US006423327B1

(12) **United States Patent**
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(10) **Patent No.: US 6,423,327 B1**
(45) **Date of Patent: Jul. 23, 2002**

(54) **TREATMENT OF SKIN WITH ADENOSINE OR ADENOSINE ANALOG**

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(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **09/672,348**

(22) Filed: **Sep. 28, 2000**

Related U.S. Application Data

(63) Continuation of application No. 09/179,006, filed on Oct. 26, 1998, now abandoned.

(51) **Int. Cl.**[7] **A61K 7/00**

(52) **U.S. Cl.** **424/401**; 424/447; 424/448; 424/449; 514/46

(58) **Field of Search** 424/407, 447, 424/448, 449; 514/46

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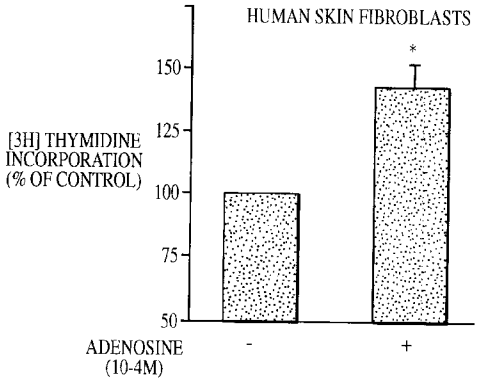
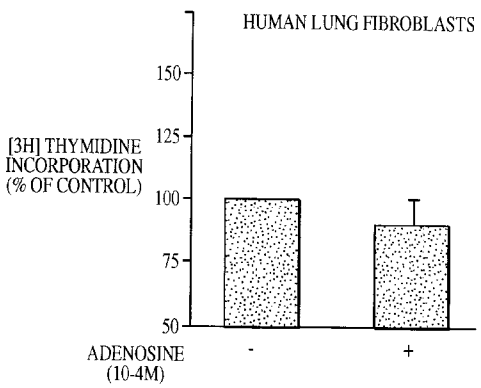
(57) **ABSTRACT**

Methods for enhancing the condition of non-diseased skin by application of compositions containing adenosine or an adenosine analog are disclosed. Also disclosed are methods for increasing DNA synthesis or protein synthesis in dermal cells, and methods for increasing dermal cell size, by application of compositions containing adenosine.

10 Claims, 2 Drawing Sheets





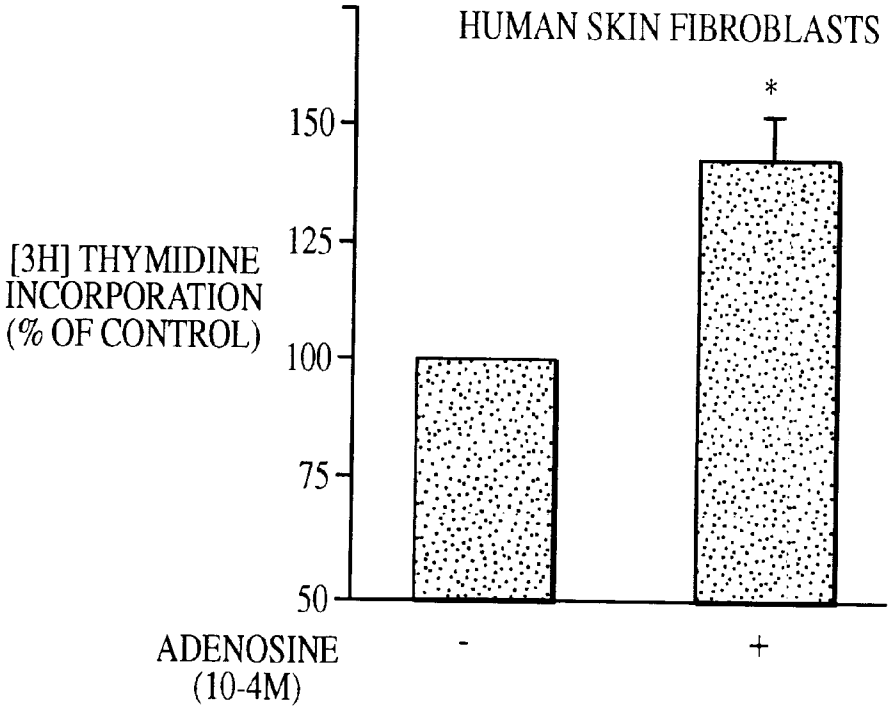


FIG. 1A

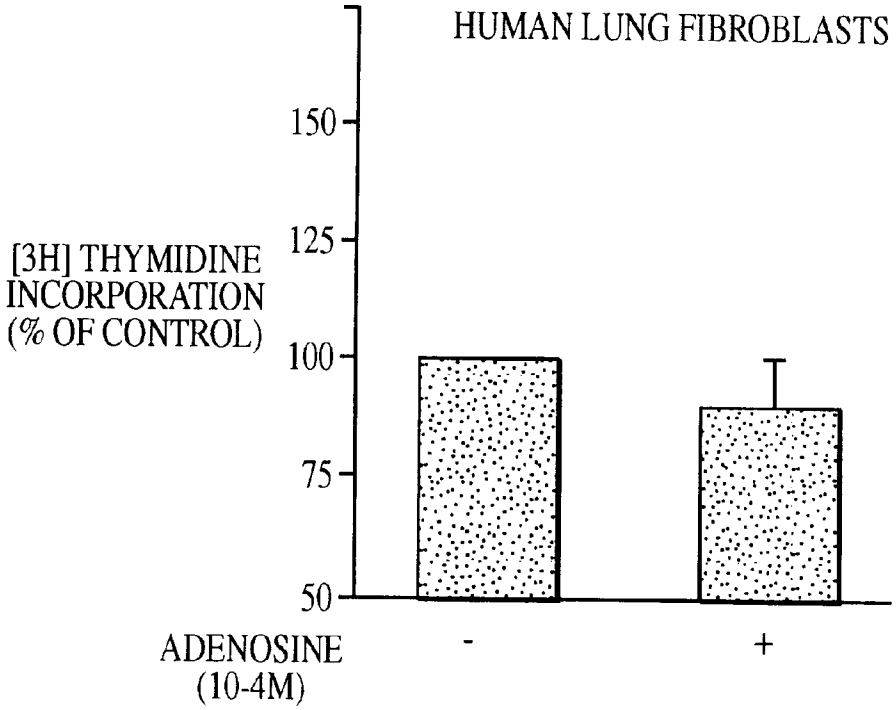


FIG. 1B

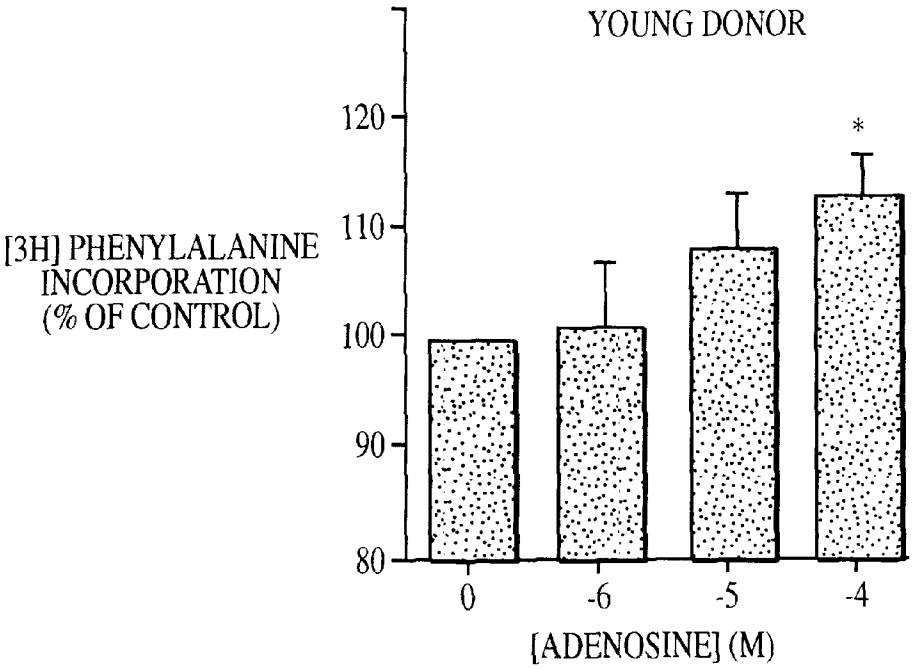


FIG. 2A

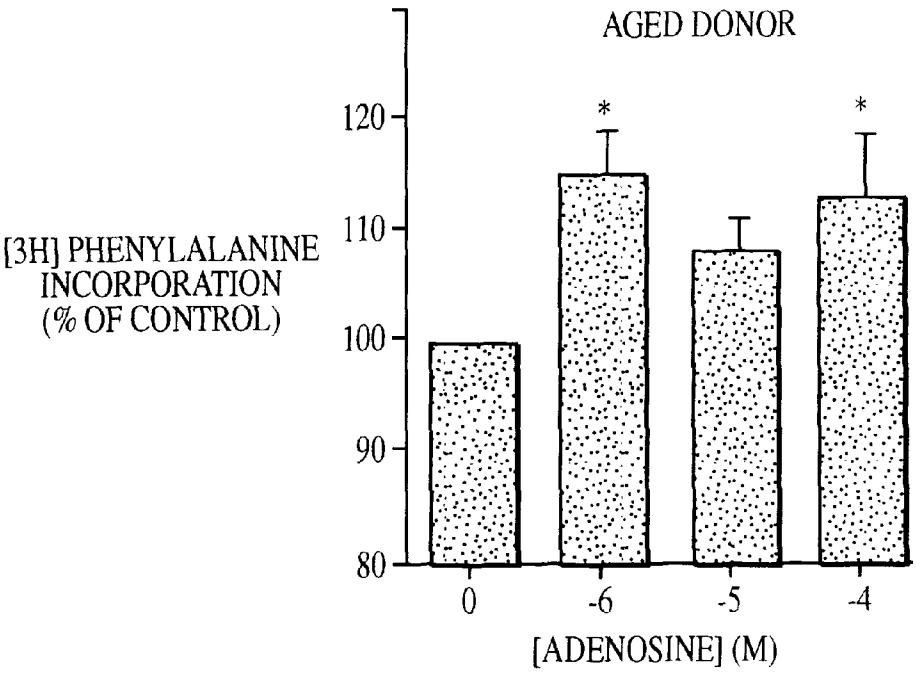


FIG. 2B

TREATMENT OF SKIN WITH ADENOSINE OR ADENOSINE ANALOG

This application is a continuation of application Ser. No. 09/179,006, filed Oct. 26, 1998, now abandoned.

STATEMENT AS TO FEDERALLY SPONSORED RESEARCH

Work on this invention was supported by funds from the United States government (Public Health Service Grants HL-22828 and AG-11491). The government therefore has certain rights in this invention.

FIELD OF THE INVENTION

This invention relates to dermatology and cell biology.

BACKGROUND OF THE INVENTION

Skin includes a surface layer, known as the epidermis, and a deeper connective tissue layer, known as the dermis. The epidermis undergoes continuous turnover as the outermost cells are exfoliated and replaced by cells that arise from inner dermal layers. The dermis is composed of a variety of cell types, including fibroblasts.

Skin thickness begins to decline in humans after the age of 20 as the dermis becomes thinner and the number of skin fibroblasts declines. As skin ages, or is exposed to UV light and other environmental insults, changes in the underlying dermis can lead to the functional and morphological changes associated with damaged skin. Decreases in the abundance and function of products of the fibroblasts, which include collagen and proteoglycans, are believed to play major roles in wrinkled and damaged skin.

SUMMARY OF THE INVENTION

We have discovered that adenosine stimulates DNA synthesis, increases protein synthesis, and increases cell size in cultures of human skin fibroblasts. Based on this discovery, the invention provides methods and compositions for enhancing the condition of skin.

In general, the invention provides a method for enhancing the condition of non-diseased skin of a mammal, e.g., a human. The method includes topically applying a therapeutically effective amount of a composition including adenosine or an adenosine analog to non-diseased skin of the mammal.

The invention also provides a method for promoting healing of broken, non-diseased skin in a mammal by topically administering a composition including a therapeutically effective amount of adenosine or an adenosine analog to the mammal.

Also included in the invention is a method for increasing DNA synthesis in a dermal cell of non-diseased skin of a mammal. The method includes topically administering a therapeutically effective amount of adenosine or an adenosine analog to a region of non-diseased skin of the mammal containing dermal cell. The adenosine is added so that it does not cause proliferation of the dermal cell.

The invention also features a method of increasing protein synthesis in a dermal cell of non-diseased skin of a mammal. The method includes topically administering a composition including a therapeutically effective amount of adenosine or an adenosine analog to a region of skin of the mammal containing the dermal cell. The adenosine or adenosine analog does not cause proliferation of the dermal cell.

Also provided in the invention is a method of increasing cell size in a dermal cell in non-diseased skin of a mammal, e.g., a human. The method includes topically administering a composition including a therapeutically effective amount of adenosine to a region of skin of the mammal containing the dermal cell, wherein addition of the adenosine does not cause proliferation of the dermal cell, wherein addition of the adenosine does not cause proliferation of the dermal cell.

The invention also includes a method for enhancing skin condition in a mammal, e.g., a human. The method includes providing fibroblasts from the mammal ex vivo, culturing the fibroblasts in the presence of adenosine, and reintroducing the fibroblasts into the mammal.

The therapeutically effective amount of adenosine used in the above-described methods is preferably 10^{-3} M to 10^{-7} M, more preferably 10^{-3} M to 10^{-6} M, and most preferably about 10^{-4} M.

The composition used in the above-described methods can include a second agent in addition to adenosine. The second agent can be, e.g. an agent that promotes binding of adenosine or an adenosine analog to an adenosine receptor, an angiogenic factor such as vascular endothelial cell growth factor (VEGF), basic fibroblast growth factor (BFGF), an agent that itself enhances skin condition, such as tretoinin or another known conditioning agent such as an emollient, a humectant, or an occlusive agent.

In preferred embodiments of the invention, the adenosine or an adenosine analog does not promote skin cell proliferation.

The invention also provides a composition including about 10^{-3} M to about 10^{-7} M adenosine and a therapeutically effective amount of an angiogenesis factor. In some embodiments, the composition of the adenosine is about 10^{-4} M.

As. used herein, "enhancement of skin condition" means a noticeable decrease in the amount of wrinkling, roughness, dryness, laxity, sallowness, or pigmentary mottling in skin.

As used herein, a "therapeutically effective amount" of adenosine or an adenosine analog means an amount that enhances skin condition when applied to skin.

As used herein, "non-diseased skin" means skin free of any proliferative disorder observable by visual inspection.

The present invention advantageously allows for enhancement of skin condition. This results in skin that shows a less wrinkled, rough, or dry complexion. For example, the invention provides for enhancing the condition of skin damaged due to exposure to the sun or skin whose condition has deteriorated due to normal aging.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

Other features and advantages of this invention will be apparent from the following description of-the preferred embodiments thereof, and from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGS. **1A** and **1B** are histograms showing the effect of adenosine on [^{3}H]thymidine incorporation in cultures of

normal human skin (FIG. **1**A) and lung fibroblasts (FIG. **1**B). After incubation in serum-free medium for 24 hours, cells were exposed to 10^{-4} M adenosine for 18 hours. Medium was replaced with serum-free medium without adenosine, and [^{3}H]thymidine was added. Results are expressed as percent [^{3}H]thymidine incorporation compared to control cultures without adenosine and are means±SEM for 4–5 experiments. "*" denotes value was significantly different from control value without adenosine.

FIGS. **2**A and **2**B are histograms showing concentration responses of adenosine-stimulated protein synthesis in human skin fibroblasts from a young (FIG. **2**A) and aged (FIG. **2**B) donor. Cells were grown to 75% confluence. Medium was then replaced with serum-free medium with or without adenosine. After 48 hours, [^{3}H]phenylalanine incorporation was determined as described. Results are expressed as % [^{3}H]phenylalanine incorporation compared to control cultures without adenosine and are means±SEM for 6–25 experiments. "*" denotes value was significantly different from control value without adenosine.

DETAILED DESCRIPTION

The invention is suitable for treating skin of a mammal, e.g., a human, for which promotion of fibroblast-associated dermal functions is desired. For example, promotion of fibroblast-associated functions is desirable in enhancing the condition of aged skin, which is associated with a decrease in dermal cell function and is characterized by increased dryness or roughness, or both. The method can also be used on subjects having otherwise damaged skin, e.g., wrinkled skin and skin with a non-proliferative disorder. The method can may further be used prophylactically on a subject to minimize deterioration of skin condition associated with aging or environmental factors, such as photodamage.

Adenosine and suitable adenosine analogs are suitable for use in enhancing skin condition. Adenosine analogs such as adenosine agonists, adenosine receptor agonists, and compounds that increase intracellular or extracellular adenosine levels are suitable for use in the invention.

Agonists of adenosine include 2'-deoxyadenosine; 2', 3'-isopropoylidene adenosine; toyocamycin; 1-methyladenosine; N-6-methyladenosine; adenosine N-oxide; 6-methylmercaptopurine riboside; 6-chloropurine riboside, 5'adenosine monophosphate, 5'-adenosine diphosphate, or 5'-adenosine triphosphate. Adenosine receptor agonists include phenylisopropyl-adenosine ("PIA"), 1-Methylisoguanosine, ENBA (S(-), N^6-Cyclohexyladenosine (CHA), N^6-Cyclopentyladenosine (CPA), 2-Chloro-N6-cyclopentyladenosine, 2-chloroadenosine, and adenosine amine congener (ADAC), all of which are agonists for the adenosine A_1 receptor. Other receptor agonists include 2-p-(2-carboxy-ethyl) phenethyl-amino-5'-N-ethylcarboxamido-adenosine (CGS-21680), N-ethylcarboxamido-adenosine (NECA) and napthyl-substituted aralkoxyadenosine (SHA-082), 5' (N-Cyclopropyl)-carboxamidoadenosine, DPMA (PD 129, 944), Metrifudil, which are agonists for the adenosine A_2 receptor. Other adenosine receptor agonists include those which preferentially bind the A_1 receptor relative to the A_2 receptor, such as 2-Chloroadenosine, N_6-Phenyladenosine, and N^6-Phenylethyladenosine; and those which preferentially bind the A_2 receptor relative to the A_1 receptor, such as 2-Phenylaminoadenosine and MECA.

Also suitable for use are compounds that increase intracellular adenosine concentration by inhibiting the cellular uptake of adenosine or the breakdown of adenosine. One pathway of adenosine metabolism is the conversion of adenosine to inosine by adenosine deaminase. An example of an adenosine deaminase inhibitor is erythro-9-(2-hydroxy-3-nonyl) adenine ("EHNA"). Adenosine kinase inhibitors can also be used. Adenosine kinase converts adenosine to adenosine monophosphate by adenosine kinase. An example of an adenosine kinase inhibitor is iodotubercidin. Other suitable compounds include those that inhibit the dipyridamole-sensitive nucleoside transporter, which exports adenosine from the cytoplasm, and agents that promote the activity of a 5'-nucleotidase, e.g., the ATP-activated 5'-nucleotidase, which forms adenosine. Compounds that increase tissue adenosine and ATP levels include acadesine (AICA-riboside), which is described in Gruber et al., Circulation 80:1400–1411 (1989).

Adenosine can be also be administered with a second compound. The second compound can enhance the action of adenosine or the adenosine analog, e.g., by enhancing binding of adenosine or an adenosine analog to an adenosine receptor. An example of such a compound is PD 81, 728, which is described in Kollias-Baker et al. J. Pharmacol. Exp. Ther. 281:761–68. Alternatively, the second agent can itself act to enhance skin condition. Examples of these types of agents include tretinoin, a recognized skin conditioning agent (see, e.g., Olsen et al., J. Amer. Acad. Dermatol. 37:217–26, 1997), an angiogenic factor such as vascular endothelial cell growth factor (VEGF) or basic fibroblast growth factor (BFGF), or a conditioning agent.

The second compound can also be a conditioning agent such as an emollient, humectant, or occlusive agent. Numerous examples of particular conditioning agents are provided in the CTFA Cosmetic Ingredient Handbook (Cosmetic Toiletries and Fragrances Association, Washington, D.D., 1988). Emollients help to maintain the soft, smooth, and pliable appearance of skin and function by remaining on the skin surface or in the stratum corneum to act as lubricants, to reduce flaking, and to improve the skin's appearance. Examples of emollients include acetyl trioctyl citrate, cetyl alcohol, butyl myristate, cetyl alcohol, and mineral oil.

Humectants act to increase the water content of the top layers of the skin. Humectants include, e.g., acetamide MEA, fructose, and xylitol. Occlusive agents inhibit the evaporation of water from skin, thereby increasing the water contend of the skin. Acetylated castor oil, mineral oil, and lauryl stearate are examples of occlusive agents.

A subject can be treated by applying adenosine or an adenosine analog in a pharmaceutical composition in an effective amount and for a period of time sufficient to improve the condition of the skin.

The pharmaceutical composition may be formulated using conventional methods to prepare pharmaceutically useful compositions. Such compositions preferably include at least one pharmaceutically acceptable carrier, such as those described in Remington's Pharmaceutical Sciences (E. W. Martin). In addition, the compositions preferably include a pharmaceutically acceptable buffer, preferably phosphate buffered saline, together with a pharmaceutically acceptable compound for adjusting isotonic pressure, such as, for example, sodium chloride, mannitol, or sorbitol.

Adenosine or an adenosine agonist can also be provided in carriers and adjuvants such as ion exchangers, alumina, aluminum stearate, lecithin, serum proteins, such as human serum albumin, buffer substances, such as phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes, such as protamine sulfate, disodium hydrogen

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