(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
12 June 2003 (12.06.2003)

PCT

(10) International Publication Number
WO 03/047589 A1

(51) International Patent Classification[7]: A61K 31/505

(21) International Application Number: PCT/US02/36412

(22) International Filing Date:
14 November 2002 (14.11.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
2764-2001 15 November 2001 (15.11.2001) CL

(71) Applicant *(for all designated States except US)*: **MICRO ALGAE CORPORATION** [US/US]; 350 E. Las Olas Boulevard, Suite 1700, Fort Lauderdale, FL 33301 (US).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: **LAGOS, Nestor, Antonio, Lagos** [CL/CL]; 602 Peru Avenue, Department 1104, Recoleta, Santiago (CL).

(74) Agents: **AMOS, Ahaji** et al.; Thompson Coburn, LLC, One U.S. Bank Plaza, St. Louis, MO 63101 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 03/047589 A1

(54) Title: PHARMACEUTICAL COMPOSITIONS CONTAINING 3,4-PROPINOPERHYDROPURINES AND USES THEREOF FOR BLOCKING NEURONAL TRANSMISSION

(57) Abstract: Pharmaceutical compositions comprising tricyclic 3,4-propinoperhydropurines and uses thereof for blocking neuronal transmission are provided.

PHARMACEUTICAL COMPOSITIONS CONTAINING 3,4-PROPINOPERHYDROPURINES AND USES THEREOF FOR BLOCKING NEURONAL TRANSMISSION.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

Not applicable.

REFERENCE TO MICROFICHE APPENDIX

Not applicable.

FIELD OF THE INVENTION

This invention relates to pharmaceutical compositions containing heterocyclic guanidine-type compounds and uses thereof for blocking neuronal transmission. More specifically, this invention relates to tricyclic 3,4-propinoperhydropurines and uses thereof for blocking neuronal transmission.

BACKGROUND OF THE INVENTION

The presence of wrinkles in the neck and face of people are seen as negative aesthetic effects by social groups. These marks reflect face aging and increase the subjective awareness of the age of people. Since the beginning of civilization, natural or synthetic chemical compounds have been used and procedures have been developed (i.e. plastic surgery) to alleviate this problem. For example, plastic surgeons and cosmetic centers have been experimenting with and using Botulin A toxin as a pharmaceutical preparation that produces facial rejuvenation by removing face wrinkles. Botulin A toxin is a neurotoxin that acts by chemiodenervation, or blocking the presynaptic release of the neurotransmitter acetylcholine in the neuromuscular plate, thus interfering with neuromuscular transmission, paralysing the muscle and preventing its contraction for a period of up to 4 months. Applied locally in the face of people, its effect is a facial rejuvenation that appears within 7-10 days after the toxin is applied, and has a duration of approximately 4 months. Botulin A toxin has been used for the treatment of diseases associated with muscular spasm, focal dystonia, sphincter relaxation (achalasia and anal fissure), hyperhydrosis and urinary bladder relaxation.

While Botulin A toxin is effective as a facial rejuvenate, it is an enzyme that is inherently unstable. This instability makes its use and handling complicated and less desirable. In fact, it requires freezing before using and must be used within four hours of opening the container. Because it is an enzyme, Botulin A toxin also generates antibodies that prevent its use in consecutive injections and can also induce an allergic response. In addition, its results are delayed 7-10 days, which is undesirable for patients wanting an immediate result. Accordingly, a need exists for a facial rejuvenate that is stable, fast-acting and that is not an enzyme.

SUMMARY OF THE INVENTION

Disclosed herein are compositions and methods and in particular, pharmaceutical compositions for interfering with neuronal transmission comprising an effective amount of at least one tricyclic 3,4-propinoperhydropurine.

Disclosed herein are preparations for facial rejuvenation comprising an effective amount of the composition of the invention and a facial cream.

Disclosed herein are methods of interfering with neuronal transmission comprising contacting a neuron with an effective amount of the pharmaceutical compositions of the invention.

Disclosed herein are methods of interfering with muscle contracting comprising contacting a muscle with an effective amount of the composition of the invention.

In a first aspect, there is provided a pharmaceutical composition for interfering with neuronal transmission, the composition consisting of an effective amount of at least two compounds selected from Gonyautoxin 1, Gonyautoxin 2, Gonyautoxin 3, Gonyautoxin 4 and Gonyautoxin 5 and a pharmaceutically acceptable carrier.

In a second aspect, there is provided a use of the composition as defined above, for the manufacture of a medicament for interfering with neuronal transmission.

In a third aspect, there is provided a use of the composition as defined above, for the manufacture of a medicament for interfering with muscle contraction.

In a fourth aspect, there is provided a use of the composition as defined above, for the manufacture of a medicament for the treatment of anal fissure.

In a fifth aspect, there is provided a use of a composition including at least one compound selected from Gonyautoxin 1, Gonyautoxin 2, Gonyautoxin 3, Gonyautoxin 4 and Gonyautoxin 5, for the manufacture of a medicament.

In a sixth aspect, there is provided a use of a composition including at least one compound selected from Gonyautoxin 1, Gonyautoxin 2, Gonyautoxin 3, Gonyautoxin 4 and Gonyautoxin 5, for the manufacture of a medicament for the treatment of anal fissure.

In a seventh aspect, there is provided a use of a composition including at least one compound selected from Gonyautoxin 1, Gonyautoxin 2, Gonyautoxin 3, Gonyautoxin 4 and Gonyautoxin 5, for the manufacture of a medicament for interfering with neuronal transmission.

In an eighth aspect, there is provided a use of a composition including at least one compound selected from Gonyautoxin 1, Gonyautoxin 2, Gonyautoxin 3, Gonyautoxin 4 and Gonyautoxin 5, for the manufacture of a medicament for interfering with muscle contraction.

In a ninth aspect, there is provided a method of reducing wrinkles including the step of administering a cosmetic formulation including the composition as defined above.

In a tenth aspect, there is provided a method for facial rejuvenation including the step of administering a cosmetic formulation including the composition as defined above.

DETAILED DESCRIPTION OF THE DRAWINGS

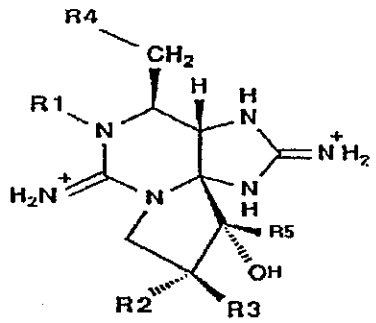
Figure 1 is an illustration of an application pattern for the treatment of a human face.

DETAILED DESCRIPTION OF THE INVENTION

In accordance with the present invention, it has been discovered that compositions comprising heterocyclic guanidine-type compounds, and more specifically tricyclic 3,4-propinoperhydropurines, can be used for many cosmetic or clinical applications, without any surgery, side effects, allergies, immune rejection or hematoma. The compositions of the invention may be used to treat conditions including, but not limited to blepharospasm, strabismus, focal dystonia, sphinceter relaxation (achalasia and anal fissure), hyperhydrosis, urinary bladder relaxation, muscular spasm-related pain management, muscular spasms,

improved wound healing and facial wrinkling. In accordance with the present invention, muscular relaxation is immediate, usually occurring in less than five minutes.

The compositions of the invention comprise an effective amount of at least one tricyclic 3,4-propinoperhydropurine represented by the following structure:



wherein R_1 and R_5 are independently H or OH; R_2 and R_3 are independently H or OSO_3; and R_4 is independently $COONH_2$, OH, H, $COONHSO_3$ or $COOCH_3$; and a pharmacologically acceptable carrier. These tricyclic 3,4-propinoperhydropurines may be purified from dinoflagellates, cyanobacterias and also may be accumulated by highly contaminated mollusks, which are also temporary muscular relaxants when locally injected. Any pharmacologically acceptable carrier may be used, including but not limited to water. The compounds of the invention are generally diluted in a solution of acetic acid or 0.09% sodium chloride.

As used herein, "an effective amount" is that amount sufficient to interfere with neuronal transmission by blocking the presynaptic release of the neurotransmitter acetylcholine in the neuromuscular plate, thus interfering with transmission, paralysing the muscle and preventing it from contracting, or producing a relaxation of contracted muscles. For example, an effective amount of the compositions of the invention may be a 100 to 800 microliter dose of forty units per milliliter solution of tricyclic 3,4-propinoperhydropurines.

The unit of activity is the amount of the composition of the invention necessary to block the muscular contractions of the crural biceps of a mouse leg for 1.5 to 2.0 hours. The compounds of the invention are generally diluted with a 10mM (millimolar), pH 4 solution of acetic acid. In one specific embodiment of the invention, the compositions of the invention comprise 40 units per milliliter of a 10mM, pH 4 solution of acetic acid.

In one embodiment of the invention, and with reference to Table 1 below, the pharmaceutical compositions of the invention comprise Saxitoxin. In a second embodiment of the invention, the pharmaceutical compositions of the invention comprise Gonyautoxin 2. In a third embodiment of the invention, the pharmaceutical compositions of the invention comprise Gonyautoxin 3. In a forth embodiment of the invention, the pharmaceutical compositions of the invention comprise Gonyautoxin 4. In a fifth embodiment of the invention, the pharmaceutical compositions of the invention comprise Gonyautoxin 5. In a sixth embodiment of the invention, the pharmaceutical compositions of the invention comprise neoSaxtoxin. In a seventh embodiment of the invention, the pharmaceutical compositions of the invention comprise Gonyautoxin 1. In an eighth embodiment of the invention, the pharmaceutical compositions of the invention comprise Decarbamoyl-Saxitoxin.

Table 1 - Tricyclic 3,4-propinoperhydropurines

Compound	R_1	R_2	R_3	R_4	R_5
Saxitoxin	H	H	H	$COONH_2$	OH
neoSaxitoxin	OH	H	H	$COONH_2$	OH
Decarbamoyl-Saxitoxin	OH	H	H	OH	OH
Gonyautoxin 1	OH	H	OSO^-_3	$COONH_2$	OH
Gonyautoxin 2	H	H	OSO^-_3	$COONH_2$	OH
Gonyautoxin 3	H	OSO^-_3	H	$COONH_2$	OH
Gonyautoxin 4	OH	OSO^-_3	H	$COONH_2$	OH
Gonyautoxin 5	H	H	H	$COONHSO^-_3$	OH

In specific embodiments of the invention, the pharmaceutical compositions comprise a mixture of tricyclic 3,4-propinoperhydropurines. The compounds are purified and used individually and/or mixed together. In one preferred embodiment, the pharmaceutical compositions of the invention comprise a mixture of Gonyautoxin 2, Gonyautoxin 3, and Saxitoxin. In another preferred embodiment, the pharmaceutical compositions of the invention comprise a mixture of Gonyautoxin 4, Gonyautoxin 1, Gonyautoxin 5, Gonyautoxin 3 and Gonyautoxin 2. In yet another preferred embodiment, the pharmaceutical compositions of the invention comprise neoSaxitoxin and Gonyautoxin 2. In still another preferred embodiment, the pharmaceutical compositions of the invention comprise a mixture of Saxitoxin, neoSaxitoxin, Decarbamoyl-Saxitoxin, Gonyautoxin 3 and Gonyautoxin 2. It should be understood by those of skill in the art that other mixtures and combinations of tricyclic 3,4-propinoperhydropurines are within the scope of this invention.

In one embodiment of the invention, the compounds of the invention are used in combination with an effective amount of Botulin A toxin. In this embodiment, the pharmaceutical compositions of the invention comprise an effective amount of Botulin A

toxin and an effective amount of at least one tricyclic 3,4-propinoperhydropurine. The combination may be used in any cosmetic or clinical application in which the compounds of the invention, or Botulin A toxin are used, for example, blepharospasm, strabismus, focal dystonia, sphincter relaxation (achalasia and anal fissure), hyperhydrosis, urinary bladder relaxation, muscular spasm-related pain management, muscular spasms, improved wound healing, and facial wrinkle removal.

These pharmaceutical compositions of the invention may be used for many cosmetic and clinical applications including, but not limited to blepharospasm, strabismus, focal dystonia, sphincter relaxation (achalasia and anal fissure), hyperhydrosis, urinary bladder relaxation, muscular spasm-related pain management, muscular spasms, improved wound healing, and facial wrinkle removal. Generally, the pharmaceutical compositions of the invention are applied locally in the form of preparations. To form preparations, an effective amount of the pharmaceutical compositions of the invention are added to a facial cream complete with its coadjuvants. Unlike Botulin A toxin, these preparations are stable at room temperature, do not require refrigeration, are sterilizable, do not generate antibodies, are not peptide in nature, act immediately, and may be injected repeatedly without any side effects.

Without being bound by theory, when applied locally, these compounds carry out their antispasmodic action by blocking the spreading of nervous impulse, or neuronal transmission, by reversibly binding to the sole biological molecular receptor, i.e. the voltage gated sodium channel, present in all neurons and excitable cells. By binding to this channel, there is no entry of sodium to the neuronal cell; depolarization does not occur and, therefore, propagation of the impulse is stopped. This action mechanism blocks the presynaptic release of the neurotransmitter acetylcholine in the neuromuscular plate, thus interfering with neuromuscular transmission, paralysing the muscle and preventing it from contracting, or producing a relaxation of muscles contracted by pathological problems. This mechanism is

particularly efficient for cosmetic purposes, as it does not let some facial muscles contract, all of them associated with and responsible for the formation of wrinkles, thus producing the sought-after effect of facial rejuvenation.

The pharmaceutical preparations of the invention are applied locally around the muscle that is to be paralysed or prevented from contracting. The application should be in amounts of no more than one milliliter in different places around the muscle, particularly around the areas of greatest innervations. The unit of activity is the amount of the compositions of the invention necessary to block the contractions of the crural biceps of a mouse leg for 1.5 to 2 hours. The preferred dosage rate is 100 to 800 microliters per injected point, depending on the size, irrigation and anatomical location of the muscle, while maintaining a concentration of 20-40 units/milliliter. The effect is immediately apparent, generally occuring a maximum of thirty seconds after injection. The maximum effect may be seen within 15 minutes. Its effective duration depends on the dose administered, the muscle in question, as well as the volume and specific composition administered. This is the pattern for all clinical applications and pathologies. The injection may be accomplished by using a 1 milliliter, tuberculin-type disposable syringe with a twenty-seven to thirty gauge needle. In the case of strabismus, a dose of twenty to forty units in a volume of 50-100 microliters may be injected in the orbicular muscle. Use of the pharmaceutical preparations of the invention is limited to individuals over twelve years old. There is no contraindication for pregnant women.

The advantageous properties of this invention can be observed by reference to the following example, which is meant to illustrate, and not limit, the invention in any way.

EXAMPLE

Before the application, a photographic record is made of the person to be treated, first with her face resting and relaxed, and then, frowning and producing a maximum facial

contraction. The person then places ice on their forehead and on the two lateral zones where the preparation is to be injected. With reference to Figure 2, the application should follow a specific pattern. A volume is injected alongside each black-dot injection point shown in Figure 2. A pharmaceutical composition comprising a (2:1:1 volume/volume) mixture of Gonyautoxin 2, Gonyautoxin 3 and Saxitoxin mixture is applied at a dose of 40 units/milliliter. Each injection is made with a 1 milliliter, tuberculin-type disposable syringe with a 27-30 gauge needle. After injecting, the point of injection is disinfected with a gauze soaked in bi-alcohol or in any other disinfectant. The total amount required to complete the face treatment is 1.7 milliliters.

The expected result is an immediate inability to frown and to show lines when the face is resting. The person experiences a feeling of facial stretching similar to that felt when applying a facial cream mask. After that, ice is applied on the injected zones for five minutes. Thirty minutes after the application, the patient gets used to the feeling of facial stretching. At that time, the person walks out with no discernible wrinkles, a rejuvenated facial look, no face marks or hematoma, and completely normal. The face recovers its normal color within twenty minutes, depending on how relaxed the injected patient has become. The whole application procedure takes ten minutes at the most, and produces a very slight pain from the needle and the injected solution. The pain disappears as soon as the syringe is withdrawn. There are no traumas of any kind, nor any sequelae. The patient may be checked the next day and every fifteen days thereafter. At this dose, the effect lasts for one month. After the first month, the treatment may be repeated as often as necessary.

In view of the above, it will be seen that all the objects and features of the present invention are achieved, and other advantageous results obtained. The examples and description of the invention contained herein is illustrative only, and is not intended in a limiting sense.

CLAIMS

1. A pharmaceutical composition for interfering with neuronal transmission, the composition consisting of an effective amount of at least two compounds selected from Gonyautoxin 1, Gonyautoxin 2, Gonyautoxin 3, Gonyautoxin 4 and Gonyautoxin 5 and a pharmaceutically acceptable carrier.

2. The composition of claim 1, wherein the composition includes a mixture of Gonyautoxin 2 and Gonyautoxin 3.

3. The composition of claim 1, wherein the composition includes a mixture of Gonyautoxin 4, and Gonyautoxin 1.

4. The composition of claim 1, wherein the composition includes Gonyautoxin 1.

5. The composition of any one of claims 1 and 4, wherein the composition includes Gonyautoxin 2.

6. The composition of any one of claims 1 and 4-5, wherein the composition includes Gonyautoxin 3.

7. The composition of any one of claims 1 and 4-6, wherein the composition includes Gonyautoxin 4.

8. The composition of any one of claims 1-7, wherein the carrier is water.

9. The composition of any one of claims 1-8, wherein the compound is diluted in a solution selected from acetic acid and aqueous sodium chloride solution.

10. The composition of any one of claims 1-9, wherein the composition includes about twenty to about forty units of the compound per milliliter of total solution.

11. The composition of any one of claims 1-10, further including Botulin A toxin.

12. The composition of any one of claims 1-11, wherein the composition includes at least three different gonyautoxins.

13. The composition of any one of claims 1-11, wherein the composition includes at least four different gonyautoxins.

14. The composition of any one of claims 1-11, wherein the composition includes at least five different gonyautoxins.

15. The composition of any one of claims 1-14, further including a facial cream.

16. The composition of claim 15, wherein the effective amount is from 4 to 32 units of activity of said composition, per dose of facial cream, and a unit of activity is an amount of the composition effective to block the muscular contractions of the crural biceps of a mouse leg for 1.5 to 2.0 hours.

17. The composition of any one of claims 1-16, wherein the effective amount is 20-40 units of activity, and a unit of activity is an amount of the composition effective to block the muscular contractions of the crural biceps of a mouse leg for 1.5 to 2.0 hours.

18. The composition of claim 17, wherein the 20-40 units of activity are present in 50-100 microliters of the composition.

19. The composition of claim 17, wherein the 20-40 units of activity are present in 100-800 microliters of the composition.

20. Use of the composition of any one of claims 1-19, for the manufacture of a medicament for interfering with neuronal transmission.

21. Use of the composition of any one of claims 1-19, for the manufacture of a medicament for interfering with muscle contraction.

22. Use of the composition of any one of claims 1-19, for the manufacture of a medicament for the treatment of anal fissure.

23. Use of a composition including at least one compound selected from Gonyautoxin 1, Gonyautoxin 2, Gonyautoxin 3, Gonyautoxin 4 and Gonyautoxin 5, for the manufacture of a medicament.

24. Use of a composition including at least one compound selected from Gonyautoxin 1, Gonyautoxin 2, Gonyautoxin 3, Gonyautoxin 4 and Gonyautoxin 5, for the manufacture of a medicament for the treatment of anal fissure.

25. Use of a composition including at least one compound selected from Gonyautoxin 1, Gonyautoxin 2, Gonyautoxin 3, Gonyautoxin 4 and Gonyautoxin 5, for the manufacture of a medicament for interfering with neuronal transmission.

26. Use of a composition including at least one compound selected from Gonyautoxin 1, Gonyautoxin 2, Gonyautoxin 3, Gonyautoxin 4 and Gonyautoxin 5, for the manufacture of a medicament for interfering with muscle contraction.

27. A method of reducing wrinkles including the step of administering a cosmetic formulation including the composition of any one of claims 1-19.

28. A method for facial rejuvenation including the step of administering a cosmetic formulation including the composition of any one of claims 1-19.

29. The method as claimed in any one of claims 27-28, wherein 50-100 microliters of said cosmetic formulation is administered per treatment.

30. The method as claimed in any one of claims 27-28, wherein 100-800 microliters of said cosmetic formulation is administered per treatment.

31. The method as claimed in any one of claims 27-30, wherein the step of administering said cosmetic formulation includes contacting an effective amount of the cosmetic formulation with a neuron.

32. The method as claimed in any one of claims 27-31, wherein the step of administering the cosmetic formulation includes injecting the cosmetic formulation into a neuron or an area proximate to the neuron.

33. The method as claimed in any one of claims 27-29, wherein the step of administering the cosmetic formulation includes injecting the cosmetic formulation into a muscle or an area proximate to a muscle.

34. The method as claimed in any one of claims 27-30, wherein the step of administering said preparation includes topically applying an effective amount of the preparation to an area proximate to a neuron.

35. The method as claimed in any one of claims 27-29, wherein the step of administering said preparation includes topically applying an effective amount of the preparation to an area proximate to a muscle.