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(71) Applicant (for all designated States except US): **OTSUKA PHARMACEUTICAL CO., LTD.** [JP/JP]; 9, Kanda-Tsukasamachi 2-chome, Chiyoda-ku, Tokyo, 1018535 (JP).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **SHINOHARA, Shigeo** [JP/JP]; c/o OTSUKA PHARMACEUTICAL CO., LTD., 1-7-1, Doshomachi, Chuo-ku, Osaka-shi, Osaka, 5410045 (JP). **IGARASHI, Sachiyo** [JP/JP]; c/o OTSUKA PHARMACEUTICAL CO., LTD., 1-7-1, Doshomachi, Chuo-ku, Osaka-shi, Osaka, 5410045 (JP). **KAWAMURA, Mitsuki** [JP/JP]; c/o OTSUKA PHARMACEUTICAL CO., LTD., 1-7-1, Doshomachi, Chuo-ku, Osaka-shi, Osaka, 5410045 (JP). **TANAKA, Masahiko** [JP/JP]; c/o OTSUKA PHARMACEUTICAL CO., LTD., 1-7-1, Doshomachi, Chuo-ku, Osaka-shi, Osaka, 5410045 (JP). **FURUTA, Yasuo** [JP/JP]; c/o OTSUKA PHARMACEUTICAL CO., LTD., 1-7-1, Doshomachi, Chuo-ku, Osaka-shi, Osaka, 5410045 (JP). **WAKAMATSU, Kosaburo** [JP/JP]; c/o OTSUKA PHARMACEUTICAL CO., LTD., 1-7-1, Doshomachi, Chuo-ku, Osaka-shi, Osaka, 5410045 (JP). **HARANO,**

Fumiki [JP/JP]; c/o OTSUKA PHARMACEUTICAL CO., LTD., 1-7-1, Doshomachi, Chuo-ku, Osaka-shi, Osaka, 5410045 (JP). **TAKASU, Osamu** [JP/JP]; c/o OTSUKA PHARMACEUTICAL CO., LTD., 1-7-1, Doshomachi, Chuo-ku, Osaka-shi, Osaka, 5410045 (JP).

(74) Agent: **Saegusa & Partners**; Kitahama TNK Building, 1-7-1, Doshomachi, Chuo-ku, Osaka-shi, Osaka, 5410045 (JP).

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(54) Title: AGENT FOR PREVENTING OR TREATING ABNORMALITY IN SKIN WATER PERMEATION FUNCTION

(57) Abstract: An object of the present invention is to provide a skin water permeation function enhancer that can adjust TEWL according to the skin condition to thereby normalize skin water permeation function, thus enhancing skin water content regulation effect. Purine nucleic acid have an action of properly adjusting TEWL according to the skin condition, and are therefore used as an active ingredient of a skin water permeation function enhancer.



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DESCRIPTION

Title of Invention: AGENT FOR PREVENTING OR TREATING ABNORMALITY
IN SKIN WATER PERMEATION FUNCTION

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TECHNICAL FIELD

The present invention relates to an agent for preventing or treating an abnormality in skin water permeation function. The present invention further relates to an agent for adjusting TEWL (transepidermal water loss) value, the amount of water evaporated from the skin, to normal levels. Further, the present invention relates to a method for preventing or treating an abnormality in skin water permeation function, for adjusting TEWL value, for preventing or treating abnormal TEWL in skin, or for improving skin water permeation function; and uses thereof.

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BACKGROUND ART

The skin plays several roles. The principal roles of the skin are providing protection from UV rays, foreign substances, microorganisms, etc., and providing water content adjustment function of the outermost skin layer, which contribute to skin homeostasis.

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The skin normally has an appropriate level of elasticity, flexibility, and strength, and can protect itself against physical force. However, for this ability to function, the skin must contain an appropriate amount of water, and be flexible.

25

However, because the skin is located as the outermost layer, it is very likely to become dry. The outer skin layer contains natural moisturizing factors (NMF) and ceramides, whose combined effects enable the entire skin to maintain an appropriate water content and keep the skin flexible.

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It is known that when an excessive amount of water is evaporated from the skin, the skin is dried, which causes aging, wrinkles, etc. of the skin.

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Further, it is known that outer skin layer thickening or deposition also roughens the skin surface and causes drying. This is because reduction of cell function causes outer skin layer thickening or deposition, and thus reduces skin water permeability. Therefore, in recent years, to ameliorate outer layer thickening or deposition, the skin has been peeled off by chemical peeling etc. However, such peeling often removes an excessive amount of the skin, and causes skin damage.

It is also known that aging reduces water permeability and thus causes senile xerosis, which is accompanied by itching, chapped skin, etc.

However, temporary symptomatic therapy, such as coating the skin to retain water, has been widely used for the above-mentioned skin disorders. Since such a therapy causes reduction of TEWL by inhibiting skin water permeation function, the water content regulation function inherent in the skin is impaired. As described above, a wide variety of research has been conducted to develop a technique for retaining water in the outer skin layer. However, there has been substantially no research in the prior art on a component that can increase or decrease skin water permeation function according to the skin condition to thereby improve skin water content regulation function and thus enable the skin to exhibit effective function that is inherent in the skin.

In general, when the TEWL value rises, water evaporation from the skin increases, which causes dryness of the skin. On the other hand, moisture retention involves the suppression of the TEWL value and the inhibition of water permeation through the skin to thereby control water evaporation from the skin. The human skin has portions that are susceptible to dryness, eczema, or the like. Each skin site has different properties. However, it is difficult to selectively use, according to the skin site, a preparation that is effective for increasing or reducing the TEWL value. Therefore, the development of a single preparation that can both increase and decrease the

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TEWL value according to the skin site to adjust the TEWL value to an appropriate level has long been desired.

With such prior art as the background, the development of a component that can adjust the TEWL of the skin to appropriate levels and improve skin water permeation function has been long desired.

CITATION LIST

PATENT LITERATURE

PTL 1: Japanese Unexamined Patent Publication No. 2007-246459

SUMMARY OF INVENTION

TECHNICAL PROBLEM

An object of the present invention is to provide an agent for preventing or treating an abnormality in skin water permeation function that can adjust TEWL according to the skin condition to thereby normalize skin water permeation function and enhance skin water content regulation effect. Another object of the present invention is to provide an agent for adjusting TEWL value that appropriately evaporates water from the epidermis, while maintaining an appropriate skin water content. Further, another object of the present invention is to provide an agent for preventing or treating abnormal TEWL in skin, or for improving skin water permeation function.

SOLUTION TO PROBLEM

The present inventors conducted extensive research to achieve the above objects. As a result, the inventors found that purine nucleic acid has an effect of appropriately adjusting TEWL value according to the skin condition. More specifically, the inventors found that purine nucleic acid can increase TEWL value to normal levels when the skin has reduced TEWL, and can also decrease TEWL to normal levels when the skin has excessive TEWL; accordingly, purine nucleic acid can prevent or treat an abnormality in skin water permeation function. As a result, the

inventors found that the adjustment of TEWL value by purine nucleic acid can prevent or ameliorate abnormal skin conditions, such as rough skin, itching, and chapped skin; and is also effective against skin diseases such as hyperkeratosis and senile xerosis. The inventors further found that purine nucleic acid has an effect of appropriately evaporating water from the epidermis, while maintaining an appropriate water content of the skin. The present inventors conducted further research based on these findings, and accomplished the present invention.

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More specifically, the present invention provides an agent for preventing or treating an abnormality in skin water permeation function having the following features.

Item 1-1. An agent for preventing or treating an abnormality in skin water permeation function comprising, at least one purine nucleic acid as an active ingredient.

Item 1-2. The agent for preventing or treating an abnormality in skin water permeation function according to Item 1-1 wherein the at least one purine nucleic acid is selected from the group consisting of adenosine monophosphates and salts thereof.

Item 1-3. The agent for preventing or treating an abnormality in skin water permeation function according to Item 1-1 containing the purine nucleic acid in an amount of 0.1 to 20 wt.%.

Item 1-4. The agent for preventing or treating an abnormality in skin water permeation function according to Item 1-1 which is used to adjust TEWL (transepidermal water loss) value.

Item 1-5. The agent for preventing or treating an abnormality in skin water permeation function according to Item 1-1 which is used to prevent or treat a skin condition or a skin disease with abnormal TEWL value.

Item 1-6. The agent for preventing or treating an abnormality in skin water permeation function according to Item 1-1, wherein the abnormality in skin water permeation function is due to aging.

Item 1-7. The agent for preventing or treating an abnormality in skin water permeation function according to Item 1-1 which is

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used to prevent or treat rough skin, itching, chapped skin, hyperkeratosis, or senile xerosis.

Item 1-8. The agent for preventing or treating an abnormality in skin water permeation function according to Item 1-1 which is in
5 the form of a cosmetic, or a pharmaceutical or quasi-drug for external application to the skin.

Item 1-9. The agent for preventing or treating an abnormality in skin water permeation function according to Item 1-1 which is used for non-therapeutic purpose.

10 Item 1-10. Use of at least one purine nucleic acid for manufacture of an external composition for preventing or treating an abnormality in skin water permeation function.

Item 1-11. Use of at least one purine nucleic acid, in an external composition, for preventing or treating an abnormality
15 in skin water permeation function.

Item 1-12. A purine nucleic acid for preventing or treating an abnormality in skin water permeation function.

Item 1-13. A method of preventing or treating an abnormality in skin water permeation function, comprising administering at least
20 one purine nucleic acid to a subject in need of preventing or treating an abnormality in skin water permeation function.

Further, the present invention provides an agent for adjusting TEWL (transepidermal water loss) value having the
25 following features.

Item 2-1. A agent for adjusting for TEWL (transepidermal water loss) value comprising t least one purine nucleic acid as an active ingredient.

Item 2-2. The agent for adjusting TEWL value according to Item 2-
30 1 wherein the purine nucleic acid is selected from the group consisting of adenosine monophosphate and salts thereof.

Item 2-3. The agent for adjusting TEWL value according to Item 2-1 containing the purine nucleic acid in an amount of 0.1 to 20 wt.%.

35 Item 2-4. The agent for adjusting TEWL value according to Item 2-

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1 which is used to improve skin water permeation function.

Item 2-5. The agent for adjusting TEWL value according to

Item 2-1 which is used to prevent or treat a skin condition or skin disease with abnormal TEWL value.

5 Item 2-6. The agent for adjusting TEWL value according to Item 2-1 which is used to prevent or treat rough skin, itching, chapped skin, hyperkeratosis, or senile xerosis.

Item 2-7. The agent for adjusting TEWL value according to Item 2-1 which is in the form of a cosmetic, or a pharmaceutical or
10 quasi-drug for external application to the skin.

Item 2-8. The agent for adjusting TEWL value according to Item 2-1 which is used for non-therapeutic purpose.

Item 2-9. Use of at least one purine nucleic acid for manufacture of an external composition for adjusting TEWL (transepidermal
15 water loss) value.

Item 2-11. Use of at least one purine nucleic acid, in an external composition, for adjusting TEWL (transepidermal water loss) value.

Item 2-12. A purine nucleic acid for adjusting TEWL
20 (transepidermal water loss) value.

Item 2-13. A method for adjusting TEWL (transepidermal water loss) value, comprising administering at least one purine nucleic acid to a subject in need of adjusting TEWL (transepidermal water loss) value.

25

Further, the present invention also provides following agents, use of the compounds, and methods.

Item 3-1. A agent for preventing or treating abnormal TEWL (transepidermal water loss) in skin comprising at least one
30 purine nucleic acid as an active ingredient.

Item 3-2. Use of at least one purine nucleic acid for manufacture of an external composition for preventing or treating abnormal TEWL (transepidermal water loss) in skin.

Item 3-3. Use of at least one purine nucleic acid, in an external
35 composition, for preventing or treating abnormal TEWL

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(transepidermal water loss) in skin.

Item 3-4. A purine nucleic acid for preventing or treating abnormal TEWL (transepidermal water loss) in skin.

Item 3-5. A method for preventing or treating abnormal TEWL
5 (transepidermal water loss) in skin, comprising administering at least one purine nucleic acid to a subject in need of preventing or treating abnormal TEWL.

Item 3-6. An agent for improving skin water permeation function comprising least one purine nucleic acid as an active ingredient.

10 Item 3-7. Use of at least one purine nucleic acid for manufacture of an external composition for improving skin water permeation function.

Item 3-8. Use of at least one purine nucleic acid, in an external composition, for improving skin water permeation function.

15 Item 3-9. A purine nucleic acid for improving skin water permeation function.

Item 3-10. A method of improving skin water permeation function, comprising administering at least one purine nucleic acid to a subject in need of restoring or maintaining skin water permeation
20 function..

ADVANTAGEOUS EFFECTS OF INVENTION

According to the present invention, TEWL value can be adjusted according to the skin condition to thereby normalize
25 skin water permeation function, thus enhancing skin water content regulation effect. Further, according to the present invention, skin conditions and skin diseases with abnormal skin water permeability, such as rough skin, itching, chapped skin, hyperkeratosis, and senile xerosis, can be prevented or treated.

30

BRIEF DESCRIPTION OF DRAWINGS

Fig. 1 shows the TEWL measurement results obtained in Test Example 3, indicating the changes in TEWL value at the skin site where a test sample containing AMP was applied and at the
35 skin site where the test sample was not applied.

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Fig. 2 shows the *stratum corneum* water content measurement results obtained in Test Example 3, indicating the changes in over time at the AMP-containing test sample application skin site, and at the non-application skin site.

5 Fig. 3a is a photograph of the skin surface condition of the non-application skin site, which was observed after 7 days from the start of the test in Test Example 3.

Fig. 3b is a photograph of the skin surface condition of the test sample application skin site, which was observed
10 after 7 days from the start of the test in Test Example 3.

DESCRIPTION OF EMBODIMENTS

A mode for carrying out the invention will be described below.

15 A feature of the agent for preventing or treating an abnormality in skin water permeation function of the present invention is containing at least one purine nucleic acid as an active ingredient. Hereinafter, "the agent for preventing or treating an abnormality in skin water permeation function of the
20 present invention" may be referred to as "the agent of the invention".

The "purine nucleic acid", used as an active ingredient in the present invention, is a general term that includes purine *per se*, various purine derivatives having a purine nucleus as the
25 skeleton, and salts thereof. purine nucleic acid as used herein are not particularly limited insofar as they are pharmaceutically or cosmetically acceptable. Examples of purine nucleic acid include adenine, guanine, deaminated adenine (i.e., hypoxanthine), deaminated guanine (i.e., xanthine), adenosine, guanosine,
30 inosine, adenosine phosphates (e.g., adenosine monophosphates such as adenosine 2'-monophosphate, adenosine 3'-monophosphate, and adenosine 5'-monophosphate; adenosine diphosphates such as adenosine 5'-diphosphate; adenosine triphosphates such as adenosine 5'-triphosphate), guanosine phosphates (i.e., guanosine
35 monophosphates such as guanosine 3'-monophosphate, and guanosine

5'-monophosphate; guanosine diphosphate such as guanosine 5'-diphosphate; and guanosine triphosphate such as guanosine 5'-triphosphate), adenylosuccinic acid, xanthic acid, inosinic acid, flavin adenine dinucleotide (FAD), nicotinamide adenine dinucleotide (NAD), and the like. In view of an excellent TWEL adjustment effect, adenosine phosphates are preferable, and adenosine monophosphates, particularly adenosine 5'-monophosphate (AMP), are more preferable. These purine nucleic acids can be used singly, or in a combination of two or more.

Salts used as the purine nucleic acid are not particularly limited insofar as they are pharmaceutically or cosmetically acceptable. Examples of such salts include alkali metal salts such as sodium salts and potassium salts; alkaline earth metal salts such as magnesium salts, calcium salts, and barium salts; basic amino acid salts such as arginine salts and lysine salts; ammonium salts such as ammonium salts and tricyclohexylammonium salts; various kinds of alkanolamine salts such as monoethanolamine salts, diethanolamine salts, triethanolamine salts, monoisopropanolamine salts, diisopropanolamine salts, and triisopropanolamine salts; etc. Among these salts, alkali metal salts are preferable, and sodium salts are particularly preferable.

The agent of the invention is applied to the skin to improve the water permeation function of the *stratum corneum*, and enhance skin water content regulation effect. Accordingly, the agent of the invention is provided as an external composition (an external preparation), such as a cosmetic, or a pharmaceutical or quasi-drug for external application to the skin, which is used to prevent or treat an abnormality in skin water permeation function, or adjust TEWL value.

The amount of the purine nucleic acid in the external composition can be suitably selected according to the form of the preparation, application site, desired effect, etc. More specifically, the amount of the purine nucleic acid in the external composition is 0.1 to 20 wt.%, preferably 0.5 to 10 wt.%,

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and more preferably 1 to 5 wt.%, based on the total amount of the external composition. The external composition, which contains a purine nucleic acid in the above-mentioned proportion, can exhibit a TEWL adjustment effect etc.

5 To form the external composition using the agent of the invention, the purine nucleic acid may be formulated with various optional components that are typically used in preparations for external application to the skin, such as pharmaceutically or cosmetically acceptable carriers and additives. Such carriers and
10 additives are known in the art, and amounts thereof can be suitably selected.

 Furthermore, the pH and osmotic pressure of the external composition can be suitably selected from the range that does not adversely affect low irritation to the skin or mucosa,
15 and good skin feel upon application.

 The form of the external composition is not particularly limited, insofar as it is in a form externally applicable to the skin, such as a cosmetic, or pharmaceutical or quasi-drug for external application to the skin. For example, the
20 agent of the invention can be provided as an external preparation in the form of a paste, mousse, gel, liquid, emulsion, suspension, cream, ointment, sheet, aerosol, spray, liniment, or like desired forms, by adding the above-mentioned optional components and further optionally adding other solvents, and bases or carriers
25 that are typically used in external preparations. Among these forms, liquids, emulsions, suspensions, and creams are preferable, and liquids and emulsions are particularly preferable. These products can be prepared by methods commonly used in the art.

 The agent of the invention is used to apply to the skin
30 of a mammal (including a human) having an abnormality in skin water permeation function. Examples of the abnormality in skin water permeation function include skin diseases and skin conditions with excess TEWL value or reduced TEWL value. Specific examples of the abnormality in skin water permeation function
35 include rough skin, itching, chapped skin, hyperkeratosis, senile

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xerosis, etc. Further, in the present invention, preferable examples of the abnormality in skin water permeation function include that caused by aging. In relating to examples of TEWL values, reference can be made to Hachiro Tagami, "Kousho-kaishi (Journal of Cosmetic Science)" 27 (2003): 158.

Further, the agent of the invention can be used to apply to the target skin of a mammal (including a human) in need of restoring or maintaining TEWL value at normal levels, preventing or treating abnormal TEWL in skin, restoring or maintaining skin water permeation function, or adjusting TEWL value ability.

The agent of the invention can prevent or treat an abnormal skin water permeation function.

Further, the agent of the invention can normalize the skin water permeation function in mammals such as humans by adjusting TEWL value to normal levels. Therefore, the agent of the invention can be used for providing a skin moisturizing effect and a skin water penetration enhancement effect, or regulating or restoring skin water content. More specifically, when applied to the skin with reduced TEWL value, the agent of the invention increases TEWL to thereby restore TEWL value to normal levels. When applied to the skin with excess TEWL value, the agent of the invention reduces TEWL value to thereby restore TEWL value to normal levels. Therefore, the agent of the invention is effective to prevent or treat skin conditions and skin diseases with abnormal TEWL value. For example, the agent of the invention is effective for preventing or treating skin diseases and skin conditions (such as dry skin, itching, chapped skin, hyperkeratosis, and senile xerosis) with reduced TEWL value. The agent of the invention is also effective for preventing or treating skin diseases and skin conditions (such as rough skin) with excess TEWL value. The agent of the invention can adjust the skin with an excessive water content to an appropriate level, and can also restore the skin with a low water content to an appropriate level. Therefore, the agent of the invention is also

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effective for restoring deteriorated water retention ability.

There is no limitation on the amount and frequency of application of the agent of the invention. For example, the agent may be applied in an appropriate amount to the skin once to
5 several times per day according to the kind and concentration of the active ingredient, the user's age, sex, severity of skin condition, application form, desired effect, etc. The dose of the agent of the invention is such that the amount of the purine nucleic acid applied per application is 0.0001 to 1 mg, and
10 preferably about 0.001 to about 1 mg, per cm² of the skin.

The agent of the invention can adjust TEWL value to normal levels to thereby maintain skin water content at appropriate levels and appropriately evaporate water from the epidermis, thus appropriately adjusting TEWL value ability.
15 Therefore, the agent of the invention can be used as an agent for improving skin water permeation function or an agent for adjusting TEWL value ability.

Further, the agent of the invention can normalize TEWL value in the skin. Therefore, the agent of the invention can be
20 used as an agent for preventing or treating abnormal TEWL (transepidermal water loss) in skin.

EXAMPLES

The present invention will be described below in more
25 detail with reference to Test Examples and Examples. However, it should be understood that the scope of the present invention is not limited to these Examples. In the Examples etc. below, percentages expressed using "%" to indicate the amount of component used are all by weight, unless otherwise specified.

30

Test Example 1: Examination 1 of skin water permeation-promoting effect of adenosine phosphate

A test was conducted to investigate the promoting effect of an adenosine phosphate on skin water permeation. In
35 this test, 12 healthy human adults (10 males and 2 females;

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average age: 41.2 years old) were used as subjects. More specifically, 9 cm x 8 cm areas of the upper front right and left arms were used as test sites. An appropriate amount of a 20% hydrous ethanol solution containing 3% adenosine 5'-sodium
5 monophosphate (AMP) (test sample) was applied to a test site on one arm, whereas the same amount of a 20% hydrous ethanol solution not containing AMP (reference sample) was applied to a test site on the other arm. The application was performed twice per day (in the morning and in the evening) for 28 days. The TEWL
10 and the water content of the *stratum corneum* were measured before application (i.e., before the test) and after application (i.e., after 28 days of application).

The TEWL value ($\text{g/m}^2/\text{h}$) was measured by using a DermaLab tester (manufactured by Cortex Technology) according to
15 the instructions included with the DermaLab tester (manufactured by Cortex Technology). The average TEWL value of each subject was calculated.

To determine the water content of the *stratum corneum*, the electric conductivity (μS) was measured by using a SKICON-200
20 tester (manufactured by I.B.S Co., Ltd.) according to the instructions included with the SKICON-200 tester (manufactured by I.B.S Co., Ltd.), and used as an index for the water content of the *stratum corneum*.

Table 1 shows the TEWL measurement results. Table 1
25 shows that when the reference sample was applied, there was substantially no difference between the TEWL values before and after the test; in contrast, when the test sample was applied, a tendency toward TEWL value increase was observed after 28 days of application, thus confirming enhanced skin water permeation
30 function.

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Table 1

	Before application	After application
Reference sample (hydrous ethanol solution not containing AMP)	5.5 ± 0.5	6.0 ± 0.4
Test sample (hydrous ethanol solution containing AMP)	5.4 ± 0.5	7.3 ± 0.5

5 Average $\pm$ standard deviation (unit: g/m²/h)

Table 2 shows the *stratum corneum* water content measurement results. Table 2 shows that when the test sample was applied, a remarkable increase in the water content of the *stratum corneum* was observed after 28 days of application. Increased TEWL is generally considered to reduce the water content of the *stratum corneum*. However, the above test results revealed that AMP can increase both TEWL and the water content of the *stratum corneum*.

15 Table 2

	Before application	After application
Reference sample (aqueous ethanol solution not containing AMP)	19.5 ± 3.0	24.6 ± 5.6
Test sample (aqueous ethanol solution containing AMP)	18.3 ± 3.5	41.5 ± 5.9

Average $\pm$ standard deviation (unit: μ S)

20 The above results show that unlike conventional compounds that reduce TEWL, AMP can enhance the skin water permeation function inherent in the skin.

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Test Example 2: Examination 2 of skin water permeation-promoting effect of adenosine phosphate

Next, a test was performed in a manner similar to Test Example 1, using an emulsion, which is generally used as an external cosmetic, in place of the aqueous ethanol solution used in Test Example 1. The test conditions, such as the subject and the test period, were the same as in Test Example 1, except for the medium of the test sample and the test sites in the upper front arms. The emulsion used in this test has a general composition.

Further, cornified envelope (CE) in the *stratum corneum* was observed to investigate whether application of AMP affects skin barrier function. More specifically, to confirm the effect of application of AMP on the number of immature keratinocytes, tape was applied to the test sites so as to obtain the *stratum corneum* after 28 days of application of the test sample or the reference sample. The *stratum corneum* thus obtained was stained with a fluorescent-labeled anti-involucrin antibody, and mature keratinocytes were then stained with a fluorescent-labeled anti-mature cell antibody. Subsequently, each stained sample was observed under a fluorescence microscope to calculate the ratio of the number of immature keratinocytes to the total number of keratinocytes.

Table 3 shows the TEWL measurement results. Table 3 shows that when the reference sample was applied, there was substantially no difference between the TEWL values before and after the test; in contrast, when the test sample was applied, a significant increase in TEWL was observed after 28 days of application, thus confirming enhanced skin water permeation function.

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Table 3

	Before application	After application
Reference sample (emulsion not containing AMP)	5.0 ± 0.5	5.6 ± 0.6
Test sample (emulsion containing AMP)	5.2 ± 0.5	7.0 ± 0.3

5 Average $\pm$ standard deviation (unit: $\text{g/m}^2/\text{h}$)

Table 4 shows the *stratum corneum* water content measurement results. Table 4 shows that when the reference sample was applied, an increase in the water content of the *stratum*
 10 *corneum* was observed; when the test sample was applied, a more remarkable increase was observed.

Table 4

	Before application	After application
Reference sample (emulsion not containing AMP)	14.6 ± 2.2	61.2 ± 6.0
Test sample (emulsion containing AMP)	15.1 ± 2.5	90.9 ± 6.6

15 Average $\pm$ standard deviation (unit: μS)

The increased water content of the *stratum corneum* achieved by application of the reference sample is believed to be due to a so-called temporal *stratum corneum* water retention
 20 effect obtained by coating the skin with a film. In contrast, the increased water content of the *stratum corneum* and enhanced water permeation function achieved by application of the test sample are believed to result from enhanced *stratum corneum* function and increased ability to supply water from the deep layers of the
 25 skin to the outermost layer. Since these effects are provided by

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improving the skin's own function and ability, the effects are expected to persist even after application of the test sample is completed.

Table 5 shows the ratio of the number of immature keratinocytes to the total number of keratinocytes. It is generally believed that the observation of many immature keratinocytes in the *stratum corneum* indicates rough skin or reduced *stratum corneum* barrier function. As shown in Table 5, the results show that when the test sample containing AMP was applied, the ratio of the number of immature keratinocytes did not increase. Thus, the results confirmed that application of the test sample containing AMP does not reduce the skin barrier function.

Table 5

	Ratio of the number of immature keratinocytes to the total number of keratinocytes (%)
Reference sample (emulsion not containing AMP)	5.7 ± 1.9
Test sample (emulsion containing AMP)	4.7 ± 1.7

The above test results confirmed that an increase of TEWL is achieved only when AMP is applied, whereas an increase of the *stratum corneum* water content is achieved even when the reference sample not containing AMP is applied. The results indicate that AMP can enhance the skin water permeation function inherent in the skin. Further, the results show that the application of AMP does not change skin barrier function, which was conventionally believed to decrease with an increase of TEWL; and that AMP can be used safely.

Test Example 3: Examination of skin water permeation-inhibitory effect of adenosine phosphate

Next, a test was conducted to examine the effect of an adenosine phosphate on a skin with overly enhanced skin water

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permeation function. The test was conducted using 9 healthy human adults (9 males; average age: 43.1 years old) as subjects. More specifically, 7 cm x 4.5 cm areas on the outer right and left calves of each subject were used as test sites. A 0.25 w/v% aqueous solution of sodium dodecyl sulfate (aqueous SDS solution, manufactured by Wako Pure Chemical Industries) was applied to the test sites on both the right and left calves in such a manner as to cover the test sites with an occlusive barrier for 24 hours. After the treatment with the aqueous SDS solution, the application sites were washed and used as rough skin models. An emulsion containing 3% sodium adenosine 5'-monophosphate was used as a test sample. An appropriate amount of the emulsion containing sodium adenosine 5'-monophosphate was applied to a test site on one calf, whereas nothing was applied to a test site on the other calf. The application of the emulsion was started after the treatment with the aqueous SDS solution (day 0), and was performed twice per day for 7 days. The TEWL value and the *stratum corneum* water content were measured in the same manner as in Test Example 1, before and after treatment with the aqueous SDS solution; and after 1 day, 3 days, and 7 days of application of the test sample. Further, the surface condition of the skin after 7 days of application of the test sample was observed. The condition of the skin surface was observed using a microscope (manufactured by Scalar).

Fig. 1 shows the TEWL measurement results. As shown in Fig. 1, the treatment with the aqueous SDS solution clearly increased TEWL value, and also caused unmistakably rough skin; afterward, TEWL value was more sharply reduced at the test sample application skin site than at the non-application skin site according to observations after 3 days and 7 days of application.

Fig. 2 shows the water content of the *stratum corneum*. As shown in Fig. 2, the treatment with the aqueous SDS solution obviously reduced the water content of the *stratum corneum*; afterward, the water content of the *stratum corneum* did not increase at the non-application skin site even after 7 days,

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whereas the water content of the *stratum corneum* was remarkably increased at the skin site where the test sample was applied.

Further, Figs. 3a and 3b show the skin surface condition observation results. At the non-application site (Fig. 3a), the skin surface pattern, composed of *sulcus cutis*, *cristae cutis*, and *area cutanea*, disappeared, and flaking of the *stratum corneum* was observed. In contrast, at the AMP-containing test sample application site (Fig. 3b), a skin surface pattern was observed, and no flaking of the *stratum corneum* was observed. The above results confirmed that application of the AMP-containing test sample is effective for ameliorating rough skin caused by excessive TEWL value.

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CLAIMS

- [Claim 1] A purine nucleic acid for preventing or treating an abnormality in skin water permeation function.
- 5 [Claim 2] The purine nucleic acid according to claim 1, wherein the purine nucleic acid is selected from the group consisting of adenosine phosphates and salts thereof.
- [Claim 3] The purine nucleic acid according to claim 1, wherein the purine nucleic acid is selected from the group
10 consisting of adenosine monophosphates and salts thereof.
- [Claim 4] The purine nucleic acid according to claim 1 which is used in an amount of 0.1 to 20 wt.% in an external composition.
- 15 [Claim 5] The purine nucleic acid according to claim 1 which is used to prevent or treat a skin condition or a skin disease with abnormal TEWL value.
- [Claim 6] The purine nucleic acid according to claim 1, wherein the abnormality in skin water permeation function is
20 due to aging.
- [Claim 7] The purine nucleic acid according to claim 1, wherein the abnormality in skin water permeation function is rough skin, itching, chapped skin, hyperkeratosis, or senile xerosis.
- 25 [Claim 8] The purine nucleic acid according to claim 1 which is used as a component of a cosmetic, or a pharmaceutical or quasi-drug for external application to the skin.
- [Claim 9] The purine nucleic acid according to claim 1 which is
30 used for non-therapeutic purpose.
- [Claim 10] A purine nucleic acid for preventing or treating abnormal TEWL (transepidermal water loss) in skin.
- [Claim 11] The purine nucleic acid according to claim 10, wherein the abnormal TEWL is due to aging.
- 35 [Claim 12] Use of at least one purine nucleic acid for manufacture of an external composition for improving skin water permeation function.

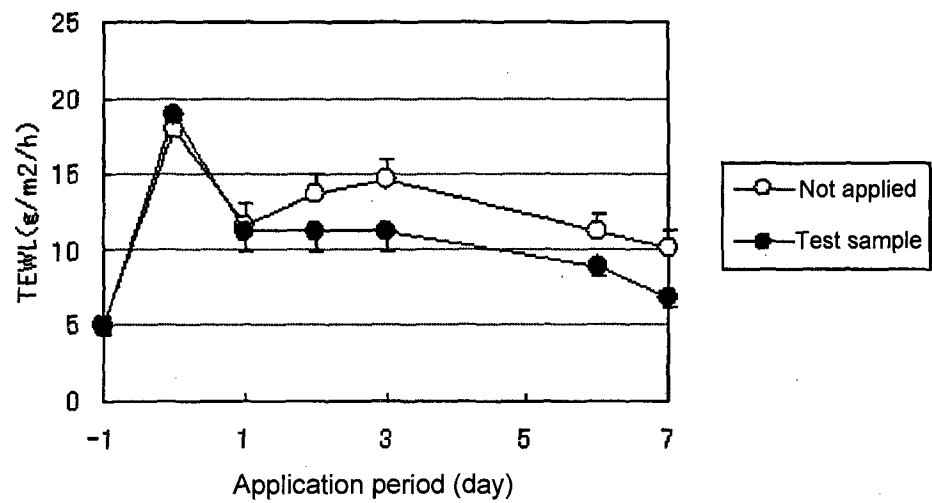
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[Claim 13] A purine nucleic acid for improving skin water permeation function.

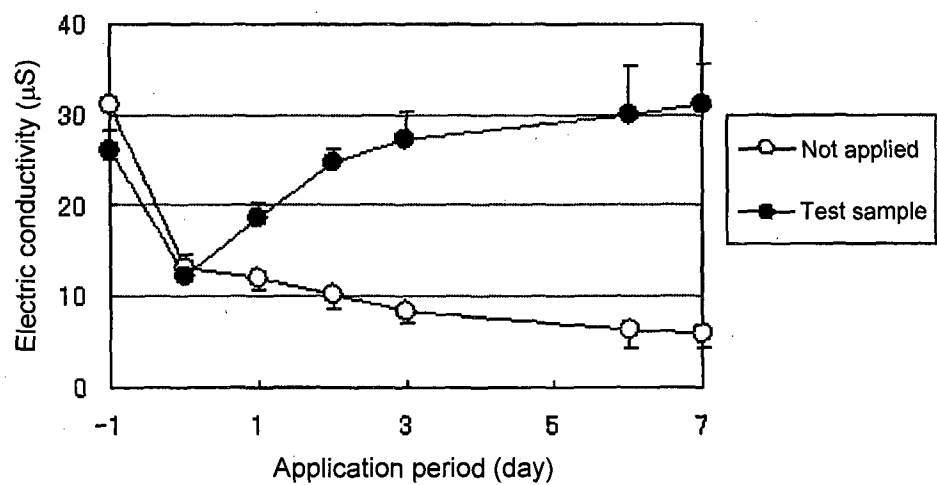
5 [Claim 14] A method of improving skin water permeation function, comprising administering at least one purine nucleic acid to a subject in need of restoring or maintaining skin water permeation function.

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[Fig. 1]



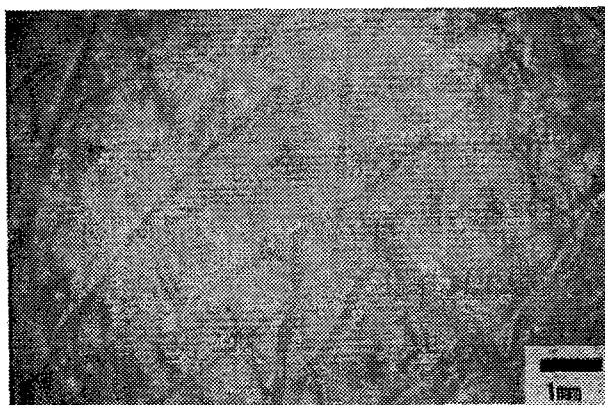
[Fig. 2]



[Fig. 3a]



[Fig. 3b]



INTERNATIONAL SEARCH REPORT

International application No
PCT/JP2010/060796

A. CLASSIFICATION OF SUBJECT MATTER INV. A61K8/60 A61K31/7076 A61K45/06 A61P17/00 ADD.		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) A61K A61P		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practical, search terms used) EPO-Internal, WPI Data, BIOSIS, MEDLINE		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JP 2005 104886 A (MARUZEN PHARMA) 21 April 2005 (2005-04-21) paragraphs [0001] - [0013] - paragraphs [0031], [0041]	1-14
X	JP 11 029457 A (KANEBO LTD; HANAOKA SHUSUKE) 2 February 1999 (1999-02-02) * abstract	1-14
X	JP 6 128140 A (KOSE CORP) 10 May 1994 (1994-05-10) * abstract	1-14
X	JP 6 065041 A (KOSE CORP) 8 March 1994 (1994-03-08) * abstract	1-14
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☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.		
* Special categories of cited documents :		
"A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art. "&" document member of the same patent family	
Date of the actual completion of the international search 30 September 2010	Date of mailing of the international search report 15/10/2010	
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Authorized officer Kling, Isabelle	

INTERNATIONAL SEARCH REPORT

International application No

PCT/JP2010/060796

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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X	WPI WORLD PATENT INF, 8 March 1994 (1994-03-08), XP002900579 * abstract -----	1-14
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