups described above, are novel compounds and form an aspect of this invention.

[0039] In one embodiment of the invention, only a positively charged carrier that has positively charged branching groups is necessary for transdermal delivery of the botulinum toxin. In certain embodiments, the positively charged carrier is a polypeptide (e.g., lysine, arginine, ornithine, homoarginine, and the like) having

multiple positively charged side-chain groups, as described above. Preferably, the polypeptide has a molecular weight of at least about 10,000. In another embodiment, the positively charged carrier is a nonpeptidyl polymer such as a polyalkyleneimine having multiple positively charged side-chain groups having a molecular weight of at least about 100,000. Such polyalkyleneimines include polyethylene- and polypropyleneimines. In either instance, for use as the sole necessary agent for transdermal delivery the positively charged carrier molecule includes positively charged branching or efficiency groups, comprising $-(gly)_{n1}-(arg)_{n2}$, , 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 from about 7 to about 17, and most preferably from about 7 to about 13, HIV-TAT or fragments thereof, or Antennapedia PTD or a fragment thereof. Preferably the side-chain or branching groups have the general formula $-(gly)_{n1}-(arg)_{n2}$ as described above. Other preferred embodiments are those in which the branching or efficiency groups are HIV-TAT fragments that have the formula $(gly)_p$-RGRDDRRQRRR-$(gly)_q$, $(gly)_p$-YGRKKRRQRRR-$(gly)_q$, or $(gly)_p$-RKKRRQRRR-$(gly)_q$, wherein the subscripts p and q are each independently an integer of from 0 to 20 and the fragment is attached to the carrier molecule via either the C-terminus or the N-terminus of the fragment. The side branching groups can have either the D- or L-form (R or S configuration) at the center of attachment. Preferred HIV-TAT fragments are those in which the subscripts p and q are each independently integers of from 0 to 8, more preferably 2 to 5. Other preferred embodiments are those in which the branching groups are Antennapedia PTD groups or fragments thereof that retain the group's activity. These are known in the art, for instance, from Console et al., J. Biol. Chem. 278:35109 (2003). Preferably, the positively charged carrier includes side-chain positively charged branching groups in an amount of at least about 0.05 %, as a percentage of the total carrier weight, preferably from about 0.05 to about 45 weight %, and most preferably from about 0.1 to about 30 weight %. For positively charged branching groups having the formula $-(gly)_{n1}-(arg)_{n2}$, the most preferred amount is from about 0.1 to about 25 %.

[0040] In another embodiment, the carrier is a polylysine with positively charged branching groups attached to the lysine side-chain amino groups. The polylysine used in this particularly embodiment can be any of the commercially

available (Sigma Chemical Company, St. Louis, Missouri, USA, e.g.) 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. However, preferably the polylysine has MW of at least about 10,000. Preferred positively charged branching groups or efficiency groups include, for example, --gly-gly-gly-arg-arg-arg-arg-arg-arg-arg (-Gly_3Arg_7), HIV-TAT or fragments of it, and Antennapedia PTD or fragments thereof.

[0041] 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. 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, more preferably less than 30,000, and most preferably, less than 25,000. When the carrier is a relatively short branched polylysine or PEI backbone, however, the backbone will have a molecular weight less than 60,000, more preferably less than 55,000, and most preferably less than 50,000. If, however, partitioning agents as described herein are included in the composition, the molecular weight of the branched polylysine and PEI backbones may be up to 75,000, while the molecular weight of the linear polylysine and PEI backbones may be up to 150,000.

[0042] The term "botulinum toxin" as used herein is meant to refer to any of the known types of botulinum toxin, whether produced by the bacterium or by recombinant techniques, as well as any such types that may be subsequently discovered including engineered variants or fusion proteins. As mentioned above, at the present time, 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 available from Sigma-Aldrich and from Metabiologics, Inc. (Madison, Wisconsin), 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® and of Ipsen having the trademark DYSPORT®, and type B is contained in preparations of Elan having the trademark MYOBLOC®.

[0043] The botulinum toxin used in the compositions of this invention can alternatively be 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 chain relative to naturally occurring or recombinant native botulinum toxins. For instance, the botulinum toxin may be a modified neurotoxin(e.g., a neurotoxin which has at least one of its amino acids deleted, modified or replaced, as compared to a native, or 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. The botulinum toxin may be any of the botulinum toxin complexes produced by the bacterium, as described above. Alternatively, the botulinum toxin may be a toxin prepared using recombinant or synthetic chemical techniques (e.g. a recombinant peptide, a fusion protein, or a hybrid neurotoxin, as prepared from subunits or domains of different botulinum toxin serotypes (see U.S. patent 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. Additionally, the botulinum toxin may be in the form of a botulinum toxin precursor, which may itself be non-toxic, for instance a nontoxic zinc protease that becomes toxic on proteolytic cleavage.

[0044] This invention also contemplates the general use of combinations and mixtures of botulinum toxins, although due to their differing nature and properties, mixtures of botulinum toxin serotypes are not generally administered at this time in the health-care or cosmetic industries.

[0045] Compositions of this invention are preferably in the form of products to be applied to the skin or epithelium of subjects or patients, i.e. humans or other mammals in need of the particular treatment. The term "in need" is meant to include both pharmaceutical or health-related needs, for example, treating conditions involving undesirable facial muscle spasms, as well as cosmetic and subjective needs,

for example, altering or improving the appearance of facial tissue. In general the compositions are prepared by mixing the botulinum toxin with the carrier, and usually with one or more additional pharmaceutically acceptable carriers or excipients. In their simplest form they may contain a simple aqueous pharmaceutically acceptable carrier or diluent, such as buffered saline. However, the compositions may contain other ingredients typical in topical pharmaceutical or cosmeceutical compositions, including a dermatologically or pharmaceutically acceptable carrier, vehicle or medium, (i.e. a carrier, vehicle or medium that is compatible with the tissues to which they will be applied.) The term "dermatologically or 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, and particularly in cosmetics and dermatology. The compositions also may include a quantity of a small anion, preferably a polyvalent anion, for example, phosphate, aspartate, or citrate.

[0046] In terms of their form, compositions of this invention may include solutions, emulsions (including microemulsions), suspensions, creams, lotions, gels, powders, or other typical solid or liquid compositions used for application to skin and other tissues where the compositions may be used. Such compositions may contain, in addition to the botulinum toxin and carrier, other ingredients typically used in such products, such as antimicrobials, moisturizers and hydration agents, penetration agents, preservatives, emulsifiers, natural or synthetic oils, solvents, surfactants, detergents, , emollients, antioxidants, fragrances, fillers, thickeners, waxes, odor absorbers, dyestuffs, coloring agents, powders, , and optionally including anesthetics, anti-itch additives, botanical extracts, conditioning agents, darkening or lightening agents, glitter, humectants, mica, minerals, polyphenols, silicones or derivatives thereof, sunblocks, vitamins, and phytomedicinals.

[0047] In particularly preferred embodiments, the compositions include gelling agents and/or viscosity-modifying agents. These agents are generally added to increase the viscosity of the composition, so as to make the application of the composition easier and more accurate. Additionally, these agents help to prevent the aqueous botulinum toxin/carrier solution from drying out, which tends to cause a

decrease in the activity of the botulinum toxin. Particularly preferred agents are those that are uncharged and do not interfere with the botulinum toxin activity or the efficiency of the toxin-carrier complexes in crossing skin. The gelling agents may be certain cellulose-based gelling agents, such as hydroxypropylcellulose (HPC) for example. In some embodiments, the botulinum toxin/carrier complex is formulated in a composition having 2-4% HPC. Alternatively, the viscosity of a solution containing a botulinum toxin/carrier complex may be altered by adding polyethylene glycol (PEG). In other embodiments, the botulinum toxin/carrier solution is combined with pre-mixed viscous agents, such as Cetaphil® moisturizer.

[0048] The compositions of this invention may optionally include partitioning agents. As used herein, a "partitioning agent" is any substance or additive that has the property of preventing or minimizing unwanted or uncontrolled aggregation of the botulinum toxin with the carriers of this invention. Partitioning agents may be useful, for example, when a concentrated botulinum toxin solution must be employed due to volume constraints. In these cases, the partitioning agent keeps the botulinum toxin dispersed, thereby preventing aggregation of the toxin that would otherwise occur without the partioning agent. Generally, a partitioning agent is (1) non-irritating, (2) does not destroy the botulinum toxin, (3) does not confer any increase in permeability, (4) affords reliable and stable particle sizes, (5) is uncharged, and (6) does not interfere with complexes of the toxin and the transdermal carrier. An example of a suitable partitioning agent is ethanol (EtOH). In preferred embodiments, the EtOH is less than 20% of the composition, and most preferably, less than 5% of the composition.

[0049] By way of example, if volume constraints require reconstituting 100 U of botulinum toxin in 0.5 ml of solution, rather than 2.5 ml, one typically observes that the botulinum toxin will exhibit undesirable aggregation, and thus lowered activity. However, by adding 1% EtOH as a dispersing agent, fully activity is maintained even after 24 hours at this concentration. As another example, Botox® at 1.0 ml 0.9% NaCl reconstitution has full activity, while reconstitution at 0.5 ml in 1% and 5% EtOH plus 0.9% NaCl produces solutions with full activity.

[0050] In certain embodiments of this invention, oligo- or polyanion bridges are added to the botulinum toxin compositions to improve the complexation of the toxin with a positively charged backbone carrier. As is well known in the art,

botulinum toxin is actually a complex of different proteins, some of which are positively charged, and some of which are negatively charged. Because the exact distribution of the components of the toxin varies depending on the source of the toxin, it may be that botulinum toxin from certain sources has a lower propensity for complexation with the positively charged backbones described herein. However, one aspect of this invention is the discovery that by adding an oligo- or polyanion bridge to such botulinum toxins, the efficiency and efficacy of topical administration is increased dramatically. Suitable examples of such oligo-/polyanion bridges include sodium phosphate (5%), PBS or 5% poly-L-aspartate (e.g., with a MW of 3000).

[0051] Compositions according to this invention may be in the form of controlled-release or sustained-release compositions, wherein the botulinum toxin and the carrier are encapsulated or otherwise contained within a material such that they are released onto the skin in a controlled manner over time. The botulinum toxin and 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 carrier may be encapsulated together (e.g., in the same capsule) or separately (in separate capsules).

[0052] Using the compositions described herein, botulinum toxin can be delivered 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 applied so as to administer an effective amount of the botulinum toxin. The term "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 or aesthetic effect, but that implicitly is a safe amount, i.e. one that is low enough to avoid serious side effects. Desired effects include the relaxation of certain muscles with the aim of, for instance, decreasing the appearance of fine lines and/or wrinkles, especially in the face, or adjusting facial appearance in other ways such as widening the eyes, lifting the

corners of the mouth, or smoothing lines that fan out from the upper lip, or the general relief of muscular tension. The last-mentioned effect, general relief of muscular tension, can be effected in the face or elsewhere. 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. Through the use of the positively charged carriers of this invention, a botulinum toxin can be administered transdermally to a subject for treating conditions such as undesirable facial muscle or other muscular spasms, hyperhidosis, acne, or conditions elsewhere in the body in which relief of muscular ache or spasms is desired. The botulinum toxin is administered topically for transdermal delivery to muscles or to other skin-associated structures. The administration may be made, for example, to the legs, shoulders, back (including lower back), axilla, palms, feet, neck, groin, dorsa of the hands or feet, elbows, upper arms, knees, upper legs, buttocks, torso, pelvis, or any other part of the body where administration of the botulinum toxin is desired.

[0054] Administration of botulinum toxin may also be carried out to treat other conditions, including treating of neurologic pain, prevention or reduction of migraine headache or other headache pain, prevention or reduction of acne, prevention or reduction of dystonia or dystonic contractions (whether subjective or clinical), prevention or reduction of symptoms associated with subjective or clinical hyperhidosis, reducing hypersecretion or sweating, reducing or enhancing immune response, or treatment of other conditions for which administration of botulinum toxin by injection has been suggested or performed.

[0055] Administration of botulinum toxin or other therapeutic proteins described herein may also be carried out for immunization-related purposes. Surprisingly, administration of botulinum toxin described herein may be carried out to reduce immune responses. More specifically, this invention allows a botulinum toxin to be delivered by an altered route of administration, thereby changing the complex antigen presentation of the agent. In this way, the invention may be useful to reduce immune response to antigens to botulinum toxin, and to facilitate repeat administration without immune-related reduction in activity. Alternatively, the complex can be prepared and applied topically to enhance an immune response, for example to provide immunizations respecting various proteins, for example, for

childhood immunizations without injections. For use in connection with immune-related activity, an "effective amount" refers to an amount of the botulinum toxin sufficient to allow a subject to mount an immune response to the botulinum toxin after application or a series of applications of it.

[0056] 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 periodic treatments over time. For transdermal delivery of botulinum toxin for the purposes mentioned above, a composition as described above is applied topically to the skin at a location or locations where the effect is desired. In embodiments were an aqueous botulinum toxin/carrier solution is applied directly to the skin, it is preferable to cover the treated area (e.g., with Cetaphil® moisturizer) or occlude the treated area with a barrier (e.g., Telfa), in order to prevent the solution from drying out, which would lead to a decrease in toxin activity. Because of its nature, most preferably the amount of botulinum toxin applied should be applied with care, at an application rate and frequency of application that will produce the desired result without producing any adverse or undesired results. Accordingly, for instance, topical compositions of the invention should be applied at a rate of from about 1U to about 20,000U, preferably from about 1U to about 10,000U botulinum toxin per cm^2 of skin surface. Higher dosages within these ranges could preferably be employed in conjunction with controlled release materials, for instance, or allowed a shorter dwell time on the skin prior to removal.

[0057] Proper preparation of the skin surface prior to the application of the botulinum toxin/carrier composition is important for maintaining the efficacy of the solution. For example, the introduction of surfactants on the surface of the skin for the purpose of cleaning off surface oils on the skin prior to application is surprisingly counterproductive, because the surfactants appear to destroy the activity of the botulinum toxin. This occurs even if the skin is subsequently washed with water several times before application of the botulinum toxin/carrier solution. Even extremely gentle surfactants, such as those found in baby wipes, appear to cause this phenomenon. Accordingly, in preferred methods of administering the compositions of this invention, the skin is pre-cleaned using water alone. Washing with only water also appears to improve the transdermal transport of the botulinum toxin moderately.

[0058] Additionally, the skin may be stripped to reduce the stratum corneum layer prior to application of the botulinum toxin/carrier complex. In principle, the process of stripping the skin should lead to enhanced efficiency of transdermal transport of botulinum toxin. However, the method used to strip the skin is important. For example, acetone-mediated reduction of the stratun corneum layer in humans or animals appears to reduce the activity of subsequently applied botulinum toxin. In contrast, tape stripping (i.e., applying tape on the surface of the skin and then removing the tape) appears to allow deeper penetation of the botulinum toxin and dosage reduction in both mouse models and humans. It is presumed that abrasion of the skin surface (e.g, via the use of abrasive pads) would cause a similar effect as tape stripping.

[0059] This invention also comprises devices for transdermal transmission of a composition that contains botulinum toxin and a carrier that has a positively charged backbone with attached branching groups as defined herein. Such devices may be as simple in construction as a skin patch, or may be more complicated devices that include means for dispensing and monitoring the dispensing of the composition, and optionally means for monitoring the condition of the subject (e.g., monitoring the reaction of the subject to the substances being dispensed).

[0060] It should be noted that the choice of materials for the construction of the device is important. Preferred materials for the construction of delivery devices are those that do not lead to a loss of activity of the botulinum toxin/carrier solution, either through degradation or unwanted adsorption of the botulinum toxin on a surface of the device. Such undesired behavior has been observed, for example, when botulinum toxin/carrier in an aqueous solution contacts polypropylene surfaces, but not when the botulinum toxin/carrier solution contacts polyvinyl chloride (PVC) surfaces.

[0061] Generally, the compositions can be pre-formulated and/or pre-installed in a device or can be prepared later, for example using a kit that houses the two ingredients (botulinum toxin and carrier) separately but provides means for combining them at or prior to the time of application. The amount of carrier molecule or the ratio of it 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.

[0062] In general, the invention also comprises a method for administering a botulinum toxin to a subject or patient in need thereof. The method includes comprising topically administering an effective amount of the botulinum toxin in conjunction with a carrier having a positively charged backbone with attached positively charged branching groups, as described herein. By "in conjunction with" is meant that the two components (botulinum toxin and carrier) are administered in a combination procedure, which may involve either combining them in a composition, which is subsequently administered to the subject, or administering them separately, but in a manner such that they act together to provide the requisite delivery of an effective amount of the therapeutic protein. For example, a composition containing the carrier may first be applied to the skin of the subject, followed by applying a skin patch or other device containing the botulinum toxin. The botulinum toxin may be incorporated in dry form in a skin patch or other dispensing device, while the positively charged carrier may be applied to the skin surface before application of the patch so that the two act together, resulting in the desired transdermal delivery. Thus, the two substances (carrier and botulinum toxin) act in combination or perhaps interact to form a composition or combination in situ. Accordingly, the invention also comprises a kit that includes both a device for dispensing botulinum toxin via the skin and a liquid, gel, cream or the like that contains the carrier or backbone, and that is suitable for applying to the skin or epithelium of a subject. Kits for administering the compositions of the inventions, either under direction of a health care professional or by the patient or subject, may also include a custom applicator suitable for that purpose.

[0063] The compositions, kits and methods of this invention allow for the delivery of a more pure botulinum toxin with higher specific activity and potentially improved pharmacokinetics. In addition, the carrier can act as a stabilizer, reducing the need for foreign accessory proteins (e.g., human serum albumin ranging from 400-600 mg or recombinant serum albumin ranging from 250-500 mg) and/or polysaccharide stabilizers, and can afford beneficial reductions in immune responses to the botulinum toxin. In addition, the compositions are suitable for use in physiologic environments with pH ranging from about 4.5 to about 6.3, and may thus

have such a pH. The compositions according to this invention may be stored either at room temperature or under refrigerated conditions.

[0064] The following are representative examples of the invention. They demonstrate delivery of functional botulinum neurotoxin complexes across skin without requiring covalent modification of the neurotoxin to be delivered.

EXAMPLES

EXAMPLE 1.

Transport of a botulinum toxin in vivo using a peptidyl carrier.

[0065] This experiment demonstrates the use of a peptidyl carrier to transport a large complex containing an intact labeled protein botulinum toxin across intact skin after a single time administration relative to controls.

Backbone selection:

[0066] The positively charged backbone was assembled by conjugating –Gly_3Arg_7 to polylysine (MW 112,000) via the carboxyl of the terminal glycine to free amines of the lysine side chains at a degree of saturation of 18% (i.e., 18 out of each 100 lysine residues is conjugated to a –Gly_3Arg_7). The modified backbone was designated "KNR". The control polycation was unmodified polylysine (designated "K", Sigma Chemical Co., St. Louis, MO) of the same size and from the same lot.

Therapeutic agent:

[0067] Botox® brand of botulinum toxin A (Allergan) was selected for this experiment. It has a molecular weight of approximately 150,000.

Preparation of samples

[0068] The botulinum toxin was reconstituted according to the manufacturer's instructions. An aliquot of the protein was biotinylated with a calculated 12-fold molar excess of sulfo-NHS-LC biotin (Pierce Chemical). The labeled product was designated "Btox-b".

[0069] In each case, an excess of polycation was employed to assemble a final complex that has an excess of positive charge as in delivery of highly negative large nucleotide complexes. A net neutral or positive charge prevents repulsion of the

protein complex from highly negative cell surface proteoglycans and extracellular matrix. Btox-b dose was standardized across all groups, as was total volume and final pH of the composition to be applied topically. Samples were prepared as follows:

[0070] Group labeled "JMW-7": 2.0 units of Btox-b per aliquot (i.e. 20 U total) and peptidyl carrier KNR at a calculated MW ratio of 4:1 were mixed to homogeneity and diluted to 200 microliters with phosphate buffered saline. The resulting composition was mixed to homogeneity with 1.8 ml of Cetaphil® lotion and aliquoted in 200 microliter portions.

[0071] Group labeled "JMW-8": 2.0 units of Btox-b per aliquot (i.e. 20 U total) and K at a MW ratio of 4:1 were mixed to homogeneity and diluted to 200 microliters with phosphate buffered saline. The resulting composition was mixed to homogeneity with 1.8 ml of Cetaphil and aliquoted in 200 microliter portions.

Animal experiments to determine transdermal delivery efficiencies after single time treatment with peptidyl carriers and labeled Btox:

[0072] Animals were anesthetized via inhalation of isoflurane during application of treatments. After being anesthetized, C57 black 6 mice (n=4 per group) underwent topical application of a metered 200 microliter dose of the appropriate treatment applied to the cranial portion of dorsal back skin (selected because the mouse cannot reach this region with mouth or limbs). Animals did not undergo depilation. At 30 minutes after the initial treatment, mice were euthanized via inhalation of CO_2, and treated skin segments were harvested at full thickness by blinded observers. Treated segments were divided into three equal portions; the cranial portion was fixed in 10% neutral buffered formalin for 12-16 hours then stored in 70% ethanol until paraffin embedding. The central portion was snap-frozen and employed directly for biotin visualization by blinded observers as summarized below. The treated caudal segment was snap frozen for solubilization studies.

[0073] Biotin visualization was conducted as follows. Briefly, each section was immersed for 1 hour in NeutrAvidin® buffer solution. To visualize alkaline phosphatase activity, cross sections were washed in saline four times then immersed in NBT/BCIP (Pierce Scientific) for 1 hour. Sections were then rinsed in saline and photographed in entirety on a Nikon E600 microscope with plan-apochromat lenses.

Data handling and statistical analysis:

[0074] Total positive staining was determined by blinded observer via batch image analysis using Image Pro Plus software (Media Cybernetics, Silver Spring, MD) and was normalized to total cross-sectional area to determine percent positive staining for each. Mean and standard error were subsequently determined for each group with analysis of significance at 95% confidence in one way ANOVA repeated measures using Statview software (Abacus, Berkeley, CA).

Results:

[0075] The mean cross-sectional area that was positive for biotinylated botulinum toxin was reported as percent of total area after single-time topical administration of Btox-b with either KNR ("EB-Btox") or K ("nl"). The results are presented in the following Table 1 and are illustrated in Figure 1. In Figure 1, the area positive for label was determined as percent of total area after three days of once daily treatment with "EB-Btox" which contained Btox-b and the peptidyl carrier KNR and "nl", which contained Btox-b with polycation K as a control. Mean and standard error are depicted for each group.

Table 1. Mean and standard error for labeled botulinum toxin area as percent of total cross-section after single time topical administration of Btox-b with KNR (JMW-7) or K (JMW-8) for 30 minutes.

Group	Mean	Std. Error
JMW-7	33	5.333334
JMW-8	8.666667	0.333334

P=0.0001 (Significant at 99%)

EXAMPLE 2.

Therapeutic efficacy of a topical botulinum toxin preparation with a peptidyl carrier.

[0076] Example 1 demonstrated that the peptidyl transdermal carrier allowed efficient transfer of botulinum toxin after topical administration in a murine model of intact skin. However, this experiment did not indicate whether the complex protein botulinum toxin was released in a functional form after translocation across skin. The

following experiment was thus constructed to evaluate whether botulinum toxin can be therapeutically delivered across intact skin as a topical agent using this peptidyl carrier (again, without covalent modification of the protein).

[0077] The positively charged backbone was again assembled by conjugating $-Gly_3Arg_7$ to polylysine (MW 112,000) via the carboxyl of the terminal glycine to free amines of the lysine side chains at a degree of saturation of 18% (i.e., 18 out of each 100 lysine residues is conjugated to a $-Gly_3Arg_7$). The modified backbone was designated "KNR". Control polycation was unmodified polylysine (designated "K", Sigma Chemical Co., St. Louis, MO) of the same size and from the same lot. The same botulinum toxin therapeutic agent was used as in Example 1, and was prepared in the same manner. Samples were prepared as follows:

[0078] Group labeled "JMW-9": 2.0 units of botulinum toxin per aliquot (i.e. 60 U total) and peptidyl carrier KNR at a calculated MW ratio of 4:1 were mixed to homogeneity and diluted to 600 microliters with phosphate buffered saline. The resulting composition was mixed to homogeneity with 5.4 ml of Cetaphil and aliquoted in 200 microliter portions.

[0079] Group labeled "JMW-10": 2.0 units of botulinum toxin per aliquot (i.e. 60 U total) and K at a MW ratio of 4:1 were mixed to homogeneity and diluted to 600 microliters with phosphate buffered saline. The resulting composition was mixed to homogeneity with 5.4 ml of Cetaphil and aliquoted in 200 microliter portions.

[0080] Group labeled "JMW-11": 2.0 units of botulinum toxin per aliquot (i.e. 60 U total) without polycation was diluted to 600 microliters with phosphate buffered saline. The resulting composition was mixed to homogeneity with 5.4 ml of Cetaphil and aliquoted in 200 microliter portions.

Animal experiments to determine therapeutic efficacy after single time treatment with peptidyl carriers and botulinum toxin:

[0081] Animals were anesthetized via inhalation of isoflurane during application of treatments. After being anesthetized, C57 black 6 mice (n=4 per group) underwent topical application of metered 400 microliter dose of the appropriate treatment applied uniformly from the toes to the mid-thigh. Both limbs were treated, and treatments were randomized to either side. Animals did not undergo depilation. At 30 minutes after the initial treatment, mice were evaluated for digital abduction

capability according to published digital abduction scores for foot mobility after botulinum toxin administration [Aoki, KR. *A comparison of the safety margins of botulinum neurotoxin serotypes A, B, and F in mice.* Toxicon. 2001 Dec; 39(12): 1815-20]. Mouse mobility was also subjectively assessed.

Data handling and statistical analysis:

[0082] Digital abduction scores were tabulated independently by two blinded observers. Mean and standard error were subsequently determined for each group with analysis of significance at 95% confidence in one way ANOVA repeated measures using Statview software (Abacus, Berkeley, CA).

Results:

[0083] Mean digital abduction scores after single-time topical administration of botulinum toxin with KNR ("JMW-9"), K ("JMW-10") or diluent without polycation ("JMW-11"), are presented in table 2 and illustrated in the representative photomicrograph of figure 2 below. The peptidyl carrier KNR afforded statistically significant functional delivery of the botulinum toxin across skin relative to both controls, which were comparable to one another. Additional independent repetitions (total of three independent experiments all with identical conclusions in statistically significant paralysis from topical botulinum toxin with KNR but not controls) of the present experiment confirmed the present findings and revealed no significant differences between topical botulinum toxin with or without K (i.e. both controls). Interestingly, the mice consistently ambulated toward a paralyzed limb (which occurred in 100% of treated animals and 0% of controls from either control group). As shown in Figure 2, a limb treated with botulinum toxin plus the control polycation polylysine or with botulinum toxin without polycation ("Botox alone") can mobilize digits (as a defense mechanism when picked up), but the limbs treated with botulinum toxin plus the peptidyl carrier KNR ("Essentia Botox lotion") could not be moved.

Table 2 : Digital abduction scores 30 minutes after single-time topical application of botulinum toxin with the peptidyl carrier KNR ("JMW-9"), with a control polycation K ("JMW-10"), or alone ("JMW-11").

Group	Mean	Std. Error

JMW-9	3.333	0.333
JMW-10	0.333	0.333
JMW-11	0.793	0.300

P=0.0351 (Significant at 95%)

Conclusions:

[0084] This experiment serves to demonstrate that the peptidyl transdermal carrier can transport a therapeutically effective amount of botulinum therapeutic across skin without covalent modification of the therapeutic. The experiment also confirms that botulinum toxin does not function when applied topically in controls.

EXAMPLE 3.

Therapeutic efficacy of a topical botulinum toxin preparation with a nonpeptidyl carrier.

[0085] This experiment demonstrates the performance of a non-peptidyl carrier in the invention.

Methods:

Backbone selection:

[0086] The positively charged backbone was assembled by conjugating –Gly_3Arg_7 to polyethyleneimine (PEI) MW 1,000,000 via the carboxyl of the terminal glycine to free amines of the PEI side chains at a degree of saturation of 30% (i.e., 30 out of each 100 lysine residues is conjugated to a –Gly_3Arg_7). The modified backbone was designated "PEIR" to denote the large nonpeptidyl carrier. Control polycation was unmodified PEI (designated "PEI", Sigma Chemical Co., St. Louis, MO) of the same size and from the same lot. The same botulinum toxin therapeutic agent was used as in example 1.

[0087] Botulinum toxin was reconstituted from the Botox product according to the manufacturer's instructions. In each case, an excess of polycation was employed to assemble a final complex that had an excess of positive charge as in delivery of highly negative large nucleotide complexes. A net neutral or positive charge prevents repulsion of the protein complex from highly negative cell surface proteoglycans and extracellular matrix. The botulinum toxin dose was standardized

across all groups as was total volume and final pH of the composition to be applied topically. Samples were prepared as follows:

[0088] Group labeled "AZ": 2.0 units of botulinum toxin per aliquot (i.e. 60 U total) and the nonpeptidyl carrier PEIR in ultrapure form at a calculated MW ratio of 5:1 were mixed to homogeneity and diluted to 600 microliters with phosphate buffered saline. The resulting composition was mixed to homogeneity with 5.4 ml of Cetaphil and aliquoted in 200 microliter portions.

[0089] Group labeled "BA": 2.0 units of botulinum toxin per aliquot (i.e. 60 U total) and PEI at a charge ratio of 5:1 were mixed to homogeneity and diluted to 600 microliters with phosphate buffered saline. The resulting composition was mixed to homogeneity with 5.4 ml of Cetaphil and aliquoted in 200 microliter portions.

Animal experiments to determine therapeutic efficacy after single time treatment:

[0090] Animals were anesthetized via inhalation of isoflurane during application of treatments. After being anesthetized, C57 black 6 mice (n=3 per group) underwent topical application of metered 400 microliter dose of the appropriate treatment applied uniformly from the toes to the mid-thigh. Both limbs were treated, and treatments were randomized to either side. Animals did not undergo depilation. At 30 minutes after the initial treatment, mice were evaluated for digital abduction capability according to published digital abduction scores for foot mobility after botulinum toxin administration [Aoki, KR. *A comparison of the safety margins of botulinum neurotoxin serotypes A, B, and F in mice.* Toxicon. 2001 Dec; 39(12): 1815-20]. Mouse mobility was also subjectively assessed.

Data handling and statistical analysis:

[0091] Digital abduction scores were tabulated independently by two blinded observers. Mean and standard error were subsequently determined for each group with analysis of significance at 95% confidence in one way ANOVA repeated measures using Statview software (Abacus, Berkeley, CA).

Results:

[0092] Mean digital abduction scores after single-time topical administration of botulinum toxin with ultrapure PEIR ("AZ"), or control polycation PEI ("BA"), are

presented in table 3 and repetition presented as table 4 (single independent repetition for this experiment). The nonpeptidyl carrier PEIR afforded statistically significant functional delivery of botulinum toxin across skin relative to controls. As before, animals were observed to walk in circles toward the paralyzed limbs.

Table 3. Digital abduction scores 30 minutes after single-time topical administration of Botox with ultrapure PEIR ("AZ"), or control polycation PEI ("BA"). Mean and standard error are presented.

Group	Mean	Std. Error
BA	0.833	0.307
AZ	3.917	0.083

P=0.0002 (Significant at 99%)

Table 4. Digital abduction scores 30 minutes after single-time topical administration of Botox with ultrapure PEIR ("AZ1"), or control polycation PEI ("BA1"). Mean and standard error are presented.

Group	Mean	Std. Error
BA1	0.333	0.211
AZ1	3.833	0.167

P=0.0001 (Significant at 99%)

Conclusions:

[0093] This experiment demonstrated that the nonpeptidyl transdermal carrier can transport therapeutic doses of botulinum toxin across skin without prior covalent modification of the botulinum toxin. These findings complement those with peptidyl transfer agents. The option of using a nonpeptidyl or a peptidyl carrier to achieve the therapeutic effect will allow tailoring to specific circumstances, environments, and methods of application and add to the breadth of the transdermal delivery platform of this invention.

EXAMPLE 4.

Therapeutic efficacy of a topical botulinum toxin preparation with peptidyl carrier for forehead hyperhidrosis and wrinkles.

[0094] This experiment demonstrates that botulinum toxin can be therapeutically delivered across intact skin as a topical agent using this peptidyl carrier for the treatment of forehead hyperhidrosis and wrinkles on human subjects.

Experimental Procedure for Forehead Hyperhidrosis and Wrinkles Study:

[0095] Baseline and post-treatment photographs of the subject's forehead were taken on a blue background using a Nikon D70 camera with Nikon TTL Macro-speedlight SB29s flash (Nikon, Inc. USA).

[0096] Baseline and post-treatment videos of the subject's forehead were taken on a blue background using a Sony Digital Handycam camcorder.

[0097] Minor's starch/iodine test was performed to visualize sweat production using 10% topical povidone iodine solution (Walgreen Co., Deerfield, Illinois) and Kingsford's 100% corn starch (ACH Food Companies, Inc., Memphis, Tennessee). The subject's forehead was painted with an iodine solution using sterile cotton balls (Johnson & Johnson Consumer Product Company, Skillman, New Jersey), and then allowed to dry completely. The area was lightly dusted with starch powder using sterile cotton balls. The sweat was induced with physical activity at ambient room temperature. Dark blue-black spots appeared as the sweat dissolved the iodine and reacted with starch powder. Baseline and post-treatment photographs of iodine-starch test were taken on a blue background using a Nikon D70 camera with Nikon TTL Macro-speedlight SB29s flash. The subject's forehead was cleansed with 70% EtOH and then deionized water.

[0098] The subject's predefined treatment area on the forehead was prepared by non-invasive tape stripping method for stratum corneum prior to treatment application. Precut tape was applied to the treatment area with firm pressure for few seconds. It was removed rapidly by pulling on one corner of the tape. The second tape was carefully applied to the same area immediately after the first tape was removed. Tape-stripping was repeated 3-5 times.

Treatment preparation:

[0099] The Botox reconstituting solution of sterile 0.9% sodium chloride (Abbott Laboratories, North Chicago, Illinois) plus 5% EtOH plus 5% short chained polyaspartate solution solution labeled A-3C (Donlar BioPolymer, Inc. Bedford Park,

Illinois) was prepared (i.e., for every 1.0 milliter solution, 900 microliters of sterile 0.9% sodium chloride plus 50 microliters of 100% EtOH plus 50 microliters of short chained polyaspartate solution). Kn21T was prepared at 1 milligram/milliliter concentration with 0.9% sodium chloride plus 5% EtOH (i.e., 500 microliters of Kn21T was aliquoted and 25 microliters of 100% EtOH was added). As used herein, Kn21T refers to a positively charged polylysine backbone having a molecular weight of 21,000 and TAT branching groups. 100 units of Botox (Allergan, Irvine, CA) was reconstituted with 1.0 milliliters of reconstituting solution using sterile 3 ml latex free syringe with $18_G1 1/2$ (Becton Dickinson & Co., Franklin Lakes, New Jersey). The reconstituted Botox was carefully mixed by inversion 8 times. 200 units of Botox were used for each subject. The "Essentia Botox solution" was prepared with 200 units of Botox and Kn21T plus 5% EtOH (ie. 2.0 milliters of Botox was added to 500 microliters of Kn21T plus 25 microliters of 100% EtOH) and sat at room temperature for 5 minutes for the complexes to form.

[0100] The control solution was prepared with reconstituting solution and Kn21T plus 5% EtOH (ie. 2.0 ml of reconstituting solution was added to 500 microliters of Kn21T plus 25 microliters of 100% EtOH) and kept at room temperature.

Treatment application:

[0101] The subject reclined on a table with protective covering around the eyes, face, and upper body. The treatment was applied evenly to the subject's forehead using a pipette and massaged into the skin in circular motion with fingers while wearing powder-free, nitrile gloves. The treatment area was covered with a thin layer of Cetaphil® moisturizing cream (Galderma, Fort Worth, TX) and incubated for 60 minutes. After 60 minute incubation, the treatment was removed with sterile gauze pads. The gauze pads and gloves were discarded in a biohazard bag.

Results:

[0102] Figure 3 depicts significant reduction in wrinkle length depth and width after topical treatment with Peptidyl carrier and botulinum combination. This experiment confirms that topically applied botulinum toxin, when combined with transdermal carrier, can afford significant muscular paralysis to afford a cosmetic

effect. Figure 4 is a Mikrosil cast of the treated skin (A) versus untreated skin (B). Wrinkles are visible on the cast of the untreated skin.

[0103] Figures 5 and 6 show the results of the Minor's starch/iodine test. Figure 5 shows photos taken two minutes after application, with panel (A) corresponding to the side treated with Essentia Botox Lotion, and panel (B) corresponding to the side treated with a control lotion containing Kn21T carrier alone. Figure 6 is the same as Figure 5, except that it was taken at four minutes. Note the more pronounced coloration on the control lotion side, indicating that the skin on that side is secreting more sweat. Also note that the sweating starts earlier on the untreated side.

Conclusions:

[0104] This example demonstrates that topically applied complexes of botulinum toxin can afford significant aesthetic benefit in reducing fine and coarse wrinkles. This transepithelial effect further confirms that muscle paralysis can be accomplished with appropriate carriers after topical application of botulinum toxin complexes such as those disclosed herein. This example thus indicates that topical application of botulinum toxin can lead to relief from muscle spasms such as blepharospasm or torticollis as well as relief of muscle spasm-related pain such as lower back pain.

EXAMPLE 5

Therapeutic efficacy of a topical botulinum toxin preparation with peptidyl carrier for axillary hyperhidrosis.

[0105] This experiment demonstrates whether botulinum toxin can be therapeutically delivered across intact skin as a topical agent using this peptidyl carrier for the treatment of axillary hyperhidrosis on human subjects (n=10 axillae per group with one axilla treated and one control per patient in a randomized double-blind fashion).

Inclusion criteria for axillary hyperhidrosis study:

[0106]

- Age: 18 years or older
- Healthy volunteers

- Informed consent given and signed by the volunteer
- Subject willing to follow instruction and return for follow-up visits.
- Subject has presence of pre-existing, subjective hyperhidrosis
- Subject is NOT pregnant or planning on becoming pregnant within the next 3 months
- Subject lives and/or work in San Francisco or near study area
- Subject has NOT had treatment for underarm sweating within the past 6 months
- Subject is NOT planning on having treatment for underarm sweating within the next 3 months

Gravimetric measurement procedures:

[0107] As a part of the gravimetric measurement procedure, the subjects were first acclimated to the testing area. Specifically, each subject sat for 15 minutes at a room temperature of 72-77 °F in the resting position.

[0108] **Axillae preparation:** (Powder-free, nitrile gloves were worn for the following procedures.) The subject changed into disposable cape and bra (if a woman) or took off all upper body garments (if a man) so as to expose both of the axillae fully. The dose area was predetermined to be the area covered by hair bearing skin, plus an area extending 1 cm beyond the hair bearing skin at each axilla. The dose area was cleaned with a pre-wet sterile gauze pad from a 50 ml conical tube by wiping with 5 long strokes from top to bottom in the same direction using one side of the gauze. This step was repeated *three more times* with a clean pre-wet gauze pad each time while being careful not to irritate or abrade the skin. The gauze pads were discarded in the trash. The same wash procedure was repeated for the other axilla. The axilla was dried with dry sterile gauze by using firm padding motion from top to bottom of the axilla while being careful not to irritate or abrade the skin. Then, the axilla was further dried by placing a filter paper under the axillary crease and allowing the filter to dwell in the test site for 5 minutes following the procedure for gravimetric assessment. The patient sat with their arms against his/her body in a resting position. The filter papers were discarded in the trash. The subject was allowed to rest for 1 minute without axilla manipulation prior to the first gravimetric assessment.

[0109] **Sweat production measurement (gravimetric measurement):** (A new pair of powder-free, nitrile gloves was donned prior to these measurements). The subject held his or her hands together at the back of head to expose axillae fully, while being partially reclined (about 45 degrees). A pre-weighed filter paper was removed from a conical storage tube and placed under the subject's axilla with the tip of the filter aligning with the center of axillary crease line. The filter paper held in place by using fingers while the subject relaxed arms to the side of the body. The subject sat with both arms held tightly against his/her trunk for five minutes. The timing started when the filter papers were securely placed under both of the axillae. Both axillae were measured simultaneously. After 5 minutes, the filter papers were removed from the axillae and placed back into the same respective conical tubes. The filter paper placed first was removed first. The caps of the conical tubes were screwed tightly to prevent the evaporation of the sweat from the tube. The sweat production was repeated two more times at one-minute intervals.

Minor's starch/iodine test:

[0110] The subject held his/her hands together at the back of head to expose the axillae fully. The iodine solution was painted onto the axilla area predetermined as before with a sterile gauze pad and allowed to air-dry. When the iodine had completely dried, a thin layer of starch was padded onto the area covered by iodine with cotton balls. The iodine was allowed to air-dry before the application of starch in order to reduce false positive and background. The subject then sat with both arms held tightly against his/her trunk. After 5 minutes, the subject raised his/her arms and held hands together at the back of head to expose axillae fully. Photographs of each axilla with left and right axilla and the date clearly labeled were taken. The axillae were cleaned with 70% EtOH and then with sterile deionized water.

Treatment preparation:

[0111] Kn21pr was prepared at 1 milligram/milliliter concentration with saline plus 5% EtOH (i.e., 500 microliters of Kn21pr was aliquoted and 25 microliters of 100% EtOH was added). As used herein, Kn21pr refers to a positively charged polylysine backbone with a molecular weight of 21,000 and branching groups comprising protected oligoarginine. 100 units of Botox® (Allergan, Irvine, CA) was reconstituted with 0.75 milliliters of 0.9% sodium choride (Abbott Laboratories,

North Chicago, Illinois) using sterile 3 ml latex free syringe with $18_G1 1/2$ (Becton Dickinson and Company, Franklin Lakes, New Jersey). The reconstituted Botox® was carefully mixed by inversion 8 times. 200 units of Botox® were used for each subject. The treatment solution was prepared with 200 units of Botox® and Kn21pr plus 5% EtOH (i.e., 1.5 milliters of Botox® was added to 500 microliters of Kn21pr plus 25 microliters of 100% EtOH) and kept at room temperature for 5 minutes to allow the complexes to form. After a 5-minute incubation period, approximately 1.0 milliters of 4% HPC (hydroxypropylcellulose) (with 1% EtOH) was added and mixed gently and thoroughly with a small metal spatula. The homogenous treatment solution was transferred into a 3 ml syringe and syringe tip cap. (Becton Dickinson and Company, Franklin Lakes, New Jersey).

[0112] The control solution was prepared with 0.9% sodium chloride and Kn21pr plus 5% EtOH (ie.1.5 milliters of 0.9% sodium chloride was added to 500 microliters of Kn21pr plus 25 microliters of 100% EtOH) and sat at room temperature for five minutes. After incubation, approximately 1.0 milliters of 4% HPC (with 1% EtOH) was added and mixed gently and thoroughly with a small metal spatula. The homogenous control solution was transferred into a 3 ml syringe and syringe tip cap.

Treatment application (wear powder-free, nitrile gloves):

[0113] The subject held his/her hands together with interlocking fingers and placed them on the back of the head to fully exposure the subject's axillae. Then, the subject reclined in a chair to an angle of about 45 degrees. As shown in Figure 7, the dose area was visually mapped out (i.e. 1 cm beyond the hair bearing skin) for application. The dose area were checked for dryness. The syringe tip cap was removed from the labeled syringe marked "L" for left and "R" for right, and prepared for application onto the subject's axilla. The treatment solution was spread evenly around the dose area with a syringe and massaged into the skin with fingers for 1 minute. The subject then placed his/her arms down along the side of the body and incubated for 60 minutes. After 60 minute incubation, the treatment was cleaned with sterile gauze pads. The gauze pads and gloves were discarded in a bio-hazard bag. The subject was discharged.

Results:

[0114] Essentia topical BT reduced sweating by 65% and illustrated in Figure 8a. Sweat production 4 weeks after treatment (randomized by side) with Kn21pr backbone alone (control) or kn21pr backbone plus 200U Botox (ratio to baseline). Statistical analyses by Wilcoxon signed ranks using NPSS with P as noted and significance at $P<0.05$. [n=10 subjects].

[0115] **Second comparison:** Baseline vs. 4 weeks (See Figure 8b). Sweat production (mg per 5 minutes) 4 weeks after axillary treatment (randomized by side) with kn21pr backbone alone (control) or kn21pr backbone plus 200U Botox (ratio of treatment to control) presented in Table 6. Statistical analyses by Wilcoxon signed ranks using NPSS with P as noted and significance at $P<0.05$. (Pr T p=0.0217) [n=10].

[0116] Figure 9 shows photographs depicting Minor's starch/iodine test before and after treatement with "Essentia Botox lotion" topically for the treatment of axillary hyperhidrosis. Starch/iodine test at Baseline vs. 2 week is shown where right axilla was treated with "Essentia Botox lotion" (a and c) and left axilla was applied with the control (b and d) for subject #12. These photographs illustrate typical benefits observed after treatment with carrier+botox in starch iodine. Although some crossover is observed on the control side (consistent with 25% reduction in gravimetric data), significant reductions are afforded with treatment (consistent with 65% reduction in gravimetric data on treated side).

Conclusions:

[0117] This example confirms that topical application of botulinum complexes formed according to the invention disclosed herein readily afford therapeutic benefit in reduction of sweating in a cohort of patients with hyperhidrosis – subjective or quantitative. This effect in reducing sweating has also been afforded in the forehead case presented above and in palmar/plantar application when combined with a glove to limit spread of formulation during dwell time. This transepithelial delivery of botulinum toxin complexes for therapeutic benefit confirms further that the approach can be extended to other cases where SNAP function or acetylcholine signals are crucial such as bladder dysfunction or spasm, gastrointestinal applications, or sebaceous gland secretions for smell reduction or acne prevention/treatment.

WHAT IS CLAIMED IS:

1. Use of 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 botulinum toxin is not covalently modified, and wherein the positively charged carrier and the botulinum toxin non-covalently and directly associate;

in the manufacture of a medicament for treating, preventing, or reducing symptoms associated with subjective or clinical hyperhidrosis in a subject in need thereof, wherein the medicament is to be topically applied to the skin or epithelium of the subject.

2. Non-therapeutic use of 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 botulinum toxin is not covalently modified, and wherein the positively charged carrier and the botulinum toxin non-covalently and directly associate;

for preventing, or reducing the severity of fine lines and/or wrinkles in a subject in need thereof, wherein the composition is to be topically applied to the skin or epithelium of the subject.

3. Use of 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 botulinum toxin is not covalently modified, and wherein the positively charged carrier and the botulinum toxin non-covalently and directly associate;

in the manufacture of a medicament for treating bladder dysfunction or spasm in a subject in need thereof, wherein the medicament is to be topically applied to the skin or epithelium of the subject.

4. Use of 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 botulinum toxin is not covalently modified, and wherein the positively charged carrier and the botulinum toxin non-covalently and directly associate;

in the manufacture of a medicament for treating neurologic pain; or preventing or reducing migraine headache pain or other headache pain; or relieving muscle spasm-related pain in a subject in need thereof, wherein the medicament is to be topically applied to the skin or epithelium of the affected area of the subject.

5. The use of a composition according to any one of claims 1-4, wherein the one or more positively charged efficiency groups is selected from:

$-(gly)_{n1}-(arg)_{n2}$ (SEQ ID NO. 1) in which the subscript n1 is an integer of from 0 to 20, and the subscript n2 is independently an odd integer of from 5 to 25;

Antennapedia PTD and fragments thereof retaining Antennapedia PTD activity; HIV-TAT; and

fragments of HIV-TAT having the amino acid sequences $(gly)_p$-RGRDDRRQRRR-$(gly)_q$ (SEQ ID NO. 2), $(gly)_p$-YGRKKRRQRRR-$(gly)_q$ (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 selected from a range of 0 to 20; a range of 0 to 8; or a range of 2 to 5.

6. The use of a composition according to claim 5, wherein the one or more positively charged efficiency groups are $-(gly)_{n1}-(arg)_{n2}$ (SEQ ID NO. 1) in which the subscript n1 is an integer of from 0 to 20 and the subscript n2 is independently an odd integer of from 5 to 25.

7. The use of a composition according to claim 6, wherein the subscript n1 is an integer of from 0 to 8.

8. The use of a composition according to claim 6, wherein the subscript n1 is an integer of from 2 to 5.

9. The use of a composition according to claim 6, wherein the subscript n2 is an odd integer of from 7 to 17.

10. The use of a composition according to claim 6, wherein the subscript n2 is an odd integer from 7 to 13.

11. The use of a composition according to claim 5, wherein the one or more positively charged efficiency groups are selected from $(gly)_p$-RGRDDRRQRRR-$(gly)_q$ (SEQ ID NO. 2), $(gly)_p$-YGRKKRRQRRR-$(gly)_q$ (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.

12. The use of a composition according to claim 11, $(gly)_p$-RKKRRQRRR-$(gly)_q$ (SEQ ID NO. 4), wherein the subscripts p and q are each independently an integer of from 0 to 20.

13. The use of a composition according to any one of claims 1-4, wherein the botulinum toxin is selected from botulinum toxin serotypes A, B, C, D, E, F and G or comprises a recombinant botulinum toxin or comprises a fusion protein.

14. The use of a composition according to claim 13, wherein the botulinum toxin is botulinum toxin A.

15. The use of a composition according to any one of claims 1-4, wherein the positively charged efficiency groups comprise at least 0.05 % by weight of the total carrier weight.

16. The use of a composition according to claim 15, wherein the positively charged efficiency groups comprise from 0.5% to 45% by weight of the total carrier weight.

17. The use of a composition according to claim 16, wherein the positively charged efficiency groups comprise from 0.1% to 30% by weight of the total carrier weight.

18. The use of a composition according to any one of claims 1-4, wherein the backbone comprises a positively charged polypeptide.

19. The use of a composition according to claim 18, wherein the positively charged polypeptide is polylysine.

20. The use of a composition according to claim 19, wherein the polylysine has a molecular weight from 10,000 to 1,500,000.

21. The use of a composition according to any one of claims 1-4, wherein the backbone comprises a positively charged nonpeptidyl carrier.

22. The use of a composition according to claim 21, wherein the positively charged nonpeptidyl carrier is a polyalkyleneimine.

23. The use of a composition according to claim 22, wherein the positively charged nonpeptidyl carrier is a polyethyleneimine.

24. The use of a composition according to claim 23, wherein the polyethyleneimine has a molecular weight from 10,000 to 2,500,000.

25. The use of a composition according to any one of claims 1-4, wherein the composition is suitable for topical application to the skin or epithelium having a pH of from 4.5 to 6.3, wherein the composition is optionally a controlled release composition, a liquid composition, cream, lotion, ointment or a gel composition, or a pharmaceutical composition or a cosmetic composition.

26. The use of a composition according to claim 25, wherein the composition is stable when stored at room temperature or under refrigerated conditions.

27. The use of a composition according to claim 25, wherein the composition is formulated to have a controlled release.

28. The use of a composition according to claim 25, wherein the composition is a liquid.

29. The use of a composition according to claim 25, wherein the composition is a gel.

30. The use of a composition according to claim 25, wherein the composition is a cream, lotion or ointment.

31. The use of a composition according to claim 25, the composition further comprising saline or saline and a pH buffer system.

32. The use of a composition according to any one of claims 1-4, wherein the one or more positively charged efficiency groups comprises $(gly)_p$-RKKRRQRRR-$(gly)_q$ (SEQ ID NO. 4), wherein the botulinum toxin is botulinum toxin A, wherein the backbone comprises a positively charged polylysine having a molecular weight from 10,000 to 1,500,000 and wherein the composition is suitable for topical application to the skin or epithelium having a pH of from 4.5 to 6.3.