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(54) Title: USE OF METABOLITES OF ARACHIDONIC ACID OR THE DERIVATIVE THEREOF

(57) Abstract: The present invention relates to a use of metabolites of arachidonic acid or derivatives thereof, more particularly to a process for inducing an itch in an animal, a process for screening an anti-itching substance and a process for evaluating the anti-itching effect of an anti-itching substance, by regulating the administration of 12(s)-HPETE or 15(s)-HPETE, which are the metabolites of arachidonic acid, or derivatives thereof. The metabolites of arachidonic acid or derivatives thereof according to the present invention are useful in understanding an itch, for screening the therapeutic candidates for itch, and for evaluating and developing such agent.

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【DESCRIPTION】**【Invention Title】**

USE OF METABOLITES OF ARACHIDONIC ACID OR THE
DERIVATIVE THEREOF

5

【Technical Field】

The present invention relates to a use of metabolites of arachidonic acid or derivatives thereof. More particularly, the present invention describes a process to induce an itch in an animal through intradermal injection of 12(s)-HPETE or 15(s)-HPETE, the metabolites of arachidonic acid, or derivatives thereof; a process to
10 screen an anti-itching substance; and a process to evaluate the anti-itching effect of the screened substance. The metabolites of arachidonic acid or derivatives thereof according to the present invention are useful for studying an itch, screening anti-itching drugs and evaluating the efficacy of a screened drug.

15

【Background Art】

Itch is an annoying sensation generally occurs in many skin diseases, and in clinics, it is defined as an unpleasant sensation provoking the desire to scratch (The Lancet, 2003; 361: 690-93). When classifying diseases that mainly induce an itch,
20 most of them are closely related to the skin diseases, with the exceptions of cholestasis, renal failure, and parasitophobia (Arch. Dermatol. 2003; 138: 1463-70). Specifically, the major skin diseases producing an itch include atopic dermatitis, hives, dry skin, seborrheic dermatitis, psoriasis and worm bite (The Lancet, 1996; 348: 938-40). Among these, in the case of atopic dermatitis, itch is being considered as the most
25 concerning factor which lowers the quality of patient's life. According to a study about atopic dermatitis, it was found that patients suffering from severe atopic dermatitis spend about 15% of their sleeping time in scratching (Br. J. Dermatol. 2001; 144: 305~). Therefore, extensive studies have been made to identify endogenous substances that induce an itch in many skin diseases, and many pruritogens have been

developed. However, the relationship between the disease and the pruritogen is still unclear, and a new animal model relating to new pruritogens is highly required.

In animal studies of itch, rodents are commonly used for the ease of execution and control of the experiments. It was found by Japanese researchers that rodents
5 show peculiar behavior such as hind paw scratching of the pruritogen-injected site, which is not usually observed in normal condition (Br. J. Dermatol. 2004; 33-8). It was also found that intradermal injection, which is an injection between epidermis and dermis, is the most effective method, because the pruritogen could be localized at the injected site. This prevents the injected pruritogens from being spread to the whole
10 body (Eur. J. Dermatol. 1995; 275: 229-23).

Suggested pruritogens conventionally used in the above prior art include kallikrein, compound 48/80, serotonin, histamine, substance P (WO 2004/027413, JP2004-132794, JP2003-313138) and corrosive organic solvents (JP2001-321016). However, when using corrosive solvents, their mechanism of action is unclear and
15 considered to be very different from that of endogenous pruritogens. In addition, histamine, serotonin and substance P are endogenous, and are more preferable than non-biological external substances in terms of toxicity, biocompatibility or similarity to clinical itch. Moreover, to induce an itch they need to be administered at high doses of 100 nmole /site, thus there is a problem in relation to clinical application.

20 Arachidonic acid is an unsaturated fatty acid whose basic skeleton is constituted with 20 carbons, and is a precursor for many bioactive materials including prostaglandins (PGs) and leukotrienes. When arachidonic acid is metabolized by cyclooxygenase-1/2 (COX-1/2), several kinds of prostaglandins are produced by way of PGG2 and PGH2 (J Leukoc Biol. 1999; 66(4): 535-41). When arachidonic acid is
25 metabolized by lipoxygenases, leukotrienes (LTs), hydroperoxyeicosatetraenoic acids (HPETEs) or hydroeicosatetraenoic acids (HETEs) are mainly produced (Clin Rev Allergy Immunol. 1999; 17 (1-2): 5-26). Thus produced metabolites of arachidonic acid are involved mainly in inflammation, and in addition to this, they take a role in neurotransmission, immunocyte proliferation, immunologic regulation and thrombosis
30 (Cell Mol Life Sci. 2002; 59 (5): 799-807). With respect to sensory nerves, it is

well-known that metabolites of arachidonic acid could induce pain.

With respect to itch, it has been reported that PGE₂, which is one of the prostaglandins, amplifies an itch (BMJ. 1973; 2: 608-09), and it is also discovered that LTB₄, which are metabolites of 5-lipoxygenase, more strongly induce an itch in
5 rodents (Eur. J. Pharm. 1998; 353: 93-6). However, the exact mechanism of the itch induced by LTB₄ is unclear.

【DISCLOSURE】

【Technical Problem】

10 The present inventors found that a composition comprising 12(s)-HPETE, 15(s)-HPETE, which is endogenous and a metabolite of arachidonic acid, or derivatives thereof, can induce sufficient itch with an extremely small amount (50pmole), which is 1/2000 times smaller amount than the required amount of histamine, serotonin, or substance P (100nmole), and thereby completed the present
15 invention.

【Technical Solution】

The object of the present invention is to provide a composition comprising metabolites of arachidonic acid or derivatives thereof, a process for inducing an itch
20 using the composition, a process for screening an anti-itching substance and a process for evaluating the effect of the screened anti-itching substance.

【Description of Drawing】

Figure 1 is a graph showing the effect for inducing an itch according to the
25 dose of 12(s)-HPETE.

Figure 2 is a graph showing the effect for inducing an itch according to the dose of 15(s)-HPETE.

Figure 3 is a graph showing the effect of capsaicin and azelastine for inhibiting an itch induced by 12(s)-HPETE.

Figure 4 is a graph showing the effect of capsaicin and azelastine for inhibiting an itch induced by 15(s)-HPETE.

【Best Mode】

5 To attain the above objects, the present invention provides a process to induce an itch to an animal using a composition comprising metabolites of arachidonic acid or derivatives thereof, a process to screen an anti-itching substance, and a process to evaluate the effect of the screened substance.

The term “itch” has the same meaning as “pruritus”, the term “pruritogen” has
10 the same meaning as “itch-inducing substance”, the term “anti-itching” has the same meaning as “anti-pruritic”.

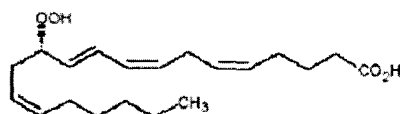
Hereinafter, the present invention is described in more detail.

In the present invention, metabolites of arachidonic acid or derivatives thereof are used as a pruritogen. Examples of such metabolites include the compounds
15 represented in Chemical Formulae 1 and 2, but are not limited thereto.

12(s)-hydroperoxyeicosa-5Z, 8Z, 10E, 14X-tetraenoic acid (hereinafter referred to as “12(s)-HPETE”), represented in Chemical Formula 1, is obtained by metabolizing arachidonic acid with 12-LO, and is known to inhibit thrombosis (P.N.A.S. 1997; 74: 3691-7).

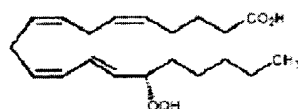
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【Chemical Formula 1】



15(s)-hydroperoxyeicosa-5Z, 8Z, 11Z, 13E-tetraenoic acid (hereinafter, referred to as “15(s)-HPETE”), represented in Chemical Formula 2, is obtained by
25 metabolizing arachidonic acid with 15-LO, and is known to inhibit generation of prostacycline and platelet aggregation (Prostaglandins 1976; 12: 715-21).

【Chemical Formula 2】



5 Therefore, the present invention provides a composition for inducing an itch comprising one or more selected from the group consisting of the above metabolites of arachidonic acid and derivatives thereof.

Further, the present invention provides a process to induce an itch through the administration of a composition comprising the above metabolites of arachidonic acid
10 represented by the above Chemical Formulae 1 and 2 to an animal, or derivatives thereof.

The animal is preferably a rodent such as a mouse, rat or guinea pig, and more preferably is ICR mouse.

The method of administering the composition comprising metabolites of
15 arachidonic acid or derivatives thereof according to the present invention is not limited particularly; however, it is preferably intradermal injection, subcutaneous injection, intravenous injection, intraperitoneal injection, intramuscular injection, topical application, patch or iontophoresis, and is more preferably intradermal injection.

With regard to the determination of itch, since itch is a completely subjective
20 sensation and the experimental animal cannot communicate its sensational experience to human, the scratching behavior of the site where the pruritogen is injected is set as an index of the itch. In particular, it has been found by many researchers that in the experiment using rodents, the behavior of lifting the hind paw to scratch the body, which is not frequently observed in general situations, is significantly increased only
25 when the pruritogen is injected, and is not increased by irritants or by pain-inducing agents (Eut. J. Pharm. 1995; 275: 229-33).

Thus, in the present invention, the extent of itch was expressed as the number

of scratching behavior which is defined as lifting the hind paw and scratching the pruritogen-injected site. In this regard, scratching behavior means scratching the site where the pruritogen was injected using the hind paw, and the behavior of scratching using the front leg or licking the site was excluded. Here, a series of behavior from
5 lifting hind paw and scratching the site to dropping the hind paw was measured as 1 occurrence of scratching, and was made as the only index of the itch.

In the present invention, the composition comprising metabolites of arachidonic acid or derivatives thereof was basically administered at once. The period of observing the scratching is not particularly limited, however, since
10 scratching is rarely observed after 30-40 minutes after injecting the composition, scratching was recorded via video camera in an unmanned environment for 60 minutes, preferably for 40 minutes, after administering the composition.

The recorded images were reproduced and observed by two or more people simultaneously. The images were observed twice and the thus obtained values were
15 averaged, in order to exclude the subjective views of the observers.

The preferred dose of the composition comprising metabolites of arachidonic acid or derivatives thereof according to the present invention as well as observation time can be confirmed by the below experiment. The preferred dose of the composition comprising metabolites of arachidonic acid or derivatives is 0.001-0.2
20 nmole. When the dose is less than 0.001 nmole, the induced scratching behavior is not significantly increased, and when the dose is more than 0.2 nmole, the scratching behavior is rather decreased.

In addition, the amount of metabolites of arachidonic acid and derivatives thereof in the composition is preferably 6.5×10^{-6} - 1.25×10^{-4} wt% with respect to the
25 total weight of the composition.

Further, video tape recording was used in the present invention to analyze repeatedly the number of scratching occurrences, which is a criterion of the itch, for accurate and effective estimation of the itch.

Further, the present invention provides a process of screening an anti-itching
30 substance by administering to an animal the metabolites of arachidonic acid

represented by Chemical Formula 1 and 2, or derivatives thereof.

The process of screening an anti-itching substance according to the present invention comprises:

- 1) inducing an itch in an animal except for human by administering the composition comprising metabolites of arachidonic acid or derivatives thereof; and
- 2) determining the number of scratching occurrences by the animal after the administration .

The animal used in the present invention is preferably a rodent such as a mouse, rat or guinea pig. More preferably it is ICR mouse.

The method of administering the composition comprising metabolites of arachidonic acid or derivatives thereof in the above step 1) is not limited particularly; however, it is preferably intradermal injection, subcutaneous injection, intravenous injection, intraperitoneal injection, intramuscular injection, topical application, patch or iontophoresis, and is more preferably intradermal injection.

The preferred administration amount for the composition comprising metabolites of arachidonic acid or derivatives is 0.001-0.2 nmole. When the administration amount was less than 0.001 nmole, the induced scratching behavior was not significantly increased, and when the amount was more than 0.2 nmole, the scratching behavior was rather decreased.

The screening process according to the present invention may further comprises orally administering an anti-itching substance prior to administering the composition; intradermal injecting an anti-itching substance at the same time as administering the composition; or topically applying an anti-itching substance at and around the site of administering the composition.

The anti-itching substance can be any conventional one used in the art, but preferably capsaicin or azelastine. Capsaicin is a pungent ingredient of red pepper and known to relieve pain and itch directly acting on the peripheral nerve, and has been formulated as topical cream or patch, and used in clinics. Azelastine is an anti-histamine agent used in clinics, and is effective in asthma, skin rashes, and atopic

dermatitis or itch.

The process of determining the number of scratching occurrences by the animal in step 2) is the same as that in the process of inducing an itch.

Further, the present invention provides a process of evaluating the anti-itching effect on the itch induced by the composition comprising metabolites of arachidonic acid or derivatives thereof.

The process comprises

1) inducing an itch in an animal except for human by administering the composition of metabolites of arachidonic acid or derivatives thereof to the animal; and

2) determining the number of scratching occurrences by the animal.

The animal used in the present invention is preferably a rodent such as a mouse, rat or guinea pig. More preferably it is ICR mouse.

The method of administering the composition comprising metabolites of arachidonic acid or derivatives thereof in the above step 1) is not limited particularly; however, it is preferably intradermal injection, subcutaneous injection, intravenous injection, intraperitoneal injection, intramuscular injection, topical application, patch or iontophoresis, and is more preferably intradermal injection.

The preferred administration amount for the composition comprising metabolites of arachidonic acid or derivatives is 0.001-0.2 nmole. When the administration amount was less than 0.001 nmole, the induced scratching behavior was not significantly increased, and when the amount was more than 0.2 nmole, the scratching behavior was rather decreased.

The evaluating process according to the present invention may further comprise orally administering an anti-itching substance prior to administering the composition; intradermally injecting an anti-itching substance at the same time as administering the composition; or topically applying an anti-itching substance at and around the site of administering the composition.

The anti-itching substance can be any conventional one used in the art, but preferably capsaicin or azelastine.

The process of determining the number of scratching occurrences by the animal in step 2) is the same as that in the process of inducing the itch.

【Mode for Invention】

5 The present invention will be described in more detail by way of the following examples. However, these examples are provided for the purpose of illustration only and should not be construed as limiting the scope of the invention, which will be apparent to one skilled in the art.

Before describing Examples, Reference Examples are described to explain the
10 criterion applied in the experimentation of the Examples.

[Reference Example 1] Process of inducing an itch

ICR mice were shaved on their upper dorsal area, and then were intradermally injected with a composition comprising metabolites of arachidonic acid or derivatives
15 thereof, and the control group was administered with the same amount of saline solution. After administration, scratching behavior of the mice was recorded by video camera for 40 minutes, and the recorded images were observed and analyzed by two or more people simultaneously at least 2 times.

20 **[Reference Example 2] Statistical analysis**

The results were shown as a mean value including its standard deviation. The significance of the results was tested by Student's t-test. When the p value, which is obtained by testing the control group and the experimental group, was less than 0.05, the difference of the experimental group and the control group was evaluated to be
25 significant.

[Reference Example 3] Evaluation of the anti-itching effect of the anti-itching substance

ICR mice were shaved on the upper dorsal area, and then were intradermally

injected with a composition comprising metabolites of arachidonic acid or derivatives thereof together with compound A (substance to be evaluated as anti-itching substance). After one administration of the composition of metabolites of arachidonic acid or derivatives thereof, and the control group was administered with the same amount of saline solution. After administration, scratching behavior of the mice was recorded by video camera for 40 minutes, and the recorded images were observed and analyzed by two or more people simultaneously at least 2 times.

[Example 1] Determination of the amount of 12(s)-HPETE for inducing an itch

32 male ICR mice (age: 5-8 weeks, weight: 25-32 g) were used in total. 8 mice were used as control and 24 mice were used as experimental. All 32 mice were shaved on the upper dorsal area 24 hours before the experiment. The 24 experimental mice were divided into 3 groups, 8 mice for each group. The drug (12(s)-HPETE) was dissolved in ethanol at concentration of 50 µg/ml. Each drug was then diluted with saline solution to be of the appropriate concentration used in the experiment. As the amount of ethanol is proportionally increased in the group where the drug of high concentration was administered, ethanol was added to the control group and to the groups where the drug in lower concentration was administered so that the same amount of ethanol was administered in all of the groups.

For adaptation to a new environment, ICR mice were left in the observatory cage for 30 minutes before injection. To the first group of ICR mice, 50 µl of solution containing 12(s)-HPETE, which was diluted to 4 µM (drug amount: 0.2 nmole/site), was intradermally injected at the shaved site. To the second group, 50 µl of solution containing 12(s)-HPETE, which was diluted to 1 µM (drug amount: 0.05 nmole/site), was intradermally injected to the shaved site. To the third group, 50 µl of solution containing 12(s)-HPETE, which was diluted to 0.2 µM (drug amount: 0.01 nmole/site), was intradermally injected to the shaved site. To the control group, the same amount of saline solution in which ethanol is diluted was intradermally injected.

After administration, animals were moved to the observatory cage, and were videotaped for 40 minutes. After recording and when the experiment was terminated,

the animals were moved to the raising cage. After 24 hours, they were examined to determine if pathological symptom was found in the injection site and then all were sacrificed. If any pathological symptom such as edema or hematoma was found, the experimentation results were invalidated.

5 The recorded images were observed and analyzed by two or more people simultaneously at least 2 times. Only the behavior of scratching the site where the pruritogen was injected using the hind paw, was set as the index of the itch, and other behavior was excluded. The results are shown in Fig. 1.

10 As can be seen in Fig. 1, all of the 12(s)-HPETE injected groups significantly elicited scratching behavior compared to the control group. When 0.05 nmole of 12(s)-HPETE was injected, scratching behavior was induced most effectively (When comparing the experimental group with the control group in Student's t-test, the p value was less than 0.01, which means significant increase).

15 [Example 2] Determination of the amount of 15(s)-HPETE for inducing an itch

32 male ICR mice (age: 5-8 weeks, weight: 25-32 g) were used in total. 8 mice were used as control and 24 mice were used as experimental. All 32 mice were shaved on the upper dorsal area 24 hours before the experiment. The 24 experimental mice were divided into 3 groups, 8 mice for each group. The drug
20 (15(s)-HPETE) was dissolved in ethanol at concentration of 50 µg/ml. Each drug was then diluted with saline solution to be of appropriate concentration used in the experiment. As the amount of ethanol is proportionally increased in the group where the drug of high concentration was administered, ethanol was added to the control group and to the groups where the drug in lower concentration was administered so
25 that the same amount of ethanol was administered in all of the groups.

For adaptation to a new environment, ICR mice were left in the observation cage for 30 minutes before injection. To the first group of ICR mice, 50 µl of solution containing 15(s)-HPETE, which was diluted to 4 µM (drug amount: 0.2 nmole/site), was intradermally injected to the shaved site. To the second group, 50 µl
30 solution containing of 15(s)-HPETE, which was diluted to 1 µM (drug amount: 0.05

nmole/site), was intradermally injected to the shaved site. To the third group, 50 μ l of solution containing 15(s)-HPETE, which was diluted to 0.2 μ M (drug amount: 0.01 nmole/site), was intradermally injected to the shaved site. To the control group, the same amount of saline solution in which ethanol is diluted was intradermally injected.

5 After administration, animals were moved to the observatory cage, and were videotaped for 40 minutes. After recording and when the experiment was terminated, the animals were moved to the raising cage. After 24 hours, they were examined to determine if pathological symptom was found in the injection site and then all were sacrificed. If any pathological symptom such as edema or hematoma was found, the
10 experimentation results were invalidated.

The recorded images were observed and analyzed by two or more people simultaneously at least 2 times. Only the behavior of scratching the site where the pruritogen was injected using the hind paw, was set as the index of the itch, and other behavior was excluded. The results are shown in Fig. 2.

15 As can be seen in Fig. 2, all of the 15(s)-HPETE injected groups significantly elicited scratching behavior compared to the control group. When 0.05 nmole of 15(s)-HPETE was injected, itch was induced most effectively (When comparing the experimental group with the control group in Student's t-test, the p value was less than 0.01, which means significant increase).

20

[Example 3] Evaluation of the anti-itching effect using 12(s)-HPETE

40 male ICR mice (age: 5-8 weeks, weight: 25-32 kg) were shaved on their upper dorsal area 24 hours before the experiment. To the first group (1% CSN), 100 μ l of solution containing 1% capsaicin (dissolved in 75% ethanol) was treated
25 topically, and 5 minutes later 0.05 nmole of saline solution of 12(s)-HPETE was intradermally injected to the shaved site. To the second group (0.05 nmole CSN), 0.05 nmole of 12(s)-HPETE and 0.05 nmole of capsaicin were simultaneously dissolved in saline solution, and this was intradermally injected to the shaved site. To the third group (30 mg/kg AZH), 30 mg/kg of azelastine was dissolved in saline
30 solution and orally administered 30 minutes before intradermal injection of 0.05 nmole

12(s)-HPETE. To the fourth group (called "12(s)-HP group"), 0.05 nmole of 12(s)-HPETE was dissolved in saline solution and was intradermally injected. To the control group, the same amount of saline solution in which the same amount of ethanol is added was intradermally injected.

5 After administration, animals were moved to the observatory cage, and videotaped for 40 minutes. After recording and when the experiment was terminated, the animals were moved to the raising cage. After 24 hours, they were examined to determine if pathological symptom was found in the injection site and then all were sacrificed. If any pathological symptom such as edema or hematoma was found, the
10 experimentation results were invalidated.

The recorded images were observed and analyzed by two or more people simultaneously at least 2 times. Only the behavior of scratching the site where the pruritogen was injected using the hind paw was set as the index of the itch, and other behavior was excluded. The results are shown in Fig. 3.

15 As can be seen in Fig. 3, the groups in which 0.05 nmole/site of 12(s)-HPETE was injected were significantly elicited an itch compared to the control group. All of the groups in which the anti-itching substances were administered topically, intradermally or orally, the itch induced by 12(s)-HPETE was effectively inhibited (When comparing the first, second, and third groups with the 12(s)-HP group in
20 Student's t-test, the p value was less than 0.05, which means the significant suppression of 12(s)-HPETE-induced scratching behavior).

[Example 4] Evaluation of the anti-itching effect using 15(s)-HPETE

25 40 male ICR mice (age: 5-8 weeks, weight: 25-32 kg) were shaved on their upper dorsal area 24 hours before the experiment. To the first group (1% CSN), 100 μ l of solution containing 1% capsaicin (dissolved in 75% ethanol) was treated topically, and 5 minutes later 0.05 nmole of saline solution in which 15(s)-HPETE was dissolved was intradermally injected to the shaved site. To the second group (0.05 nmole CSN), 0.05 nmole of 15(s)-HPETE and 0.05 nmole of capsaicin were
30 simultaneously dissolved in saline solution and was intradermally injected to the

shaved site. To the third group (30 mg/kg AZH), 30 mg/kg of azelastine was dissolved in saline solution and was orally administered 30 minutes before intradermal injection of 0.05 nmole 15(s)-HPETE. To the fourth group (called "15(s)-HP group"), 0.05 nmole of 15(s)-HPETE was dissolved in saline solution and was intradermally injected. To the control group, the same amount of saline solution in which the same amount of ethanol is added was intradermally injected

After administration, animals were moved to the observatory cage, and videotaped for 40 minutes. After recording and when the experiment was terminated, the animals were moved to the raising cage. After 24 hours, they were examined to determine if pathological symptom was found in the injection site and then all were sacrificed. If any pathological symptom such as edema or hematoma was found, the experimentation results were invalidated.

The recorded images were observed and analyzed by two or more people simultaneously at least 2 times. Only the behavior of scratching the site where the pruritogen was injected using the hind paw was set as the index of the itch, and other behavior was excluded. The results are shown in Fig. 4.

As can be seen in Fig. 4, the groups in which 0.05 nmole/site of 15(s)-HPETE was injected were significantly elicited an itch compared to the control group. All of the groups in which the anti-itching substances was administered locally, intradermally or orally, the itch induced by 15(s)-HPETE was effectively inhibited (When comparing the first, second, and third groups with the 15(s)-HP group in Student's t-test, the p value was less than 0.05, which means the significant suppression of 15(s)-HPETE-induced scratching behavior).

25 **【Industrial Applicability】**

As can be seen in the above, metabolites of arachidonic acid and derivatives thereof according to the present invention induce an itch effectively, and are useful in the experiment for evaluating anti-itching effect of drug candidates. Therefore, metabolites of arachidonic acid and derivatives thereof according to the present

invention are useful for studying an itch, searching for a drug for treating an itch and evaluating the drug effect.

【CLAIMS】**【Claim 1】**

A composition for inducing an itch comprising metabolites of arachidonic acid as effective ingredient.

5 **【Claim 2】**

The composition according to claim 1, wherein said metabolites of arachidonic acid are one or more selected from the group consisting of 12(s)-HPETE, 15(s)-HPETE and derivatives thereof.

【Claim 3】

10 A process to induce an itch in an animal except for human, comprising administering the composition of claim 1 to the animal.

【Claim 4】

The process according to claim 3, wherein said animal is a rodent selected from the group consisting of mouse, rat and guinea pig.

15 **【Claim 5】**

The process according to claim 4, wherein said animal is ICR mouse.

【Claim 6】

The process according to claim 3, wherein said administration is selected from the group consisting of intradermal injection, subcutaneous injection, intravenous
20 injection, intraperitoneal injection, intramuscular injection, topical application, patch and iontophoresis.

【Claim 7】

The process according to any one of claims 3 to 6, wherein the amount of administered composition is 0.001-0.2 nmole.

25 **【Claim 8】**

A process of screening an anti-itching substance comprising:
inducing an itch in an animal except for human by administering the composition of claim 1 to the animal; and

determining the number of occurrences of scratching behavior by the animal.

【Claim 9】

The process according to claim 8, wherein said animal is a rodent selected from the group consisting of mouse, rat and guinea pig.

5 **【Claim 10】**

The process according to claim 9, wherein said animal is ICR mouse.

【Claim 11】

The process according to claim 8, wherein said administration is selected from the group consisting of intradermal injection, subcutaneous injection, intravenous
10 injection, intraperitoneal injection, intramuscular injection, topical application, patch and iontophoresis.

【Claim 12】

The process according to claim 8, further comprising orally administering an anti-itching substance prior to administering the composition.

15 **【Claim 13】**

The process according to claim 8, further comprising intradermally injecting an anti-itching substance at the same time as administering the composition.

【Claim 14】

The process according to claim 8, further comprising, after administering the
20 composition, topically administering an anti-itching substance at and around the site of administering the composition.

【Claim 15】

The process according to any one of claims 8 to 14, wherein the amount of administered composition is 0.001-0.2 nmole.

25 **【Claim 16】**

An anti-itching substance obtained by the screening process according to any one of claims 8 to 14.

【Claim 17】

A process of evaluating the anti-itching effect of an anti-itching substance comprising:

inducing an itch in an animal except for human by administering the composition of claim 1 to the animal; and

5 determining the number of occurrences of scratching by the animal.

【Claim 18】

The process according to claim 17, wherein said animal is a rodent selected from the group consisting of mouse, rat and guinea pig.

【Claim 19】

10 The process according to claim 18, wherein said animal is ICR mouse.

【Claim 20】

The process according to claim 17, wherein said administration is selected from the group consisting of intradermal injection, subcutaneous injection, intravenous injection, intraperitoneal injection, intramuscular injection, topical application, patch
15 and iontophoresis.

【Claim 21】

The process according to claim 17, further comprising, prior to administering the composition, orally administering a substance to be evaluated for its anti-itching effect.

20 **【Claim 22】**

The process according to claim 17, further comprising, at the same time with administering the composition, intradermally injecting a substance to be evaluated for its anti-itching effect.

【Claim 23】

25 The process according to claim 17, further comprising, after administering the composition, topically administering a substance at and around the site of administering the composition to be evaluated for its anti-itching effect.

【Claim 24】

The process according to any one of claims 17 to 23, wherein the amount of

administering the composition is 0.001-0.2 nmole.

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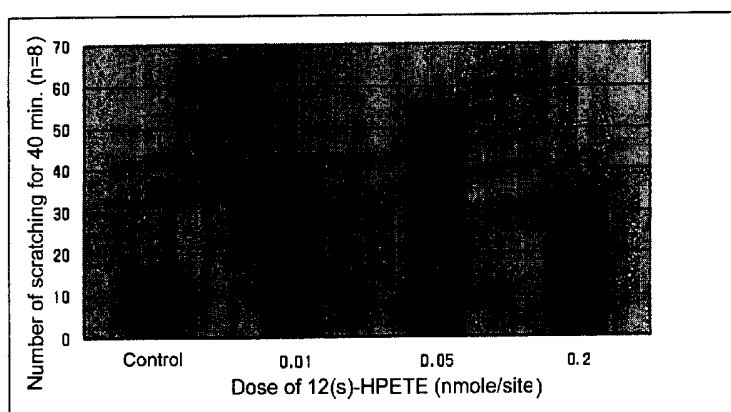
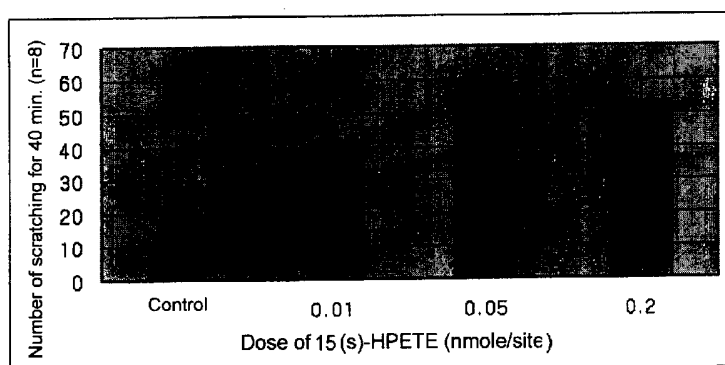
FIGURES**FIG. 1****FIG. 2**

FIG. 3

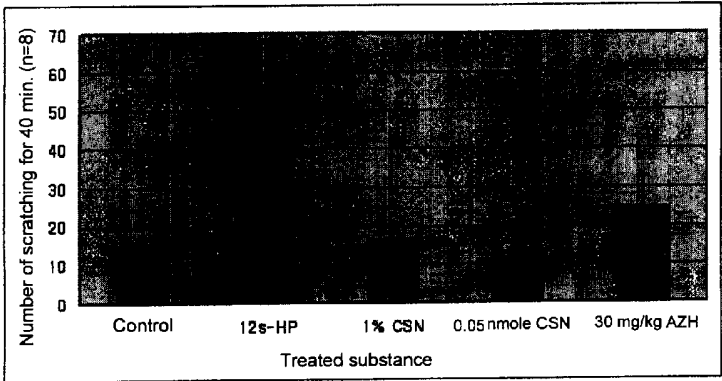
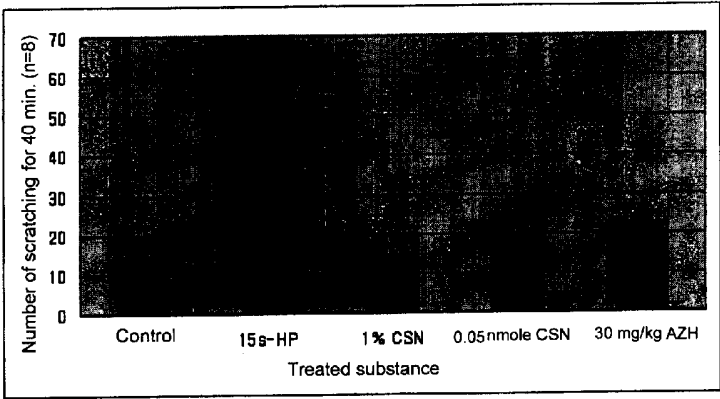


FIG. 4



INTERNATIONAL SEARCH REPORT

International application No.

PCT/KR2005/003648

A. CLASSIFICATION OF SUBJECT MATTER***A61K 31/202(2006.01)i, A61P 17/04(2006.01)i***

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)

IPC 08 A61K 31/202, A61P 17/04

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
KR, JP : IPC as aboveElectronic data base consulted during the international search (name of data base and, where practicable, search terms used)
eKIPASS, Pubmed, CA**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	GMEREK, DEBRA E., et al., 'An animal model for preclinical screening of systemic antipruritic agents', Journal of Pharmacological Methods, 1983, Vol.10, No.2, pp.107-12 See the Abstract	1-15, 17-24
A	DARSOW, U., et al., 'Skin testing of the pruritogenic activity of histamine and cytokines(interleukin-2 and tumor necrosis factor-alpha) at the dermal-epidermal junction', British Journal of Dermatology, 1997, Vol.137, No.3, pp.415-7 See the whole document	1-15, 17-24
A	HAGERMARK, OSTEN, et al., 'Pruritogenic activity of prostaglandin E2', Acta Dermato-venereologica, 1977, Vol.57, No.1, pp.37-43 See the whole document	1-15, 17-24
A	ANDOH, TSUGUNOBU, et al., 'Intradermal leukotriene B4, but not prostaglandin E2, induces itch-associated responses in mice', European Journal of Pharmacology, 1998, Vol.353, No.1, pp.93-6 See the whole document	1-15, 17-24
A	DAVIES, M.G., et al., 'Sensory responses of human skin to synthetic histamine analogs and histamine', British Journal of Clinical Pharmacology, 1980, Vol.9, No.5, pp.461-5 See the whole document	1-15, 17-24



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 application or patent 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 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

09 JANUARY 2006 (09.01.2006)

Date of mailing of the international search report

10 JANUARY 2006 (10.01.2006)

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/KR2005/003648

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	HASHIMOTO T., et al., 'Itch-scratch responses induced by lysophosphatidic acid in mice', Pharmacology, 2004 Sep., Vol.72, No.1, pp.51-6 See the whole document	1-15, 17-24

INTERNATIONAL SEARCH REPORT

International application No.

PCT/KR2005/003648

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☒ Claims Nos.: 16
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

The scope of the claim is so broad and indefinite that the International Search Authority could not carry meaningful search on the said claim.
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.