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(54) Title: USE OF SPHINGOSYLPHOSPHORYLCHOLINE OR THE DERIVATIVE THEREOF

(57) Abstract: The present invention relates to a use of sphingosylphosphorylcholine 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 sphingosylphosphorylcholine. Sphingosylphosphorylcholine or derivatives thereof according to the present invention are useful in understanding an itch, for screening the curing agents for an itch, and for evaluating and developing such agent.

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**【DESCRIPTION】****【Invention Title】**

USE OF SPHINGOSYLPHOSPHORYLCHOLINE OR THE DERIVATIVE  
THEREOF

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**【Technical Field】**

The present invention relates to a use of sphingosylphosphorylcholine (SPC) or derivatives thereof. More particularly, the present invention relates to a process to induce an itch in an animal by administering sphingosylphosphorylcholine; a process  
10 to screen an anti-itching substance; and a process to evaluate the anti-itching effect of the screened substance. Sphingosylphosphorylcholine 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, most of them are closely related to the skin diseases, with the exceptions of cholestasis,  
20 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 concerning factor which lowers the quality of patient's life. According to a study  
25 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 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 body (Eur. J. Dermatol. 1995; 275: 229-23).

10 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 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 Lysosphingolipids are the structures of sphingolipids in which a fatty acid moiety is absent. Sphingosylphosphorylcholine (SPC) is an N-deacylated form of sphingomyelin (SM). It is structurally similar to sphingosine-1-phosphate, but is characterized by the structure in which choline is bonded to phosphate, and its molecular weight is 465.6.

Lysophosphatidic acid (LPA), which is a similar lipid substance to SPC, is a hydrophilic phospholipid and is involved in proliferation, differentiation, survival and movement of various cells (TRENDS in Biochemical Sciences. 2003: 28: 377-83).

A recent study revealed that LPA induces an itch, which is a result of the study finding that an itch induced by histamine or substance P is related to LPA (Pharmacology. 2004; 72: 51-56).

30 However, there have been no studies on the correlation between

sphingosylphosphorylcholine and an itch.

It was found that sphingosylphosphorylcholine induced release of  $\text{Ca}^{2+}$  from endoplasmic reticulum in various cells, inhibits or promotes cell growth or cell proliferation, and is deeply involved in cell death. In addition, it plays various roles in various cells by regulating the reconstruction of the cell skeleton and the cell migration. For example, it shows a wound healing effect in fibroblast present in skin, and induces inflammation, Keratinocytes differentiation and melanin formation in melanocytes. Especially, it acts as a strong cell segmentation stimulator in Swiss 3T3 fibroblast. Furthermore, it has been reported that sphingosylphosphorylcholine activates mitogen-associated protein kinase (MAP kinase) via several G-protein coupled receptors (GPCR) and protein kinase C (PKC), and thereby it promotes cell proliferation and cell differentiation.

The most important fact is that sphingosylphosphorylcholine takes on an aspect of substantial increase in the skin of patients suffering from atopic dermatitis (AD) (Journal of Lipid research, 2003: 44: 93-102). This could be confirmed by the fact that the activity of sphingomyelin deacylase is increased in or around the damaged site of the skin of AD patients (Biochem. J. 2000; 350: 747-756).

It has been reported that the increased activity of sphingomyelin deacylase reduces sphingomyelin in cells, which causes the decrease of ceramide formation by sphingomyelinase, finally leading to functional disorder in the skin barrier (J. invest. Dermatol. 2000; 115: 406-413). The pathological role of sphingosylphosphorylcholine in AD patients is still unclear; however, the present invention will suggest a scientific base for the hypothesis that the increase of sphingosylphosphorylcholine can be a cause of an itch in AD patients.

#### 【DISCLOSURE】

#### 【Technical Problem】

The present inventors found that sphingosylphosphorylcholine, which is endogenous, can induce sufficient itch at an extremely low concentration (100  $\mu\text{M}$ ),

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which is 1/200 times smaller amount than the required amount of histamine, serotonin, or substance P (2mM), and thereby completed the present invention.

#### 【Technical Solution】

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

#### 10      【Advantageous Effects】

According to the present invention regarding Sphingosylphosphorylcholine or derivatives thereof effectively induce an itch, and can be used in an experiment for evaluating the anti-itching effect.

#### 15      【Description of Drawings】

Figure 1 is a conceptual diagram showing the metabolism of sphingosylphosphorylcholine.

Figure 2 is a graph showing the effect of sphingosylphosphorylcholine inducing an itch according to the dose.

20       Figure 3 is a graph showing the effect of capsaicin, azelastine and cyproheptadine for inhibiting an itch induced by sphingosylphosphorylcholine.

Figure 4 is a graph showing experimental results on cell proliferation of sphingosylphosphorylcholine (The proliferation induced by sphingosylphosphorylcholine was determined by [6-H<sup>3</sup>]thymidine incorporation).

25       Figure 5 is a graph showing experimental results on cell proliferation of sphingosylphosphorylcholine (The proliferation induced by sphingosylphosphorylcholine was determined by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)).

Figure 6 is a graph showing the search result for new substances that inhibits

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the biological effect of sphingosylphosphorylcholine.

Figure 7 is a graph showing the result of applying Substance #6 to the animal model in which an itch is induced by SPC.

# 5   【Best Mode】

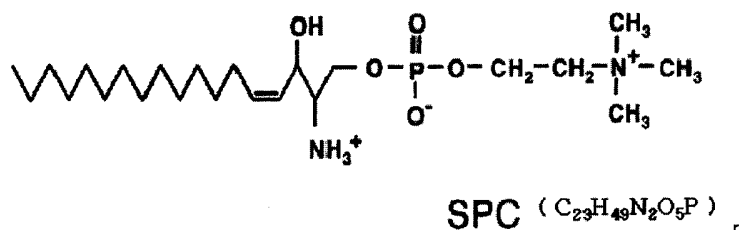
To attain the above objects, the present invention provides a process for inducing an itch to an animal using sphingosylphosphorylcholine, a process for screening an anti-itching substance, and a process for evaluating the effect of the screened substance.

10       The term “itch” has the same meaning as “pruritus”, the term “pruritogen” has 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.

15       The present invention provides a composition for inducing an itch comprising sphingosylphosphorylcholine represented in Chemical Formula 1, or derivatives thereof as an itch-inducing substance.

## 【Chemical Formula 1】



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Sphingosylphosphorylcholine used in the present invention is formed from sphingomyelin by the enzyme called sphingomyelin N-deacylase (Biochem J. 2000; 350: 747-56). It acts as a strong mitogen in various cell types, and is known to promote secretion of specific cytokines (Biochim Biophys Acta. 2002; 1582: 178-89).

The above metabolism process of sphingosylphosphorylcholine is shown in Fig. 1. In addition, it has been reported that a large amount of sphingosylphosphorylcholine is found in the epidermis of AD patients (J. Lipid Res. 2003; 44: 93-102).

The present invention provides a process for inducing an itch by administering  
5 to an animal a composition comprising sphingosylphosphorylcholine 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  
10 sphingosylphosphorylcholine 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.

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

25 Thus, in the present invention, the extent of the itch induced was determined by the number of occurrences of the behavior of lifting the hind paw and scratching the site. In this regard, the behavior of scratching means scratching the site where the itch-inducing substance 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  
30 behavior from scratching the site using the hind paw to dropping the leg was measured

as 1 occurrence of scratching, and was made as the only index of the itch.

In the present invention, the composition comprising sphingosylphosphorylcholine or derivatives thereof was basically administered once. The period of observing the scratching is not particularly limited; however, since  
5 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 by each person and the thus  
10 obtained values were averaged, in order to exclude the subjective views of the observers.

The preferred dose of the composition comprising sphingosylphosphorylcholine or derivatives thereof according to the present invention as well as observation time can be confirmed by the following experiment. The  
15 preferred dose of the composition comprising sphingosylphosphorylcholine or derivatives is 0.01~1000 nmole/site (injection of 50 $\mu$ l of 0.2 $\mu$ M~20mM sphingosylphosphorylcholine). When the dose is less than 0.01 nmole/site, the induced scratching behavior is not significantly increased, and when the dose is more than 1000 nmole/site, a lesion such as hematoma was generated.

20 In addition, the amount of sphingosylphosphorylcholine or derivatives thereof in the composition is preferably  $10^{-5} \sim 1$  wt% (  $2 \times 10^{-7} \sim 2 \times 10^{-2}$  wt%/site) with respect to the 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  
25 accurate and effective estimation of the an itch.

Further, the present invention provides a process of screening an anti-itching substance by administering to an animal the composition comprising sphingosylphosphorylcholine or derivatives thereof.

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

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

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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 sphingosylphosphorylcholine 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 sphingosylphosphorylcholine or derivatives is 0.01~1000 nmole/site (injection of 50 $\mu$ l of 0.2 $\mu$ M~20mM sphingosylphosphorylcholine). When the administration amount was less than 0.01 nmole/site, the induced scratching behavior was not significantly increased, and when the amount was more than 1000 nmole/site, a lesion such as hematoma was generated.

The screening 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 administering 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, azelastine or cyproheptadine. Capsaicin is effective against an itch, especially in incurable an itch in topical areas. Azelastine is a second-generation anti-histamine agent, and cyproheptadine has anti-serotonin action and is widely used in atopic dermatitis patients when they suffer from severe an itch at night.

The process of determining the number of scratching occurrences by the

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animal in step 2) is the same as that in the process of inducing an itch.

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

- 5 1) using the composition of claim 1 to a cell line that induces cell proliferation of said composition;
- 2) determining the optimal concentration for the cell proliferation of the composition in the cell line; and
- 10 3) applying a proposed anti-itching substance to the cell line in which the optimum concentration of the composition is applied and confirming the effect of the proposed substance.

The cell line used in the above process is selected from the group consisting of NIH 3T3 fibroblast, BALB/c 3T3 fibroblast, 3T3L1 adipocyte and Swiss 3T3 fibroblast originated from mice; melanocyte and keratinocyte from humans; and  
15 neonatal cardiac myocytes from rats (Desai NN *et al.*, *Biochem Biophys Res Commun*, 1991 Nov 27; 181(1): 361-6; Seufferlein T *et al.*, *J. Biol. Chem.* 1995 Oct 13; 270(41): 24334-42; Higuchi K. *et al.*, *Pigment Cell Res.* 2003 Dec; 16(6): 670-8; Wakita, H. *et al.*, *J. Invest. Dermatol.* 110: 253-258, 1998; and K. Sekiguchi *et al.*, *Circ. Res.* 85(1999) 1000-1008). The other conditions are the same as those in the above  
20 screening process.

Further, the present invention provides a process of evaluating the anti-itching effect of the anti-itching substance which is screened.

The process comprises

- 25 1) inducing an itch in an animal except for human by administering the composition of sphingosylphosphorylcholine or derivatives thereof to the animal except for human; 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,  
30 rat or guinea pig. More preferably it is ICR mouse.

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The method of administering the composition comprising sphingosylphosphorylcholine 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 sphingosylphosphorylcholine or derivatives is 0.01~1000 nmole/site. When the administration amount was less than 0.01 nmole/site, the induced scratching behavior was not significantly increased, and when the amount was more than 1000 nmole/site, a lesion such as hematoma was generated.

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 administering 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, azelastine or cyproheptadine.

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.

#### 【Mode for Invention】

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 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 sphingosylphosphorylcholine or derivatives thereof. After one administration of the composition of sphingosylphosphorylcholine or derivatives thereof, the scratching behavior of the mice for 40 minutes was recorded by video camera and observed. The control group was administered with saline solution in the same amount. Recorded images were observed by two or more people simultaneously at least 2 times, and the mean value was obtained.

[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 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 sphingosylphosphorylcholine or derivatives thereof together with compound A (substance to be evaluated as anti-itching substance). After one administration of the composition of sphingosylphosphorylcholine or derivatives thereof, the scratching behavior of the mice for 40 minutes was recorded by video camera and observed. The control group was administered with saline solution in the same amount. Recorded images were observed by two or more people simultaneously at least 2 times, and the mean value was obtained.

[Example 1] Determination of the amount of sphingosylphosphorylcholine for inducing an itch

40 male ICR mice (age: 5-8 weeks, weight: 25-32 g) were used in total. 8 mice were used as control and 32 mice were used as experimental. All 40 mice were

shaved on the upper dorsal area 24 hours before the experiment. The 32 experimental mice were divided into 4 groups, 8 mice for each group. The drug (sphingosylphosphorylcholine) was mixed with PBS (pH 7.4) to be a solution at concentration of 100  $\mu$ M. Each drug was then diluted with saline solution to be of  
5 the appropriate concentration used in the experiment. As the amount of PBS is proportionally increased in the group where the drug of high concentration was administered, PBS was added to the control group and to the groups where the drug in lower concentration was administered so that the same amount of PBS was administered in all of the groups.

10 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  $\mu$ l of solution containing sphingosylphosphorylcholine, which was diluted to 100 nM (drug amount: 5 pmole/site), was intradermally injected to the site between the back and neck. To the second group, 50  $\mu$ l of solution containing  
15 sphingosylphosphorylcholine, which was diluted to 1  $\mu$ M (drug amount: 50 pmole/site), was intradermally injected to the site between the back and neck. To the third group, 50  $\mu$ l of solution containing sphingosylphosphorylcholine, which was diluted to 1 mM (drug amount: 50 nmole/site), was intradermally injected to the site between the back and neck. To the fourth group, 50  $\mu$ l of solution containing  
20 sphingosylphosphorylcholine, which was diluted to 3 mM (drug amount: 150 nmole/site), was intradermally injected to the site between the back and neck. To the control group, the same amount of saline solution in which PBS is diluted was intradermally injected.

After administration, animals were moved to the observatory cage, and were  
25 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 experimental results were invalidated.

30 The recorded images were observed by two or more people simultaneously.

The images were observed 2 times and the mean value was obtained. Only the behavior of scratching the site where the itch-inducing substance 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.

5 As can be seen in Fig. 2, all of the sphingosylphosphorylcholine injected groups were significantly induced to an itch compared to the control group. When 50 nmole of sphingosylphosphorylcholine was injected, an itch was induced most effectively (When testing the experimental group and the control group with Student's t-test, the p value was less than 0.01, which means significant increase).

10

[Example 2] Evaluation of the effect of inhibiting itchiness induced by sphingosylphosphorylcholine

48 male ICR mice (age: 5-8 weeks, weight: 25-32 kg), which had been left in the observatory cage for 30 minutes for adaptation, were shaved on the upper dorsal area 24 hours before the experiment. To the first group (1% CSN), 100  $\mu$ l of solution containing 1% capsaicin (dissolved in 75% ethanol) was treated topically, and 5 minutes later 50 nmole of saline solution of sphingosylphosphorylcholine was intradermally injected to the sheared site. To the second group (0.05 nmole CSN), 50 nmole of sphingosylphosphorylcholine and 0.05 nmole of capsaicin were simultaneously dissolved in saline solution, and this was intradermally injected to the site. To the third group (30 mg/kg AZH), 30 mg/kg of azelastine was dissolved in saline solution and orally administered 30 minutes before intradermally injecting 50 nmole of sphingosylphosphorylcholine. To the fourth group (30 mg/kg, CYP), 30 mg/kg of cyproheptadine was dissolved in saline solution and orally administered 30 minutes before intradermally injecting 50 nmole of sphingosylphosphorylcholine. To the Fifth group (called "SPC group"), 50 nmole of sphingosylphosphorylcholine was dissolved in saline solution and was intradermally injected. 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 case, 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.

5       The recorded images were observed by two or more people simultaneously. The images were observed 2 times and the mean value was obtained. Only the behavior of scratching the site where the itch-inducing substance 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.

10       As can be seen in Fig. 3, the groups in which 50 nmole/site of sphingosylphosphorylcholine was injected were significantly induced to an itch compared to the control group. In all of the groups in which the anti-itching substance was topically, intradermally or orally administered, the itch induced by SPC was effectively inhibited (When testing the groups in which the anti-itching substance  
15       was injected and the group in which sphingosylphosphorylcholine was solely injected with Student's t-test, the p value was less than 0.05, which means the significant difference).

[Example 3] Determination of the optimum concentration for the cell proliferation  
20       induction using sphingosylphosphorylcholine

      This determination method was established to develop a novel substance that can inhibit the effect of sphingosylphosphorylcholine on the induction of an itch and other causes of disease. The cell proliferation of SPC was induced using NIH 3T3 cell line; thereby the optimum concentration was obtained. The results are shown in  
25       Figs. 4 and 5. In Fig. 4, the proliferation of sphingosylphosphorylcholine was determined by [ $^3\text{H}$ ] thymidine incorporation. In Fig. 5, the proliferation of sphingosylphosphorylcholine was determined by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide).

      The details of the above experiment are as follows: First,  $1 \times 10^5$  (normally  $1 \times 10^4 \sim 10^6$ ) NIH 3T3 cells were inoculated on a culture plate, and cultivated. They  
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were then synchronized by serum starvation, then treated with sphingosylphosphorylcholine to induce cell proliferation. The cell proliferation induction was done by [6-H<sup>3</sup>] thymidine incorporation and by MTT (3-(4,5-dimethylthiazol-2-yl)- 2,5-diphenyltetrazolium bromide). As can be seen with the above two determination methods, the cell proliferation of SPC was accomplished in the concentration of 0.4~20 mM. The most effective concentration range was found to be 3~15 mM. (When testing the group in which sphingosylphosphorylcholine was injected and a non-treated group with Student's t-test, the p value was less than 0.05, which means the significant difference).

Among the above concentration range, it was concluded that about 7 mM is the optimum concentration, and this concentration was used in the following experiments. In addition, as the results of both [6-H<sup>3</sup>] thymidine incorporation and MTT (3-(4,5-dimethylthiazol-2-yl)- 2,5-diphenyltetrazolium bromide) method were similar to each other, the MTT method, which is methodologically simpler and faster, was used in the following experiments.

[Example 4] Screening the SPC inhibiting substance using the effect of the cell proliferation induced by sphingosylphosphorylcholine

The following experiment was carried out in order to screen substances which inhibit the function of SPC, using the result of the SPC induced cell proliferation. This experiment is the method (MTT determination method) for screening substances that inhibit the cell proliferation effect of SPC, and using this method, novel proposed substances which inhibit SPC can be searched (Fig. 6). As can be seen in Fig 6, at 0.1 ppm, the effect of substance #6 was excellent among the substances which apparently inhibit the function of SPC. At 1 ppm, substance #6 shows the best effect. At 10 ppm, substances #1, #4, #6, #7 and #8 show the best effect (When testing the group in which sphingosylphosphorylcholine was solely injected and the groups in which both sphingosylphosphorylcholine and the novel substance were injected together with Student's t-test, the p value was less than 0.05, which means the significant difference). By applying the substances screened by this method to the

model in which an itch was induced by SPC, substances which are proposed to have an anti-an itch effect can be primarily screened. In addition, by applying to other diseases in which SPC is increased, new proposed drugs can be rapidly screened.

- 5 [Example 5] Application of proposed an itch-inhibition substances to animal model where an itch was induced by SPC

Among the substances #1, #4, #6, #7 and #8 screened in Example 4, substance #6 was applied to the animal model in which an itch was induced by SPC. Substance #6 was dissolved in saline solutions, and one of 1 mg/kg or 10 mg/kg of the solution  
10 was administrated orally to each ICR mouse. After 1 hour, SPC was intradermally injected (50 mmole/site). After injection, the scratching behavior of the mice was observed for 30 minutes. The results are shown in Fig. 7.

As can be seen in Fig. 7, it can be confirmed that substance #6 inhibits an itch induced by SPC in a concentration-dependent way. At 10 mg/kg, an itch induced by  
15 SPC was decreased by about 65%. (When testing the group in which sphingosylphosphorylcholine was solely injected and the groups in which SPC was injected after treating with the substance #6 with Student's t-test, the p value was less than 0.05, which means the significant difference).

Therefore, rapid screening of the proposed substances that can inhibit the  
20 effect of SPC in cell level is possible, and by way of confirming them in the animal model in which an itch was induced by SPC, effective selection of substances having anti-an itch effect is possible.

#### **【Industrial Applicability】**

As can be seen in the above, sphingosylphosphorylcholine and derivatives  
25 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 one or more selected from the group consisting of sphingosylphosphorylcholine and derivatives thereof as effective  
5 ingredient.

**【Claim 2】**

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

**【Claim 3】**

10 The process according to claim 2, wherein said animal is a rodent selected from the group consisting of mouse, rat and guinea pig.

**【Claim 4】**

The process according to claim 3, wherein said animal is ICR mouse.

**【Claim 5】**

15 The process according to claim 2, wherein said administration is selected from the group consisting of intradermal injection, subcutaneous injection, intravenous injection, intraperitoneal injection, intramuscular injection, topical application, patch and iontophoresis.

**【Claim 6】**

20 The process according to any one of claims 2 to 5, wherein the amount of administered composition is 0.01-1000 nmole/site.

**【Claim 7】**

A process of screening an anti-itching substance comprising:

- 25 1) inducing an itch in an animal except for human by administering the composition of claim 1 to the animal; and  
2) determining the number of occurrences of scratching by the animal.

**【Claim 8】**

The process according to claim 7, wherein said animal is a rodent selected from the group consisting of mouse, rat and guinea pig.

**【Claim 9】**

The process according to claim 8, wherein said animal is ICR mouse.

**【Claim 10】**

5 The process according to claim 7, wherein said administration is selected from the group consisting of intradermal injection, subcutaneous injection, intravenous injection, intraperitoneal injection, intramuscular injection, topical application, patch and iontophoresis.

**【Claim 11】**

10 The process according to claim 7, further comprising orally administering an anti-itching substance prior to administering the composition.

**【Claim 12】**

The process according to claim 7, further comprising intradermally injecting an anti-itching substance at the same time as administering the composition.

**【Claim 13】**

15 The process according to claim 7, further comprising, after administering the composition, topically administering an anti-itching substance at and around the site of administering the composition.

**【Claim 14】**

20 The process according to any one of claims 7 to 13, wherein the amount of administered composition is 0.01-1000 nmole/site.

**【Claim 15】**

An anti-itching substance obtained by the screening process according to any one of claims 7 to 13.

**【Claim 16】**

25 A process of screening an anti-itching substance comprising:

- 1) using the composition of claim 1 to a cell line, which induces cell proliferation of the composition;
- 2) determining the optimum concentration of the composition for the cell proliferation in the cell line; and

3) applying a proposed anti-itching substance to the cell line in which the optimum concentration of the composition is applied, and confirming the effect of the proposed anti-itching substance.

**【Claim 17】**

5           The method according to claim 16, wherein said cell line is selected from the group consisting of NIH 3T3 fibroblast, BALB/c 3T3 fibroblast, 3T3L1 adipocyte and Swiss 3T3 fibroblast which are originated from mouse; melanocytes and keratinocyte from human; and neonatal cardiac myocytes from rat.

**【Claim 18】**

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

- 1) inducing an itch in an animal except for human by administering the composition of claim 1 to the animal; and
- 2) determining the number of occurrences of scratching by the animal.

15           **【Claim 19】**

          The process according to claim 18, wherein said animal is a rodent selected from the group consisting of mouse, rat and guinea pig.

**【Claim 20】**

          The process according to claim 19, wherein said animal is ICR mouse.

20           **【Claim 21】**

          The process according to claim 18, wherein said administration is selected from the group consisting of intradermal injection, subcutaneous injection, intravenous injection, intraperitoneal injection, intramuscular injection, topical application, patch and iontophoresis.

25           **【Claim 22】**

          The process according to claim 18, further comprising, prior to administering the composition, orally administering a substance to be evaluated for its anti-itching effect.

**【Claim 23】**

The process according to claim 18, further comprising, at the same time as administering the composition, intradermally injecting a substance to be evaluated for its anti-itching effect.

**【Claim 24】**

5       The process according to claim 18, 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.

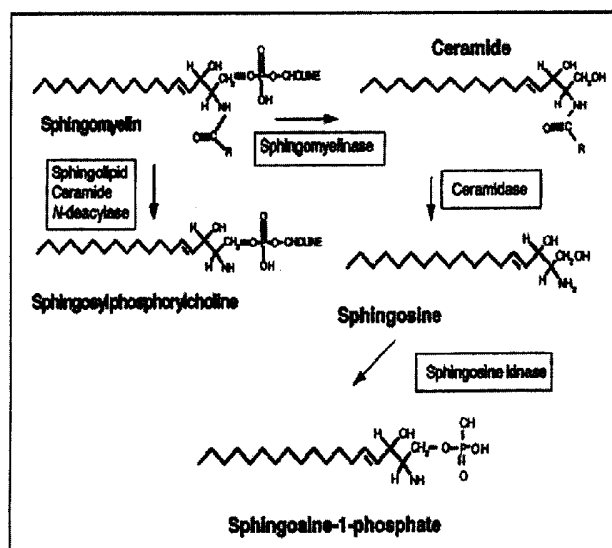
**【Claim 25】**

10       The process according to any one of claims 18 to 24, wherein the amount of the composition administered is 0.01-1000 nmole/site.

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## FIGURES

FIG. 1



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FIG. 2

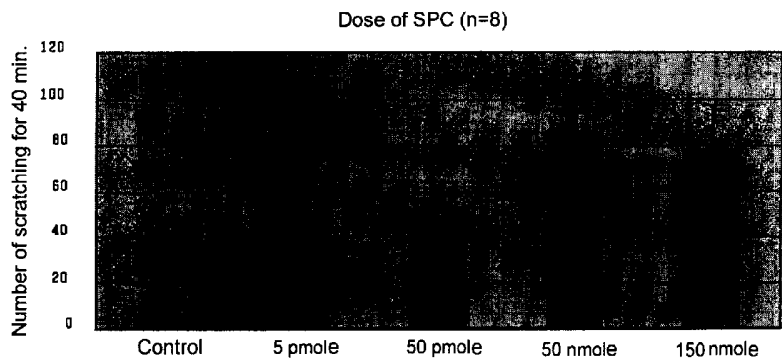
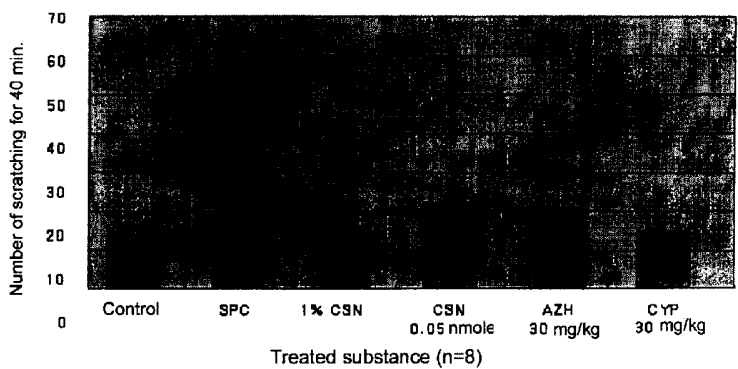


FIG. 3



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FIG. 4

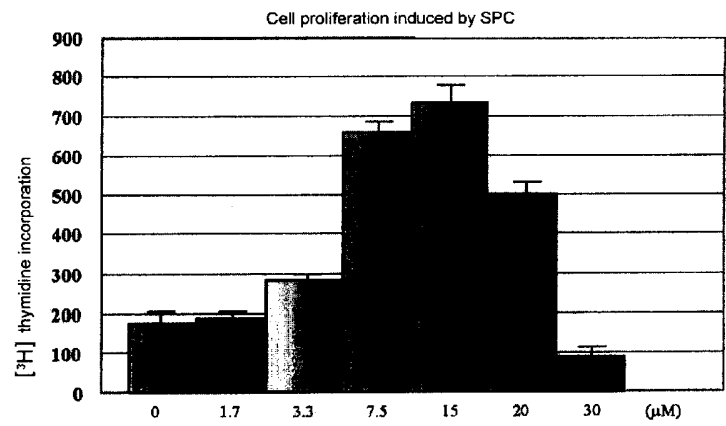


FIG. 5

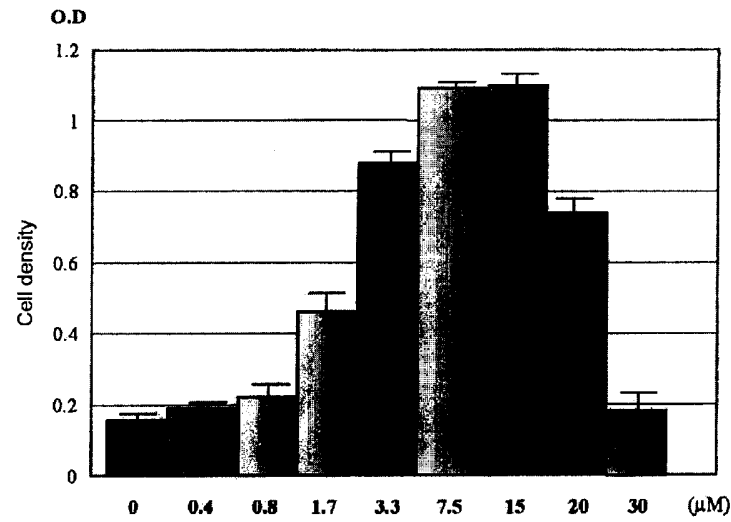


FIG. 6

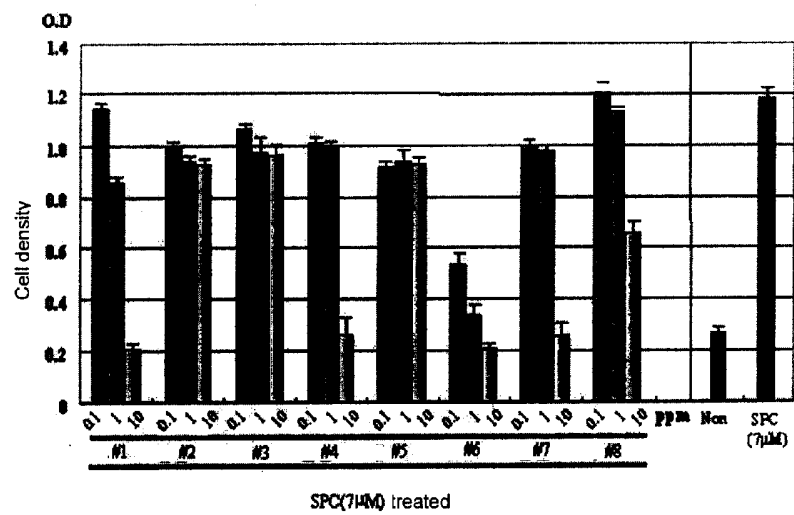
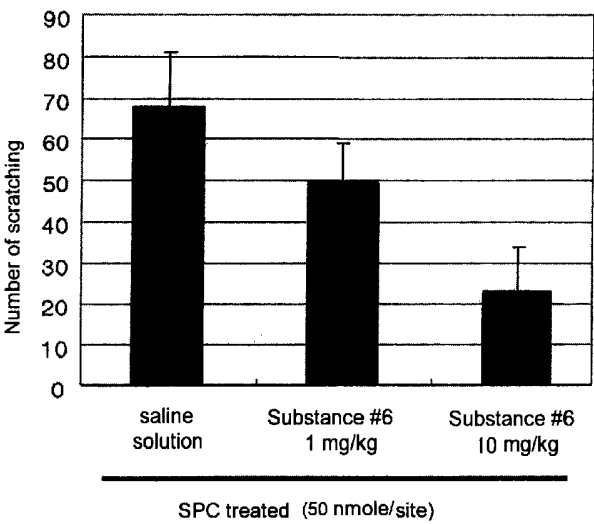


FIG. 7



## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/KR2005/003711

**A. CLASSIFICATION OF SUBJECT MATTER****IPC7 A61K 31/685, A61P 17/04**

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)

IPC7 A61K 31/685, 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 above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

eKIPASS, Pubmed [ sphingosylphosphorylcholine AND ( itch(y) OR atopy OR AD)]

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category* | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                            | Relevant to claim No. |
|-----------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| A, D      | OKAMOTO et al., 'Sphingosylphosphorylcholine is upregulated in the stratum corneum of patients with atopic dermatitis', Journal of Lipid Research, 2003, Vol.44, pp.93-102<br>See the whole document.                         | 1 - 14, 16 - 25       |
| A, D      | HASHIMOTO et al., 'Itch-scratch responses induced by lysophosphatidic acid in mice', Pharmacology, Sep. 2004, Vol.72(1), pp.51-56<br>See the whole document.                                                                  | 1 - 14, 16 - 25       |
| A, D      | HARA et al., 'High-expression of sphingomyelin deacylase is an important determinant of ceramide deficiency leading to barrier disruption in atopic dermatitis', Sep. 2004, Vol.115(3), pp.406-413<br>See the whole document. | 1 - 14, 16 - 25       |
| A         | US 5714478 A (SARAH SPIEGEL) 3 Feb. 1998<br>See the whole document.                                                                                                                                                           | 1 - 14, 16 - 25       |



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

"&amp;" document member of the same patent family

Date of the actual completion of the international search

20 DECEMBER 2005 (20.12.2005)

Date of mailing of the international search report

**20 DECEMBER 2005 (20.12.2005)**

Name and mailing address of the ISA/KR



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Facsimile No. 82-42-472-7140

Authorized officer

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Telephone No. 82-42-481-5601



**INTERNATIONAL SEARCH REPORT**

International application No.

PCT/KR2005/003711

**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.: 15  
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.

**INTERNATIONAL SEARCH REPORT**

Information on patent family members

International application No.

PCT/KR2005/003711

| Patent document<br>cited in search report | Publication<br>date | Patent family<br>member(s) | Publication<br>date |
|-------------------------------------------|---------------------|----------------------------|---------------------|
| US 5714478 A                              | 03.02.1998          | WO 97/11706 A1             | 03.04.1997          |
|                                           |                     | EP 0862438 A1              | 09.09.1998          |
|                                           |                     | DE 69607854 T2             | 17.08.2000          |
|                                           |                     | CA 2233331 AA              | 03.04.1997          |