

S P E C I F I C A T I O N

TITLE OF INVENTION: Hair Growth Agent

TECHNICAL FIELD

[0001] The present invention relates to a hair growth agent. More particularly, it relates to a hair growth agent that contains palmitoyl dipeptide-5 diaminobutyloyl hydroxythreonine and palmitoyl dipeptide-5 diaminohydroxybutyrate, and phytosphingosine.

BACKGROUND ART

[0002] Growth of hair is such that growth and loss repeatedly occur in accordance with the hair cycle (hair life cycle) which comprises anagen, catagen, and telogen. Symptoms of male pattern hair loss are such that the balance which would otherwise exist in the hair cycle is lost for one reason or another, the length of anagen is reduced, increasing the fractional percentage represented by telogen, and causing the hair to become vellus hair (downy hair). Histologically, there are reports that the size of the anagen hair follicle is smaller than would be the case with a healthy scalp, development of dermal papillae is poor, and there is reduction in the capillary network which surrounds the hair follicle (Nonpatent Reference No. 1).

[0003] There has therefore been increasing demand for hair growth agents and other such topical agents that will improve hair type and/or hair quality and hair growth effect in mammals including humans. To improve hair type and/or hair quality and hair growth effect, active ingredients which contribute to regulation of the hair cycle, i.e., the hair life cycle, have been proposed and are in the process of coming onto the market in the form of hair growth agents.

[0004] Whereas vascular endothelial growth factor (VEGF) is a growth factor with action specific for vascular endothelial cells that was isolated from pituitary foliculo-stellate (folliculostellate) cell culture medium and that is known to act to increase vascular permeability and promote neovascularization such as by causing growth of vascular endothelial cells, it is clear that adenosine, when allowed to act on human dermal papilla cells, promotes expression of vascular endothelial growth factor (VEGF) (see Nonpatent Reference No. 2).

[0005] For example, use of minoxidil as an active ingredient in a hair growth agent has been proposed (see Patent Reference Nos. 1 through 3 and so forth), and hair growth agents employing minoxidil as active ingredient have undergone clinical trials in humans and are on the market. It is clear that minoxidil, when allowed to act on dermal papilla cells, promotes expression of vascular endothelial growth factor (VEGF) (see Nonpatent Reference No. 3). However, for reasons such as the fact that the pharmaceutical use thereof within Japan is limited to male alopecia prematura, it has not adequately satisfied the broad needs of consumers who desire hair growth effect and hair type and/or hair quality improvement effect. Furthermore, with respect to adenosine, because there have been no reports of major side effects in human clinical trials, and because the mechanism for improvement of thinning of hair does not directly involve regulation of sex hormones, utilization of adenosine as an active ingredient in a hair growth agent that may be safely used by both males and females has been proposed, and it has been placed on the market (Nonpatent Reference No. 4). However, because there have been marked differences in the benefit in terms of the improvement produced thereby depending on individual, it has not adequately satisfied the broad needs of consumers who desire hair growth effect and hair type and/or hair quality improvement effect, as a result of which there has been demand for improvement.

[0006] The inventor(s) have therefore engaged in intensive research and have discovered that palmitoyl dipeptide-5 diaminobutyloyl hydroxythreonine and palmitoyl dipeptide-5 diaminohydroxybutyrate cause significant increase in maximum hair shaft length and hair shaft elongation rate, exhibit excellent hair growth effects such as promotion of expression of vascular endothelial growth factor (VEGF) in dermal papilla cells, and are useful as active ingredients in hair growth agents and scalp care agents (see Patent Reference No. 4).

[0007] Phytosphingosine is known as a component in raw materials for cosmetics (see Patent Reference No. 5). However, there are no reports related to a hair growth effect of phytosphingosine.

PRIOR ART REFERENCES

PATENT REFERENCES

[0008] Patent Reference No. 1: Specification of U.S. Patent No. 4,139,619

Patent Reference No. 2: Japanese Patent Application Publication Kokai No. S63[1988]-150211

Patent Reference No. 3: Japanese Patent Application Publication Kokai No. S63[1988]-145217

Patent Reference No. 4: International Patent Application Publication No. 2021/075219

Patent Reference No. 5: Japanese Patent No. 3220434

Nonpatent Reference No. 1: Daisaburo YOKOYAMA, J. Jpn. Oil Chem. Soc., 1995, Vol. 44, No. 4, p. 266-273

Nonpatent Reference No. 2: KYUNG-A HWANG et al., Int J Mol Med., 2012, Feb; 29 (2): 195-201

Nonpatent Reference No. 3: Lachgar S, Charveron M, Gall Y and Bonafe JL: Br J Dermatol 138, 407-411 (1998)

Nonpatent Reference No. 4: Ritsuko EHAMA et al., J. Soc. Cosmet. Chem. Jpn., 2011, Vol. 45, No. 1: 35-40.

SUMMARY OF INVENTION

PROBLEM TO BE SOLVED BY INVENTION

[0009] It is an object of the present invention to provide a hair growth agent that possesses excellent hair growth action.

MEANS FOR SOLVING PROBLEM

[0010] As a result of intensive and repeated research for the purpose of solving the foregoing problems, the present inventor(s) discovered that use of phytosphingosine as active ingredient made it possible to attain hair growth activity, and moreover that use of phytosphingosine with palmitoyl dipeptide-5 diaminobutyloyl hydroxythreonine and palmitoyl dipeptide-5 diaminohydroxybutyrate as active ingredients permitted attainment of stronger hair growth activity than use of phytosphingosine or of palmitoyl dipeptide-5 diaminobutyloyl hydroxythreonine and palmitoyl dipeptide-5 diaminohydroxybutyrate, which culminated in the present invention.

[0011] A first means in accordance with the present invention for solving the foregoing problems is a hair growth agent characterized in that it contains

palmitoyl dipeptide-5 diaminobutyloyl hydroxythreonine and palmitoyl dipeptide-5 diaminohydroxybutyrate, and phytosphingosine, as active ingredients.

[0012] A second means in accordance with the present invention for solving the foregoing problems is a hair growth agent for use in combination with phytosphingosine, characterized in that it contains palmitoyl dipeptide-5 diaminobutyloyl hydroxythreonine and palmitoyl dipeptide-5 diaminohydroxybutyrate as active ingredients.

[0013] A third means in accordance with the present invention for solving the foregoing problems is a hair growth agent for use in combination with palmitoyl dipeptide-5 diaminobutyloyl hydroxythreonine and palmitoyl dipeptide-5 diaminohydroxybutyrate, characterized in that it contains phytosphingosine as active ingredient.

[0014] A fourth means in accordance with the present invention for solving the foregoing problems is the hair growth agent of any one among the first through third means in accordance with the present invention for use in causing new hair growth or hair shaft growth promotion.

[0015] A fifth means in accordance with the present invention for solving the foregoing problems is the hair growth agent of any one among the first through fourth means in accordance with the present invention used for causing improvement in hair shaft elongation rate.

[0016] A sixth means in accordance with the present invention for solving the foregoing problems is the hair growth agent of any one among the first through fourth means in accordance with the present invention used for causing improvement in maximum hair shaft length.

[0017] A seventh means in accordance with the present invention for solving the foregoing problems is the hair growth agent of any one among the first through fourth means in accordance with the present invention used for causing increase in hair shaft diameter.

[0018] An eighth means in accordance with the present invention for solving the foregoing problems is the hair growth agent of any one among the first through fourth means in accordance with the present invention used for causing increase in number of hairs.

- [0019] A ninth means in accordance with the present invention for solving the foregoing problems is the hair growth agent of any one among the first through eighth means in accordance with the present invention in liquid solution form.
- [0020] A tenth means in accordance with the present invention for solving the foregoing problems is the hair growth agent of any one among the first through ninth means in accordance with the present invention for use on head hair, beard, eyelashes, and/or eyebrows.
- [0021] An eleventh means in accordance with the present invention for solving the foregoing problems is a method of manufacturing a hair growth agent, characterized by presence of an operation in which palmitoyl dipeptide-5 diaminobutyloyl hydroxythreonine and palmitoyl dipeptide-5 diaminohydroxybutyrate, and phytosphingosine, are made to be contained as active ingredients in a pharmaceutical preparation.
- [0022] A twelfth means in accordance with the present invention for solving the foregoing problems is a method of manufacturing a hair growth agent for use in combination with phytosphingosine, characterized by presence of an operation in which palmitoyl dipeptide-5 diaminobutyloyl hydroxythreonine and palmitoyl dipeptide-5 diaminohydroxybutyrate are made to be contained as active ingredients in a pharmaceutical preparation.
- [0023] A thirteenth means in accordance with the present invention for solving the foregoing problems is a method of manufacturing a hair growth agent for use in combination with palmitoyl dipeptide-5 diaminobutyloyl hydroxythreonine and palmitoyl dipeptide-5 diaminohydroxybutyrate, characterized by presence of an operation in which phytosphingosine is made to be contained as active ingredient in a pharmaceutical preparation.
- [0024] A fourteenth means in accordance with the present invention for solving the foregoing problems is a hair growth agent kit that comprises a pharmaceutical preparation which contains phytosphingosine, and a pharmaceutical preparation which contains palmitoyl dipeptide-5 diaminobutyloyl hydroxythreonine and palmitoyl dipeptide-5 diaminohydroxybutyrate.
- [0025] A fifteenth means in accordance with the present invention for solving the foregoing problems is a hair growth agent kit for use in combination with

phytosphingosine, which comprises a pharmaceutical preparation that contains palmitoyl dipeptide-5 diaminobutyloyl hydroxythreonine and palmitoyl dipeptide-5 diaminohydroxybutyrate.

[0026] A sixteenth means in accordance with the present invention for solving the foregoing problems is a hair growth agent kit for use in combination with palmitoyl dipeptide-5 diaminobutyloyl hydroxythreonine and palmitoyl dipeptide-5 diaminohydroxybutyrate, which comprises a pharmaceutical preparation that contains phytosphingosine.

[0027] A seventeenth means in accordance with the present invention for solving the foregoing problems is a hair growth method characterized by administration of palmitoyl dipeptide-5 diaminobutyloyl hydroxythreonine, palmitoyl dipeptide-5 diaminohydroxybutyrate, and phytosphingosine, as active ingredients.

[0028] An eighteenth means in accordance with the present invention for solving the foregoing problems is a hair growth method comprising administering the hair growth agent of any one among the first through tenth means in accordance with the present invention to a subject.

[0029] Another means in accordance with the present invention for solving the foregoing problems is a scalp care agent that contains palmitoyl dipeptide-5 diaminobutyloyl hydroxythreonine and palmitoyl dipeptide-5 diaminohydroxybutyrate, and phytosphingosine, as active ingredients.

[0030] Moreover, another means in accordance with the present invention for solving the foregoing problems is a scalp symptom improvement method that comprises administering a scalp care agent which contains palmitoyl dipeptide-5 diaminobutyloyl hydroxythreonine and palmitoyl dipeptide-5 diaminohydroxybutyrate, and phytosphingosine, as active ingredients to a subject.

[0031] Moreover, another means in accordance with the present invention for solving the foregoing problems is an agent for promoting dermal papilla cell VEGF production that contains palmitoyl dipeptide-5 diaminobutyloyl hydroxythreonine and palmitoyl dipeptide-5 diaminohydroxybutyrate, and phytosphingosine, as active ingredients.

BENEFIT OF INVENTION

[0032] By causing palmitoyl dipeptide-5 diaminobutyloyl hydroxythreonine and palmitoyl dipeptide-5 diaminohydroxybutyrate, and phytosphingosine, to be contained as active ingredients or used in combination in a hair growth agent which is a topical agent, means in accordance with the present invention make it is possible to provide an excellent hair growth agent and scalp care agent that exhibit scalp care effect as well as effect in terms of causing increase in hair shaft diameter and improving maximum hair shaft length and improving hair shaft elongation rate and hair shaft growth promotion at head hair, beard, eyelashes, eyebrows, and/or other such hair.

BRIEF DESCRIPTION OF DRAWINGS

[0033] FIG. 1 contains a graph showing change in amount of expression of the VEGF gene in human dermal papilla cells as a result of stimulation for 72 hours with palmitoyl dipeptide-5 diaminobutyloyl hydroxythreonine, palmitoyl dipeptide-5 diaminohydroxybutyrate, and phytosphingosine; and with palmitoyl dipeptide-5 diaminobutyloyl hydroxythreonine and palmitoyl dipeptide-5 diaminohydroxybutyrate and phytosphingosine.

EMBODIMENTS FOR CARRYING OUT INVENTION

[0034] Embodiments for carrying out the present invention are described below. Note that the present invention is not limited to these examples alone, it being of course possible to make any number of changes thereto without departing from the gist of the present invention.

[0035] The active ingredient(s) of a hair growth agent and a scalp care agent associated with the present invention comprise substance(s) containing palmitoyl dipeptide-5 diaminobutyloyl hydroxythreonine and palmitoyl dipeptide-5 diaminohydroxybutyrate together with phytosphingosine; or comprise substance(s) in which palmitoyl dipeptide-5 diaminobutyloyl hydroxythreonine and palmitoyl dipeptide-5 diaminohydroxybutyrate, and adenosine, are used in combination therein.

[0036] Concentrations of palmitoyl dipeptide-5 diaminobutyloyl hydroxythreonine, palmitoyl dipeptide-5 diaminohydroxybutyrate, and phytosphingosine, which constitute active ingredient(s) in a hair growth agent or scalp care agent in accordance with the present invention, are 0.001 wt% to 20 wt% of the entirety of

the hair growth agent or scalp care agent. More specifically, they are 0.005 wt% to 10 wt%.

[0037] While hair growth agents and scalp care agents in accordance with the present invention may be used in the form of pharmaceutical preparations having any of a wide variety of modes of dosage forms serving as topical agents such as ointments, poultices, liniments, lotions, liquids for topical use, dusting powders, creams, gels, emulsions, hair tonics, hair sprays, microneedles, and so forth as cosmetics including cosmetics for the scalp and cosmetics for the eyelashes and/or eyebrows, beard, head hair, quasi-pharmaceutical agents, pharmaceutical agents, and so forth, there is no limitation with respect thereto.

[0038] Hair growth agents and scalp care agents in accordance with the present invention may be manufactured as a result of performance of operations which cause palmitoyl dipeptide-5 diaminobutyloyl hydroxythreonine, palmitoyl dipeptide-5 diaminohydroxybutyrate, and/or phytosphingosine to be contained therein as active ingredient(s). Furthermore, in accordance with the present invention, hair growth agents and scalp care agents for use in combination with palmitoyl dipeptide-5 diaminobutyloyl hydroxythreonine and/or palmitoyl dipeptide-5 diaminohydroxybutyrate may be manufactured as a result of performance of operations which cause phytosphingosine to be contained as active ingredient therein; moreover, hair growth agents and scalp care agents for use in combination with phytosphingosine may be manufactured as a result of performance of operations which cause palmitoyl dipeptide-5 diaminobutyloyl hydroxythreonine and/or palmitoyl dipeptide-5 diaminohydroxybutyrate to be contained as active ingredient(s) therein. Moreover, in addition to the foregoing operations, operations may be added which cause additive(s) to be contained therein for the purpose of formulation as pharmaceutical preparation(s).

[0039] In the context of hair growth agents and scalp care agents in accordance with the present invention, where palmitoyl dipeptide-5 diaminobutyloyl hydroxythreonine, palmitoyl dipeptide-5 diaminohydroxybutyrate, and/or phytosphingosine are to be contained in separate pharmaceutical preparations that will be provided, the dosage forms for those pharmaceutical preparations may be such that they are a combination of different dosage forms or the same dosage

form, and they may also be provided in the form of a kit for providing pharmaceutical preparation(s) having such dosage form(s).

[0040] Furthermore, to the extent that it does not interfere with the hair growth effect and scalp care effect of the present invention, additives and/or other such components, presence of which would ordinarily be permitted in cosmetics including cosmetics for the scalp and cosmetics for the eyelashes and/or eyebrows, beard, head hair, quasi-pharmaceutical agents, pharmaceutical agents, and so forth, may be additionally blended therein. As such additives and/or other such components, while excipients, stabilizers, corrigents, vehicle, dispersants, diluents, anionic surface active agents, amphoteric surface active agents, nonionic surface active agents, cationic surface active agents, anionic polymers, nonionic polymers, ethylene oxide - propylene oxide block copolymer, alcohols, emulsifiers, percutaneous absorption promoters, pH adjustors, preservatives, colorants, lipids, mineral oils, and other such oily components, moisturizing agents, thickeners, polymers, film-forming agents, ultraviolet light absorbers, cell activators, moisturizing agents, inorganic salts, functional beads and capsules, silicones, metal chelating agents, antioxidants, antiseptic agents, fresheners, deodorants, pigments, dyes, fragrances, sugars, amino acids, vitamins, organic acids, organic amines, plant extracts, clay minerals, various polymers, and other such viscosity modifiers, and so forth may be cited as examples, there is no limitation with respect thereto.

[0041] Hair growth agents and scalp care agents in accordance with the present invention may additionally contain known components having new hair growth effect, hair growth effect, hair tonic effect, and/or the like.

[0042] Administration dosage of active ingredient(s) per dose of a hair growth agent and scalp care agent of a means in accordance with the present invention may be adjusted so as to cause effect(s) of the hair growth agent and scalp care agent in accordance with the present invention to be exhibited. In addition, such administration dosage might for example be 0.005 mg to 200 mg, might more specifically be 0.05 mg to 100 mg, and might still more specifically be 0.5 mg to 10 mg.

[0043] So as to cause effect(s) of the hair growth agent and scalp care agent in accordance with the present invention to be exhibited, the number of administrations of a hair growth agent and scalp care agent in accordance with the present invention might be one administration or might be multiple administrations. In addition, the number of administrations of a hair growth agent and scalp care agent in accordance with the present invention might for example be 1 to 6 times per day. In addition, more specifically this might be 1 to 3 times per day, and still more specifically this might be 1 to 2 times per day.

[0044] Hair growth agents and scalp care agents in accordance with the present invention relate to hair shaft growth promotion, new hair growth, and hair loss prevention, and preferably relate to hair shaft growth promotion and new hair growth.

[0045] In the present specification, the term “hair shaft growth promotion” means improving hair shaft elongation rate, improving maximum hair shaft length, and/or increasing hair shaft diameter.

[0046] In the present specification, the term “new hair growth” means promoting growth of new hair and increasing number of hairs at follicle pores where new hair growth capability has been lowered or where new hair growth has stopped at a location where there are a small number of hairs or where there is no hair (no hair shaft extends to the exterior from the epidermis), and more specifically means shortening the telogen phase of the hair cycle and/or restarting a stopped hair cycle.

[0047] In the present specification, “to have hair shaft growth promotion effect” means acting in a way such as will be advantageous for promotion of hair shaft growth, and the quality by which hair shaft growth promotion effect is indicated is referred to as “hair shaft growth promotion activity”. Furthermore, “to have new hair growth effect” means acting in a way such as will be advantageous for new hair growth, and the quality by which new hair growth effect is indicated is referred to as “new hair growth promotion activity”.

[0048] In the present specification, the term “hair loss” means the phenomenon whereby the hair shaft comes free from the follicle pore, and more specifically means increase in inhibitory cytokines or the like which interfere with cell growth,

and to cell death resulting therefrom. The quality by which hair loss prevention effect is indicated is referred to as “hair loss prevention activity”. Furthermore, “to have hair loss prevention effect,” which is a physiological phenomenon different from the qualities by which hair shaft growth promotion and/or new hair growth effect are indicated, means decreasing the number of hair shafts that come free from follicle pores as a result of reduction in or interference with inhibitory cytokines and suppression of cell death.

[0049] In the present specification, the term “scalp symptoms” means dandruff, roughness of the scalp, dryness of the scalp, erythema, itchiness, acne, and/or other such symptoms. In addition, in the present specification, the term “improvement of scalp symptoms” means improvement or suppression of dandruff, roughness of the scalp, dryness of the scalp, erythema, itchiness, acne, and/or the like.

[0050] A hair growth agent in accordance with the present invention may be used to improve hair shaft elongation rate and/or maximum hair shaft length. In addition, with respect to hair shaft elongation rate, as compared with hair shaft elongation rate pursuant to hair cycle reference data, it may for example cause a maximum improvement of on the order of 110%, more specifically it may cause improvement on the order of 25% to 110%, and still more specifically it may cause improvement on the order of 33% to 110%. Furthermore, with respect to maximum hair shaft length, as compared with maximum hair shaft length pursuant to hair cycle reference data, it may for example cause a maximum improvement of on the order of 49%, more specifically it may cause improvement on the order of 1% to 49%, and still more specifically it may cause improvement on the order of 2% to 49%.

[0051] A hair growth agent in accordance with the present invention may be used to increase hair shaft diameter.

[0052] A hair growth agent in accordance with the present invention may be used to promote growth of new hair and increase the number of hairs at follicle pores where new hair growth capability has been lowered or where new hair growth has stopped at a location where there are a small number of hairs or where there is no hair (no hair shaft extends to the exterior from the epidermis), and more

specifically may be used to shorten the telogen phase of the hair cycle and/or restart a stopped hair cycle.

- [0053] Hair growth agents and scalp care agents in accordance with the present invention may be used not only for humans but also for domesticated animals, animal pets, and/or other such animals. One aspect of the present invention provides a scalp symptom improvement method and/or a hair growth method that includes administration of topical agent(s) which contain phytosphingosine and adenosine to subject(s) which may include human(s), domesticated animal(s), animal pet(s), and/or other such nonhuman animal(s).

WORKING EXAMPLES

- [0054] Exemplary Test 1: Evaluation of Human Dermal Papilla Cell VEGF Gene Expression

- [0055] The VEGF gene is expressed in dermal papilla cells, and contributes to expression of such effects as increase in hair shaft diameter and improving maximum hair shaft length and improving hair shaft elongation rate and hair shaft growth promotion at head hair, beard, eyebrows, and/or eyelashes. Human dermal papilla cells were therefore used, and evaluation was carried out with respect to increase in VEGF gene expression for the respective components.

- [0056] 1. Materials and Methods

(1) Human Dermal Papilla Cells and Culture Medium

Human dermal papilla cells (Catalog No. CA60205a; Caucasian; derived from 29-year-old male; Toyobo Co., Ltd. (Japan)) were purchased, testing and evaluation being carried out with maintenance and culture of cells being performed as described in the protocol.

- [0057] (2) Drugs

As drugs for testing, drug solutions of the following respective concentrations (final concentrations) were prepared and used.

Comparative Example 1: 0.1% solution of mixture of palmitoyl dipeptide-5 diaminobutyloyl hydroxythreonine and palmitoyl dipeptide-5 diaminohydroxybutyrate

Comparative Example 2: 3% solution of mixture of palmitoyl dipeptide-5
diaminobutyloyl hydroxythreonine and palmitoyl dipeptide-5
diaminohydroxybutyrate

Working Example 1: 0.3125 μ M phytosphingosine

Working Example 2: 1.25 μ M phytosphingosine

Working Example 3: 0.1% solution of mixture of palmitoyl dipeptide-5
diaminobutyloyl hydroxythreonine and palmitoyl dipeptide-5
diaminohydroxybutyrate and of 1.25 μ M phytosphingosine

Working Example 4: 3% solution of mixture of palmitoyl dipeptide-5
diaminobutyloyl hydroxythreonine and palmitoyl dipeptide-5
diaminohydroxybutyrate and of 1.25 μ M phytosphingosine

[0058] (3) Test Procedure

A 24-well plate was seeded with human dermal papilla cells so as to obtain 6×10^3 thereof per well. Following culture for 1 day within a CO₂ incubator (5% CO₂; 37° C), the culture medium was replaced with culture medium which contained the respective drugs for testing. The cell plate was thereafter returned to the CO₂ incubator, and this was further cultured for 72 hours. Following culture, total RNA was extracted from the respective wells and was recovered, and this was reverse-transcribed into cDNA. The cDNA that was prepared was used to measure the VEGF gene expression of each thereof in accordance with the real-time PCR method. The GAPDH gene was used as an internal standard, the amount of VEGF gene expression being calculated relative to the negative control group.

[0059] A FastGene RNA Basic Kit (Catalog No. FG-80250; Nippon Genetics Co., Ltd. (Japan)) was used to recover total RNA from cells.

[0060] 300 μ L of lysis buffer RL was added thereto per well, and the cells were lysed by pipetting. 300 μ L of 70% ethanol was added to the cell lysate, and this was mixed by pipetting. The sample solution was added to a FastGene RNA binding column, and this was centrifuged at room temperature for 1 minute at 10000 rpm. The filtrate that passed through the column was discarded from the collection tube, and after returning the FastGene RNA binding column to its original collection tube, 600 μ L of wash buffer RW1 was added to the FastGene RNA binding

column, and this was centrifuged at room temperature for 1 minute at 10000 rpm. The FastGene RNA binding column was transferred to a new collection tube that was placed thereat, 700 μ L of wash buffer RW2 was added to the FastGene RNA binding column, and this was centrifuged at room temperature for 1 minute at 10000 rpm. The FastGene RNA binding column was transferred to a new collection tube that was placed thereat, and this was centrifuged at room temperature for 1 minute at 15000 rpm. The FastGene RNA binding column was transferred to a new collection tube that was placed thereat, 50 μ L of elution buffer RE was added at the center of the membrane of the FastGene RNA binding column, and this was centrifuged at room temperature for 1 minute at 10000 rpm to recover the purified RNA. Concentration of the recovered RNA was measured using a NanoDrop Lite (Catalog No. ND-LITE; Thermo Fisher Scientific K.K.), and this was stored at -80° C until the following cDNA creation procedure.

[0061] A FastGene scriptase II cDNA synthesis 5 $\times$ Ready Mix (Catalog No. NE-LS64; Nippon Genetics Co., Ltd. (Japan)) was used to synthesize cDNA. Dilution with RNase Free Water was carried out so as to cause concentration of total RNA produced in a new tube to be 20 ng/mL, 4 μ L of FastGene scriptase II cDNA synthesis 5 $\times$ Ready Mix was added to 16 μ L of this sample solution, and this was agitated by vortexing. A MiniAmp thermal cycler (Thermo Fisher Scientific K.K.) was used to incubate this at 25° C for 10 minutes, 42° C for 60 minutes, and 85° C for 5 minutes to synthesize cDNA.

[0062] The cDNA that was synthesized in accordance with the foregoing method was used to carry out real-time PCR. At prescribed wells in a 96-well plate, respective dilute solutions of cDNA template were added, Thunderbird SYBR qPCR Mix (Catalog No. QPS-201; Toyobo Co., Ltd. (Japan)) and primer were added thereto and mixed therewith, and gene expression was analyzed using a QuantStudio 7 Flex Real-Time PCR System (Catalog No. 4485693; Thermo Fisher Scientific K.K.). The PCR reaction was such that 40 cycles of 95° C for 5 seconds, 60° C for 30 seconds, and 72° C for 30 seconds were carried out.

[0063] Primers specific for the GAPDH gene which was used as internal standard, and primers specific for the VEGF gene, these having been used for testing, are indicated below.

Primers for detecting VEGF gene expression

Forward: aggccagcacataggagaga (Sequence No. 1)

Reverse: acgcgagtctgtgttttgc (Sequence No. 2)

Primers for detecting GAPDH gene expression

Forward: catccctgcctctactggcgctgcc (Sequence No. 3)

Reverse: ccaggatgcccttgagggggccctc (Sequence No. 4)

[0064] Relative amounts of expression of the respective genes were calculated as follows.

For each gene, Ct value (number of PCR cycles) was calculated based on the intersection of the amplification curve with the threshold line. The relative amount of expression is the target gene Ct value less the internal standard GAPDH gene Ct value.

[0065] 2. Results

The change in the amount of expression of the VEGF gene after phytosphingosine, a mixture of palmitoyl dipeptide-5 diaminobutyloyl hydroxythreonine and palmitoyl dipeptide-5 diaminohydroxybutyrate, and phytosphingosine and a mixture of palmitoyl dipeptide-5 diaminobutyloyl hydroxythreonine and palmitoyl dipeptide-5 diaminohydroxybutyrate, were allowed to act on human dermal papilla cells for 72 hours was measured, the results thereof being shown in FIG. 1.

[0066] As shown in FIG. 1, it was found that causing phytosphingosine to act for 72 hours on human dermal papilla cells resulted in an increase in dosage-dependent fashion in the amount of expression of the VEGF gene as compared with the control group at which nothing had been added (Working Examples 1 and 2). In addition, it was found that increasing the amount of phytosphingosine that was added thereto caused the amount of expression of the VEGF gene to be significantly greater than that which was produced as a result of action of adenosine (Comparative Example 1). Moreover and in addition, it was found that causing a liquid mixture of phytosphingosine and a mixture of palmitoyl dipeptide-5 diaminobutyloyl hydroxythreonine and palmitoyl dipeptide-5 diaminohydroxybutyrate to act for 72 hours on human dermal papilla cells resulted in increase in the amount of expression of the VEGF gene that was

significantly more marked than was the case when only phytosphingosine or a mixture of palmitoyl dipeptide-5 diaminobutyloyl hydroxythreonine and palmitoyl dipeptide-5 diaminohydroxybutyrate was allowed to act thereon (Working Examples 3 and 4).

[0067] It was thus found that phytosphingosine was useful, in dermal papilla cells, as an active ingredient in a hair growth agent and scalp care agent exhibiting effects in terms of increasing expression of the VEGF gene and causing increase in hair shaft diameter and improving maximum hair shaft length and improving hair shaft elongation rate and hair shaft growth promotion at head hair, beard, eyelashes, eyebrows, and/or other such hair.

Moreover, as compared with the situation when either palmitoyl dipeptide-5 diaminobutyloyl hydroxythreonine and palmitoyl dipeptide-5 diaminohydroxybutyrate, or phytosphingosine, were respectively used alone as active ingredient(s), it was found that use of phytosphingosine in combination with a mixture of palmitoyl dipeptide-5 diaminobutyloyl hydroxythreonine and palmitoyl dipeptide-5 diaminohydroxybutyrate caused further increase in hair growth action and more marked increase in expression of the VEGF gene in dermal papilla cells, and that these were useful as active ingredients in a hair growth agent and scalp care agent exhibiting effects in terms of causing increase in hair shaft diameter and improving maximum hair shaft length and improving hair shaft elongation rate and hair shaft growth promotion at head hair, beard, eyelashes, eyebrows, and/or other such hair.

INDUSTRIAL UTILITY

[0068] As a result of causing active ingredient(s) in a hair growth agent to contain both phytosphingosine and adenosine, or for phytosphingosine and adenosine to be used in combination therein, a means in accordance with the present invention makes it possible to provide a novel scalp care agent and hair growth agent that produce more marked increase in the amount of expression of the VEGF gene in dermal papilla cells and that also exhibit scalp care effect as well as effect in terms of improving maximum hair shaft length and effect in terms of improving hair shaft elongation rate and hair

shaft growth promotion effect at head hair, beard, eyelashes eyebrows, and/or other such hair.

CLAIMS

1. A hair growth agent characterized in that it contains palmitoyl dipeptide-5 diaminobutyloyl hydroxythreonine, palmitoyl dipeptide-5 diaminohydroxybutyrate, and phytosphingosine as active ingredients.
2. A hair growth agent for use in combination with phytosphingosine, characterized in that it contains palmitoyl dipeptide-5 diaminobutyloyl hydroxythreonine and palmitoyl dipeptide-5 diaminohydroxybutyrate as active ingredients.
3. A hair growth agent for use in combination with palmitoyl dipeptide-5 diaminobutyloyl hydroxythreonine and palmitoyl dipeptide-5 diaminohydroxybutyrate, characterized in that it contains phytosphingosine as active ingredient.
4. The hair growth agent according to any one among claim 1 through claim 3 for use in causing new hair growth or hair shaft growth promotion.
5. The hair growth agent according to any one among claim 1 through claim 3 used for causing improvement in hair shaft elongation rate.
6. The hair growth agent according to any one among claim 1 through claim 3 used for causing improvement in maximum hair shaft length.
7. The hair growth agent according to any one among claim 1 through claim 3 used for causing increase in hair shaft diameter.
8. The hair growth agent according to any one among claim 1 through claim 3 used for causing increase in number of hairs.
9. The hair growth agent according to any one among claim 1 through claim 3 in liquid solution form.
10. The hair growth agent according to any one among claim 1 through claim 3 for use on head hair, beard, eyelashes, and/or eyebrows.
11. A method of manufacturing a hair growth agent, characterized by presence of an operation in which palmitoyl dipeptide-5 diaminobutyloyl hydroxythreonine and palmitoyl dipeptide-5 diaminohydroxybutyrate, and phytosphingosine, are made to be contained as active ingredients in a pharmaceutical preparation.
12. A method of manufacturing a hair growth agent for use in combination with phytosphingosine, characterized by presence of an operation in which palmitoyl

dipeptide-5 diaminobutyloyl hydroxythreonine and palmitoyl dipeptide-5 diaminohydroxybutyrate are made to be contained as active ingredients in a pharmaceutical preparation.

13. A method of manufacturing a hair growth agent for use in combination with palmitoyl dipeptide-5 diaminobutyloyl hydroxythreonine and palmitoyl dipeptide-5 diaminohydroxybutyrate, characterized by presence of an operation in which phytosphingosine is made to be contained as active ingredient in a pharmaceutical preparation.
14. A hair growth agent kit that comprises a pharmaceutical preparation which contains phytosphingosine, and a pharmaceutical preparation which contains palmitoyl dipeptide-5 diaminobutyloyl hydroxythreonine and palmitoyl dipeptide-5 diaminohydroxybutyrate.
15. A hair growth agent kit for use in combination with phytosphingosine, which comprises a pharmaceutical preparation that contains palmitoyl dipeptide-5 diaminobutyloyl hydroxythreonine and palmitoyl dipeptide-5 diaminohydroxybutyrate.
16. A hair growth agent kit for use in combination with palmitoyl dipeptide-5 diaminobutyloyl hydroxythreonine and palmitoyl dipeptide-5 diaminohydroxybutyrate, which comprises a pharmaceutical preparation that contains phytosphingosine.
17. A hair growth method characterized by administration of palmitoyl dipeptide-5 diaminobutyloyl hydroxythreonine, palmitoyl dipeptide-5 diaminohydroxybutyrate, and phytosphingosine as active ingredients.
18. A hair growth method comprising administering the hair growth agent according to any one among claim 1 through claim 3 to a subject.