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(54) Title: AGONIST PEPTIDES OF BASIC FIBROBLAST GROWTH FACTOR (bFGF) AND THE METHOD OF REDUCTION OF WRINKLE ON SKIN, DARKENING OF HAIR AND ACCELERATION OF WOUND HEALING

(57) Abstract: Synergistic therapies for the treatment of vitiligo are provided. The major therapies for the treatment of vitiligo a pigmentary disorder characterized by patchy depigmentation of skin are Psoralens plus UV-A, steroids, basic fibroblast growth factor (bFGF) peptide location or surgical procedures. Psoralens plus UV-A is effective in about 50% of cases, steroids are limitedly effective only in fast spreading cases of vitiligo and often reoccurs on stoppage of treatment. Surgical treatment is the last resort for vitiligo therapy, when all other therapies failed. It is limitedly effective. Basic fibroblast growth factor peptide(s) location was developed as a new mode therapy for the treatment of vitiligo. It is effective in 80% of stable vitiligo and in segmental vitiligo. Here data are provided and demonstrated that combinatorial treatment of vitiligo by local application of bFGF peptide(s) lotion in association with psoralens and UV-A or steroids or surgical procedures produce synergistic response and that the rate of re-pigmentation increases synergistically and more efficacious results are obtained than with any of them alone. An combinatorial therapy comprising the local application of bFGF peptide lotion on the vitiligo patch in combination with any other therapy for treatment of vitiligo appears to act synergistically.



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TITLE

Agonist peptides of basic fibroblast growth factor (bFGF) and the method of reduction of wrinkle on skin, darkening of hair and acceleration of wound healing.

5

FIELD OF INVENTION

This invention relates to agonist peptides of basic fibroblast growth factor (bFGF) and the method of reduction of wrinkles on skin, darkening of hair and acceleration of wound healing.

BACKGROUND OF THE INVENTION

10

Basic fibroblast growth factor (bFGF) also known as FGF2 no named Because it contains a high number of basic amino acid residues (Lysine, Arginine and histidine) is a potent mitogen for a variety of cell types including melanocytes, keratinocytes the major cell type in the epidermal unit and fibroblasts located in the dermis of skin. Both bovine and human bFGF were isolated and the genes expressing this product were sequenced and cloned. In addition bFGF was found to be expressed in a wide variety of tissue types including placenta, keratinocytes, fibroblasts.

20

We have earlier described that bFGF may be involved in repigmentation of vitiligo macules a pigmentary disorder characterized by patchy depigmentation of skin (Ramaiah A, Puri, N, and Majamdar.M A new hypothesis for etiology of vitiligo in Acta Derma Venereol

(Stockholm), 1989, 69,323-327). This idea was tested first on the in-vitro melanocyte cell cultures from the uninvolved areas of untreated vitiligo subjects and later on mixed cell cultures of melanocytes and keratinocytes obtained from the uninvolved areas of untreated vitiligo subjects. The results demonstrated that bFGF corrects all the abnormalities of melanocytes obtained from the uninvolved areas of untreated vitiligo subjects. An animal model was developed to resemble vitiligo to test the efficacy of interdermal injection bFGF on the rate of repigmentation of depigmented ear lobes of Guinea pigs and depigmented ears lobes of Guinea pigs and depigmentated skin patches of Yucatan swine. They were found effective. Peptides ranging from deca peptide to 24 amino acid long peptide were tested for their efficacy to repigment the depigmented ear lobes of Guinea pigs and the depigmented skin patches of Yucatan swine. These peptides were shown to be specific since other growth factors like epidermal growth factor or peptide 1-12 of bFGF did not have any effect on the repigmenting depigmented patches on the experimental animals.

20 Patents of interest describing bFGF or peptides described above and the formulation for their penetration through intact skin include US Patent no. 6,143,723, AU Patent 722626, Indian Patents, 185613, 186437, 185703.

25 The peptides were tested on human volunteers suffering from vitiligo in the various phase of clinical trials in India and found to be successful in repigmenting about more than 80% volunteers with stable generalized vitiligo and segmental vitiligo.

5 Skin is subject to aging and is visible to the naked eyes. The aging process of skin can be divided in to chrono aging and photo aging. The former is a normal aging process and can be accelerated by the exposure of skin to sun, which is known as photo aging. In addition, skin is subject to deterioration through the dermatological disorders, environmental abuse.

10 Exposure of white/very fair skin to ultraviolet radiation results in more wrinkling of skin than the more pigmented skin. Wrinkles of skin are a reminder to the individual that he/she is looking older and therefore likes to reduce the skin wrinkles by application to skin various cosmetic creams/moisturizers or failing that to various forms of cosmetic surgery which treat or delay the visible signs of chrono aging/photo aging such as wrinkles, liens, sagging etc.

15 There is bulk of epidemiological literature relating thinning of skin to aging. Collagen-1 represents more than 70% (Uitto J. Connective tissue biochemistry of the aging dermis: Age related alterations in collagen and elastin: *Dermatol Clin* (1986) 4:433-46) of the dermis of the skin. Dermal collagen content peaks in the third decade of life and declines gradually at a rate approximately 1% per year thereafter in men and women (Artho P. Skin thickness and collagen content in some endocrine, connective tissue and skin diseases. *Acta derm. Veneroeol Suppl* (Stockh) 1972: 69:1-48. Meema HE, Sheppard RH, Roentgeno graphic visualization and measurement of skin thickness and its diagnostic application to acromegaly, *Radiology* (1964); 82:411-7, Shuster S, BlackMM, Mc Vitie

E.: The influence of age and sex on skin thickness, skin collagen and density (Br J Dermatol (1975); 93:639-43, Shuster S Bottoms E senile feneration of skin collagen in men). In women it may be so after the fifth decade of life.

- 5 Collagen is the predominant matrix skin protein and is known to impart tensile strength to skin. Decorin is proteoglycan, which is known to be important for controlled and correct deposition of collagen in the extra cellular matrix of skin. It is also known in the art that the levels of collagen and decorin in skin are significantly reduced with age and/or
- 10 photo damaged skin. Many studies had shown that the levels of collagen type I in skin are decreased with age and/or with increased photo damage (Lavaker R, Jour. Invest. Dermatol (1979); 73:59-66 Griffiths et al New. Eng J. med (1993); 329, 530-535). In the case of decorin, it has been shown that mRNA expression of the proteoglycan is greatly reduced in photo-damaged skin in vitro (Bernstein et al Lai. Invest (1995); 72:662-669). The reduction of the levels of these skin proteins is accordingly associated with collagen. Elastin also is lost with age. So the skin gets thinner with age and has tougher time getting enough moisture to the epidermis. At about the same time the fat in the subcutaneous layer (which gives skin a plump firm appearance) also begins to disappear. The epidermis begins to sag, and wrinkles form. Wrinkles are then prone in the face where facial muscle contractions have been repeated for over many years.

In addition to gradually ageing and sun exposure there are a number of other factors that can contribute to increased wrinkles. Some of these can be controlled while others cannot. The most common factors that determine wrinkle occurrence include the following:

- 5 Heredity
- Skin type
- Smoking
- Hairstyle
- Sun exposure
- 10 Drug use

Wrinkles on the face are due to repeated nerve stimulation that result in repeated facial muscle contractions, in addition to reduction in the amount of collagen, elastin and subcutaneous fat in the skin. All anti wrinkling agents which reduce the nerve stimulation and thus facial muscle contractions can help in the reduction of fine lines and wrinkles on the face.

Wrinkle reduction on the rest of the skin on the body including the skin on the face should essentially be by increasing the synthesis of collagen, retention in the skin of moisture.

- 20 It is well known in the art that retinoic acid is potent anti-aging agent and induces dermal repair of photo-damaged skin. It was shown that wrinkle effacement and dermal repair following topical treatment of skin

With retinoic acid arises through new collagen deposition and synthesis in skin (Griffith et al New, Eng. J, Med (1993); 329, 530-535).

OBJECTS OF THE INVENTION

An object of the present invention is to propose peptide(s) and composition containing said peptide for reducing the formation of wrinkles on skin.

Another object of this invention is to propose peptide(s) and a composition containing said peptide for darkening of hair.

These objects arose out of observations made during conducting the clinical trials on the safety and efficacy of the peptides in repigmenting the depigmented vitiligo patches of skin. While the clinical trials were being conducted on the vitiligo volunteers it was observed that the treated areas with the peptide in the formulation became smooth as a result of reduction of wrinkles on the skin. A representative photograph was presented in figs 1a, 1b before and after the topical application of the peptide in the formulation.

BRIEF DESCRIPTION OF THE INVENTION

According to this invention there is provided the peptides consisting of sequence 1-8 listed herein for darkening and reduce formulation of wrinkles on skin. The peptides are present in the range of 0.02-5% w/w in the composition are effective when applied topically in the formulation for reduction of wrinkles on skin, and in darkening of hair. They can be applied in the form of lotion, gels, creams the mode of administration is not restricted to the above methods.

Definitions of the specific embodiments of the invention as claimed herein follow.

According to a first embodiment of the invention, there is provided use of a composition comprising 0.02% to 5% w/w of at least one peptide selected from the group consisting of SEQ ID NOs 1 to 8 which are agonist peptides of bFGF and an acceptable carrier in the manufacture of a medicament for topical application in reducing skin wrinkles.

According to a second embodiment of the invention, there is provided a method for reducing skin wrinkles by topical application of a composition comprising:

0.02 % to 5 % w/w of at least one peptide selected from the group consisting of SEQ ID NOs 1 to 8 which are agonist peptides of bFGF; and
an acceptable carrier.

According to a third embodiment of the invention, there is provided a composition comprising 0.02 % to 5 % w/w of at least one peptide selected from the group consisting of SEQ ID NOs 1 to 8 which are agonist peptides of bFGF and an acceptable carrier when used as a method of reducing skin wrinkles by topical application of the composition.

EXAMPLE 1

[Text Continues on page 7].

ANTI WRINKLE

Based on the observations presented in Fig 1a and 1b, a planned clinical trial was conducted on 20 normal volunteers. Volunteers with clear skin were chosen for this clinical trial. The application of either placebo (formulation) on the right dorsal part of the hand or the formulation containing the peptide was topically applied

On the left dorsal hand of the volunteer once a day for 6 weeks and the pictures were taken at the end of such trial.

The results indicated that topical application of the peptide in the formulation once a day for 6 weeks resulted without exception in reduction in wrinkles. A representative photograph after 6 weeks of application of the peptide in the formulation on dorsal hand reduced wrinkles as compared to the control where only the formulation was topically applied. On stopping of the topical application of the peptide lotion, the skin returns to normal in about 2-2 and half months. This effect is thus not permanent. The reduction of wrinkles on skin by the
15 topical application of the bFGF derived peptide described above may be
Due to increased cellularity of the epidermis and also increased synthesis of collagen. Fig. 2 shows the reduction of wrinkles on left dorsal hand of a volunteer after application of the composition with the peptide for 6 weeks as compared to the dorsal right hand only with placebo.

EXAMPLE 2

20 DARKENING OF HAIR

In addition, during the clinical trial of its use for the treatment of vitiligo

It was observed that the peptides were able to repigment white hair located in the vitiligo macules. Therefore, these peptides increase the proliferation of melanocytes located not only in the dermal epidermal junction but also the melanocytes located in the hair follicles, as indicated in the photographs, (Fig3) taken before and after the topical application of these peptides for 3-6 months.

Peptides derived from bFGF may accelerate wound healing and other biological functions as native bFGF does.

Advanced wound care technologies have emerged from the shadow to become, in the last 20 years, significant products in wound treatment.

10 While conventionally made wound care products are considerable and Generally less expensive, new products and biotechnological advancements are beginning to revolutionize the wound care market. New technological products are competing in a fast track market that seeks to improve the quality of life for millions of individuals needing both acute and chronic wound care.

The subcutaneous implantation of poly-vinyl alcohol sponges had been used to generate artificial wound space. Healing process was followed with time by a combination of histological and biochemical analyze of new tissue that grows in to the interstices of the sponge material in presence and absence of recombinant basic fibroblast growth factor (rbFGF). The results of these experiments clearly indicated that recombinant basic fibroblast growth factor acts to increase neo vascularisation, the number of fibroblasts and the deposition of collagen in the wound space. Similar results were seen on wounds of diabetic mice. There were no side effects (Bernstein et al Lab. Invest. (1995); 72: 662-669).

In view of the fact that the peptides listed herein are agonist peptides of bFGF it is indeed possible that these peptides may accelerate wound healing as the rbFGF does by topical application of an effective amount of any of the peptide listed herein in a composition in sterile phosphate buffered isotonic saline solution.

The sequence listing of the peptides is given as follows:

(2) Information for Sequence ID NO: 1

(I) Sequence Characteristics:

- 10 (A) Length: 10 amino acids
- (B) Type: amino acid
- (C) Stranded ness: Not applicable
- (D) Topology: Linear
- (E) Source: Synthetic, non -bacterial source

15 (ii) Molecular Type: Peptide

(vi) Sequence Description: Sequence ID NO: 1:

All sequences of the peptides were described starting from the amino terminus of the peptide: The standard 3-letter description of the amino acid was used.

20 Tyr Arg Ser Arg Lys Tyr Ser Ser Trp Tyr amide
1 10

(2) Informations for sequence ID NO: 2:

(i) Sequence Characteristics:

(A) Length: 10 amino acids

(B) Type: amino acid

(C) Strandedness: Not applicable

(D) Topology: linear

(E) Source: Synthetic, Non-bacterial source

(ii) Molecular type: Peptide

(vi) Sequence Description: Sequence ID NO: 2: an invention Tyr Arg
Ser Arg Lys Tyr Glu Ser Tip Tyr amide

10

(2) Informations for sequence NO: 3:

(i) Sequence characteristics:

(A) Length: 10 amino acids

(B) Type amino acid

(C) Strandedness: Not applicable

20

(D) Topology: Cyclic

(E) Source: Synthetic, Non Bacterial source

(ii) Molecular Type: Peptide

(vi) Sequence Description: Sequence ID NO: 3:

Cyclo (Tyr Arg Ser Arg Lys Tyr Glu Ser Trp Tyr)

(2) Informations for sequence IDNO: 4:

(i) Sequence Characteristics:

(A) Length: 11 amino acids

(B) Type: amino acid

(C) Strandedness: Not applicable

(D) Topology: cyclic

(E) Source: Synthetic, Non-bacterial source

(ii) Molecular type: Peptide

(vi) Sequence Description Sequence IDNO: 4

Cyclo (Gly Tyr Arg Ser Arg Lys Tyr Ser Ser Arg Tyr)

(2) Informations for sequence ID NO: 5:

(i) Sequence Characteristics:

(A) Length: 15 amino acids

(B) Type amino acid

(C) Strandedness: Not applicable

(D) Topology: Linear

(E): Source: Synthetic, Non-bacterial source (ii)

(iv) Molecular Type: Peptide

(vi) Sequence Description: Sequence ID NO: 5:

Tyr Arg Ser Arg Lys Tyr Ser Ser Trp Tyr Val Ala Leu Lys Arg
1 10 15

(2) Informations for seq. ID NO: 6:

(i) Sequence Characteristics:

(A) Length: 24 amino acids

5

(B) Type: amino acid

(C) Strandedness: Not applicable

(D) Topology: Linear

(E) Source: Synthetic, Non-bacterial source

(ii) Molecular type: Peptide

10

(vi) Sequence Description: seq. ID NO: 6:

Pro Ala Leu Pro Glu Asp Gly Gly Ser Gly Ala Phe Pro Pro Gly His

Phe Lys Asp Pro Lys Arg Leu Tyr

20

24

(vi) Sequence description: Sequence ID NO: 7

(2) Informations for sequence NO: 7

15

(i) Sequence characteristics

(A) Length: 10 amino acid residues

(B) Type: amino acid

(C) Strandedness: Not applicable

5

(D) Topology: linear

(E) Source: Synthetic, Non bacterial source

(ii) Molecular type: Peptide

(vi) Sequence description: Sequence ID NO: 7

para hydroxy phenyl propionic-amide -Tyr Arg Ser Arg Lys Tyr Ser Ser
Trp Tyr amide

(2) Informations for Sequence NO:8

(i) Sequence characteristics

(A) Length: 10 amino acids

(B) Type: amino acid

(C) Strandedness: Not applicable (D)

Topology: linear (ii) Molecular type:
Peptide

(vi) Sequence description IDNO: 8

Tyr Arg Ser Arg Lys Tyr Ser Ser Trp Tyr-NH(CH₂)₂C₆H₄OHp

The term "comprise" and variants of the term such as "comprises" or "comprising" are used herein to denote the inclusion of a stated integer or stated integers but not to exclude any other integer or any other integers, unless in the context or usage an exclusive interpretation of the term is required.

Any reference to publications cited in this specification is not an admission that the disclosures constitute common general knowledge in Australia.

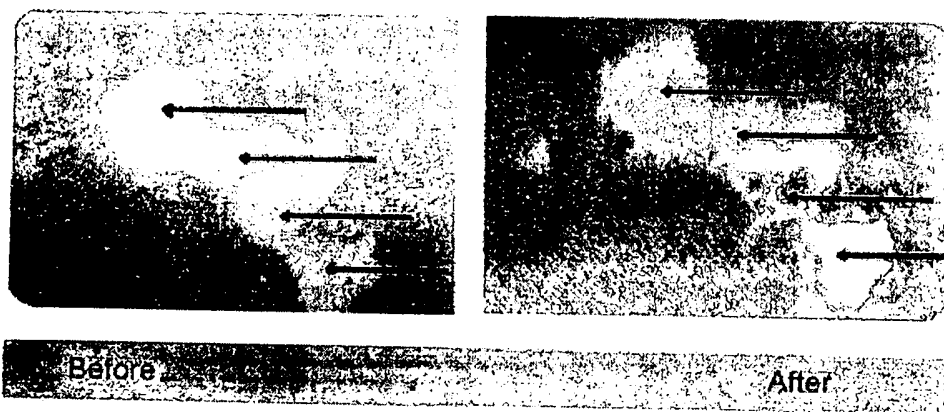
It is to be understood that the present invention is susceptible to, changes, adaptations by those skilled in art. Such modifications, changes and adaptations are intended to be within the scope of the present invention, which is further set forth under the following claims:

The claims defining the invention are as follows:

1. Use of a composition comprising 0.02% to 5% w/w of at least one peptide selected from the group consisting of SEQ ID NOs 1 to 8 which are agonist peptides of bFGF and an acceptable carrier in the manufacture of a medicament for topical application in reducing skin wrinkles.
2. A method for reducing skin wrinkles by topical application of a composition comprising:
0.02 % to 5 % w/w of at least one peptide selected from the group consisting of SEQ ID NOs 1 to 8 which are agonist peptides of bFGF; and
an acceptable carrier.
3. A composition comprising 0.02 % to 5 % w/w of at least one peptide selected from the group consisting of SEQ ID NOs 1 to 8 which are agonist peptides of bFGF and an acceptable carrier when used as a method of reducing skin wrinkles by topical application of the composition.
4. Use of a composition as defined in claim 1 and substantially as described herein with reference to EXAMPLE 1.
5. A method as defined in claim 2 and substantially as described herein with reference to EXAMPLE 1.

Date: 9 November 2011

1/2



1a

1 b



2a (After application for 6 weeks)

Control (formulation only applied for 6 weeks)

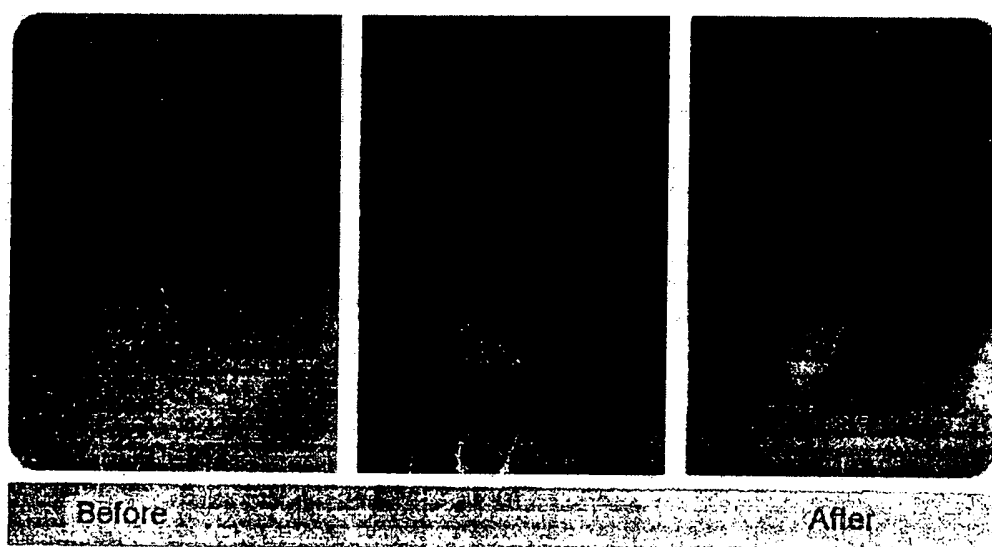


Fig-3