Topical Surfactant-Induced Pruritus: Involvement of Histamine Released from Epidermal Keratinocytes

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ABSTRACT
Surfactants, an important component of cleansers, often cause itch in humans. Topical application of sodium laurate and N-lauroylsarcosine sodium salt to the skin of mice immediately (for 1–1.5 hours) increased scratching, and the former increased scratching again between 2 and 3 hours after application. Thus, we examined the mechanisms of sodium laurate-induced delayed scratching. Sodium laurate (0.1%–10%) increased delayed scratching and skin surface pH in a concentration-dependent manner. N-lauroylsarcosine sodium salt had no effect on these parameters, and sodium hydroxide solution did not increase delayed scratching. Sodium laurate-induced delayed scratching was markedly inhibited by the H1 histamine receptor antagonist terfenadine, but it was not affected by mast cell deficiency. Sodium laurate application had no effect on the number of total and degranulated mast cells, and did not induce plasma extravasation or the infiltration of inflammatory cells in the skin. Sodium laurate application increased the histamine content of the epidermis, but not that of the dermis, in normal and mast cell-deficient mice. Sodium laurate application increased the ratio of 53-kDa L-histidine decarboxylase (HDC, a key enzyme for histamine production) to 74-kDa HDC in the mouse epidermis and in a human keratinocyte culture. Sodium laurate increased histamine in the human keratinocyte culture, without affecting cell viability. The present results suggest that sodium laurate induced delayed scratching at an alkaline pH through the increased production of histamine in keratinocytes, which may be due to enhanced processing of 74-kDa to 53-kDa HDC.

Introduction

The use of toiletries (or cosmetics), such as soaps and shampoos, often causes adverse effects, including skin irritation, dryness, and itching (Groot 1987; Berne et al., 1996). Many cleansers contain surfactants, and repeated exposure to these surfactants can cause allergic contact dermatitis (Inoue et al., 2008) and irritant contact dermatitis (Wigger-Alberti et al., 2000). Surfactants irritate the skin through several mechanisms, including interaction with keratin (Imokawa et al., 1975) and changing lipid structure and barrier function (Wihelm et al., 1994; Ananthapadmanabhan et al., 2004). Cutaneous irritation is dependent on the duration and frequency of surfactant exposure and the concentration and type of surfactant. Surfactants are categorized, according to the charges present in their hydrophilic head, into four primary groups: anionic, cationic, amphoteric, and nonionic. Anionic surfactants, which chemically possess a negative charge on their hydrophilic head, are commonly used in soaps and detergents because of their high detergency; therefore, exposure to anionic surfactants occurs almost daily. Anionic surfactants remove essential components from the skin, such as sebum barrier, ceramide, and natural moisturizing factors. The most frequently reported subjective symptom in surfactant users is itching (Groot 1987). Repeated itch scratching damages the skin and initiates a vicious itch-scratch cycle, which worsens the cutaneous lesions due to irritation and dryness. Anionic substances, such as poly-l-lysine and morphine, are apt to degranulate mast cells, which releases histamine, an itch mediator (Church et al., 1991). In this study, we accordingly asked whether the topical application of anionic surfactants caused acute pruritus, and whether histamine is involved in the pruritogenic action.

Materials and Methods

Animals. Male Institute for Cancer Research mice (Japan SLC [Sankyo Labo Service Corporation], Shizuoka, Japan) were used at 7–8 weeks of age. In a series of experiments, male mast cell-deficient mice (WBB6F1-W/W'; Japan SLC) and their normal littermates (WBB6F1-+/+; Japan SLC) were used at 7 weeks of age. The animals were housed in a room under controlled temperature (21–23°C), humidity (45–65%), and light (lights on from 7:00 AM to 7:00 PM). Food and water were freely available. The procedures used in the animal experiments were approved by the Committee for Animal Experiments at the University of Toyama.

Materials. Sodium laurate [CH₃(CH₂)₁₀–COONa] and N-lauroylsarcosine sodium salt [CH₃(CH₂)₁₀–CO–N(CH₃)–CH₂–COONa] (Nacalai Tesque, Inc., Kyoto, Japan) were dissolved in distilled water to the desired final concentrations and stored at 4°C until use. Terfenadine (Sigma-Aldrich, St. Louis, MO) was dissolved in 0.5% sodium saline.

ABBREVIATIONS: ANOVA, analysis of variance; HDC, L-histidine decarboxylase; ICR, Institute for Cancer Research; PBS, phosphate-buffered saline.
carboxymethyl cellulose just before use. Sodium hydroxide solution (1 M; Wako Pure Chemical Industries, Ltd., Osaka, Japan) was diluted with distilled water just before use. Rabbit polyclonal anti-\( \gamma \)-histidine decarboxylase (HDC) antibody (Progen Biotechnik GmbH, Heidelberg, Germany) and rabbit polyclonal anti-\( \beta \)-actin antibody (Abcam, Tokyo, Japan) were diluted to the appropriate concentrations with the reaction solution 1 (Toyobo Co. Ltd., Osaka, Japan), and fluorophore-labeled donkey anti-rabbit IgG antibody (Invitrogen Co., Carlsbad, CA) was diluted with the reaction solution 2 (Toyobo Co. Ltd.).

**Agent Treatment.** In the in vivo experiments, the rostral part of the back was shaved, and 3 days later, sodium laurate (a 10% solution was warmed to 37°C before use because of its high viscosity at low temperatures) and \( N \)-lauroylsarcosine sodium salt were applied topically in a volume of 50 \( \mu l \); these solutions were not removed after the application. For the application of sodium hydroxide solution, thin paper (2.5 x 2 cm) saturated with 50 \( \mu l \) of 0.1 M sodium hydroxide was placed on the shaved skin for 30 seconds. In the in vitro experiments using a three-dimensional keratinocyte culture (vide infra), 100 \( \mu l \) of sodium laurate solution was applied topically to the keratinous layer of the culture for 1 minute, then immediately removed. The external aspect of the culture was washed three times with distilled water and then left to stand in 5% CO\(_2\) and air at 37°C for 3 hours. Terfenadine was administered orally 30 minutes before the start of the behavioral observations.

**Behavioral Experiments.** Mice were placed individually into an acrylic observation cage. After a 1-hour acclimation, their behaviors were videotaped with no one present. Hind-paw scratching of the rostral back was determined during playback of the video (Kurachi et al., 1995). A series of these movements was counted as a single bout of scratching (Tsujii et al., 2008).

**Histologic Staining.** Mice were transcardially perfused with phosphate-buffered saline (PBS) followed by 4% paraformaldehyde under sodium pentobarbital anesthesia (80 mg/kg, i.p.; Sigma-Aldrich) 2 hours after surfactant treatment. The skin was removed from the surfactant-treated region and postfixed in the same fixative for 24 hours. Skin specimens were embedded in paraffin and sectioned at 3 \( \mu m \). After deparaffinization, the skin sections were stained with hematoxylin and eosin or 0.1\% toluidine blue. The staining was observed using a light microscope (model BZ-8000; Keyence, Osaka, Japan). The number of mast cells was counted in nine sections (field size: 662.0 x 579.3 \( \mu m \)) randomly selected from each skin specimen, and the average was taken for each animal. Pathologic evaluation was performed in a blinded fashion.

**Measurement of Erythema, Plasma Extravasation, and Skin Surface pH.** Cutaneous erythema was observed macroscopically and scored as follows: 0 = normal, 1 = subtle erythema, 2 = mild erythema, and 3 = severe erythema. For the observation of plasma extravasation, 150 \( \mu l \) of 1% Evans blue dissolved in physiologic saline was injected into the tail vein of the mice 40 minutes after the application of surfactant. After 80 minutes, the mice were euthanized via cervical dislocation, skin was removed from the shaved region, and a circle 18 mm in diameter was punched out. The skin sample was incubated in 200 \( \mu l \) of dimethylsulfoxide overnight, and the concentration of dye extracted was determined spectrophotometrically at 620 nm (Andoh et al., 2010). The pH of the skin surface was measured using a pH meter with a flat probe (model 6261-10c; Horiba Co., Ltd., Kyoto, Japan).

**Enzyme Immunoassay for Histamine.** For the determination of cutaneous histamine content, mice were transcardially perfused with PBS under sodium pentobarbital anesthesia 2 hours after surfactant treatment. The skin was removed from the surfactant-treated region, and skin specimens 18 mm in diameter were taken using a punch. The epidermis and dermis were separated by heating at 60°C for 30 seconds and used for histamine determination.

To determine whether keratinocytes produce and contain histamine, we used the Labcyte Epi-Model system (Japan Tissue Engineering Co., Ltd., Gamagori, Japan), a three-dimensional keratinocyte culture prepared from normal human keratinocytes obtained from neonate foreskin, which was free of other epidermal cells, such as melanocytes and dendritic cells. The keratinocyte culture was placed on 500 \( \mu l \) of culture medium and maintained in an incubator in 5% CO\(_2\) and air at 37°C. Three hours after surfactant treatment, the culture medium and keratinocyte culture were used for the assay.

The epidermis and dermis samples and the keratinocyte culture were homogenized in 300, 1500, and 300 \( \mu l \) of 0.4 M perchloric acid, respectively, using a Preceylls 24 tissue homogenizer (Bertin Technologies, Montigny, France). The samples were centrifuged at 10,000g for 10 minutes at 4°C. The concentration of histamine in their supernatant and culture medium was determined using a histamine enzyme immunoassay kit (ImmunoTech, Marseilles, France), in which cross reactivities for 3-methylhistamine, methylyhistamine, and histidine were 0.038, 0.01, and less than 0.01%, respectively, and the detection limit for histamine was 0.1 nM.

**Cell Viability Assay.** To determine cell viability, the keratinocyte culture was incubated on the culture medium containing 0.5 mg/ml of 3,4,5-dimethylthiazol-2-yl-2,5-diphenyl tetrazolium bromide (Sigma-Aldrich) for 3 hours under the previously mentioned conditions. After removing the culture medium, the reaction product formazan was extracted from the culture by treating it with isopropanol, and its concentration was determined by assessing differences in the absorbance at 570 and 650 nm using a microplate reader (Molecular Devices, Sunnyvale, CA) with isopropanol used as a blank.

**Western Blotting.** Protein was extracted from murine epidermis (heat treated at 60°C for 30 seconds to separate the dermis and epidermis) and the human keratinocyte culture using a mammalian cell lysis kit (Sigma-Aldrich). Protein extracts (20 and 7 \( \mu g \) from murine epidermis and the human keratinocyte culture, respectively) underwent electrophoresis on a NuPAGE 4–12% Bis-Tris gel (Invitrogen) and were transferred to a polyvinylidene difluoride membrane. After blocking with 1\% skim milk in PBS containing 0.1\% Tween 20, the membrane was cut, at an approximately 50-kDa point, and the upper and lower parts were reacted with rabbit polyclonal anti-HDC and anti-\( \beta \)-actin antibodies (1/1000 each), respectively, overnight at 4°C. After washing with PBS containing Tween 20, the membranes were incubated with fluorophore-labeled donkey anti-rabbit IgG antibody (1/1000) for 2 hours. These membranes were then scanned using a fluorescence scanner (Typhoon; GE Healthcare, Munich, Germany), and positive bands were quantified using Scion Image (Scion Corporation, Frederick, MD).

**Statistical Analysis.** The data are presented as means ± S.D., except for the data on histamine content in the human keratinocyte culture, which are presented as geometric means ± 95% confidence limits because of low concentrations. Statistical significance was analyzed using one-way, two-way, or two-way repeated-measures analysis of variance (ANOVA) followed by Dunnett’s test, Tukey’s test, or Fisher’s least-significant difference test; \( P < 0.05 \) was considered significant. The statistical analyses were performed using the statistical software SigmaPlot (version 11; Systat Software, Inc., Chicago, IL).

**Results**

**Different Effects of Sodium Laurate and \( N \)-lauroylsarcosine Sodium Salt.** Whereas application of the vehicle (water) did not increase scratching, a single topical application of 10% sodium laurate to shaved skin markedly increased scratching in ICR mice; scratching was immediately induced (early phase), gradually decreased up to 1.5 hours after application, then increased again (delayed phase) before subsiding approximately 3 hours after application (Fig. 1A). When the pH of sodium laurate solution was decreased, sodium laurate was educed from the solution; therefore, we examined the effects of \( N \)-lauroylsarcosine sodium salt, an
almost neutral surfactant with a chemical structure similar to sodium laurate. Early-phase scratching was also observed after the topical application of 10% N-lauroylsarcosine sodium salt, but delayed-phase scratching did not occur (Fig. 1B). Since early-phase scratching was also observed after the topical application of an ointment base (Andoh T et al., unpublished observation), the scratching might be a response to the physical stimulation of the skin surface by exogenous materials. On the other hand, the delayed-phase scratching is unique to sodium laurate. In an additional experiment using different mice, delayed-phase scratching was obvious from 2 to 2.5 hours after application (unpublished data). In the subsequent experiments, therefore, the delayed effect of sodium laurate on scratching was examined from 2 to 3 hours after application. Topical applications of 0.1, 1, and 10% sodium laurate solutions (pH 7.6, 9.8, and 10.1, respectively) increased scratching in a concentration-dependent manner; a significant increase was observed after the application of 1% and 10% sodium laurate (Fig. 1C). No significant increase in scratching was observed after 24 hours (Fig. 1C). The topical application of 10% N-lauroylsarcosine sodium salt (pH 7.7) did not increase scratching 2 or 24 hours after application (Fig. 1C). Since an increase in the pH of N-lauroylsarcosine sodium salt solution results in its hydrolysis, we examined the effect of alkaline stimulation without surface action. Topical application of 0.1 mM sodium hydroxide solution (pH 10.2) did not increase scratching; scratching bouts per hour were 25.1 ± 8.0, 36.2 ± 31.6, and 25.8 ± 12.2 (n = 11 each) before, 2 hours, and 24 hours after application, respectively.

Two-hour topical application of 1% and 10% sodium laurate significantly increased the pH of the skin surface from 5.1 to 5.8 and 6.0, respectively (Fig. 1D). The pH of the skin surface 24 hours after application of 10% sodium laurate was 5.4, which was significantly higher than the normal pH (Fig. 1D). In contrast, the pH of the skin surface was almost normal (pH 5.0–5.2) 2 and 24 hours after application of 10% N-lauroylsarcosine sodium salt or 0.1% sodium laurate (Fig. 1D).

**Effect of Terfenadine on Sodium Laurate–Induced Scratching.** Topical application of 10% sodium laurate increased scratching in ICR mice 2 hours after application. This was, however, significantly suppressed by an oral after-application treatment (90 minutes after application) with the peripherally acting H1 histamine receptor antagonist terfenadine (30 mg/kg); the number of bouts of scratching was similar to that before topical application (Fig. 2).

**Effect of Mast Cell Deficiency on Sodium Laurate–Induced Scratching.** The topical application of 10% sodium laurate increased scratching even in mast cell–deficient WBB6F1-/-W/W mice; the number of scratching events was similar in these mice and their normal littermates (Fig. 3A). Skin-surface pH was increased from around 6.3 to around 7.2 by the topical application of 10% sodium laurate, and the degree of pH increase was similar in WBB6F1-/-W/W mice and their normal littermates (Fig. 3B).

**Pathologic Evaluation of the Skin.** The topical application of 10% sodium laurate did not affect the appearance of...
the treated skin or cause any erythema (erythema score = 0, n = 6) in ICR mice at 2 and 24 hours after application. It did not induce the infiltration of inflammatory cells into the dermis (hematoxylin and eosin staining) or the degranulation of mast cells (assessed with toluidine blue staining) 2 hours after application (Fig. 4). Sodium laurate (10%) did not affect the number of mast cells in the dermis; the number of mast cells per section was 62.7 ± 12.5 and 63.7 ± 7.0 (n = 3 each) in vehicle- and sodium laurate–treated groups, respectively. There was an increased tendency for plasma extravasation, but this apparent difference was not statistically significant; the amount of the extravasated Evans blue (micrograms per tissue) was 1.12 ± 0.48 (n = 4) and 1.84 ± 1.49 (n = 5) in vehicle- and sodium laurate–treated groups, respectively.

**Histamine and HDC in the Skin.** Topical application of 10% sodium laurate significantly increased the epidermal content of histamine in ICR mice 2 hours after application; this effect almost subsided by 24 hours (Fig. 5A). The effect of sodium laurate was limited to the epidermis, and the histamine content did not increase in the dermis 2 or 24 hours after application (Fig. 5A).

Topical application of 10% sodium laurate markedly increased the epidermal level of 53-kDa HDC in ICR mice 2 hours after application, whereas vehicle treatment had no effect (Fig. 5B). The ratio of 53-kDa HDC to 74-kDa HDC was significantly increased 2 hours after sodium laurate application and had almost returned to the untreated level after 24 hours (Fig. 5C).

The histamine content of the normal epidermis in the WBB6F1-W/Wv mice was similar to that of the ICR mice, and it was significantly increased by 10% sodium laurate 2 hours after topical application (Fig. 6). The histamine content of the normal dermis in the WBB6F1-W/Wv mice was approximately 1/25 of that in the ICR mice (Figs. 5A and 6). Topical application of 10% sodium laurate did not increase the dermal content of histamine in WBB6F1-W/Wv mice (Fig. 6).

**Histamine and HDC in the Human Keratinocyte Culture.** The concentration of histamine in a three-dimensional keratinocyte culture (including histamine in the medium) was around the detection limit in the untreated group and groups treated with vehicle and 0.1% sodium laurate, and was increased by a 1-minute application of 0.5% and 1% sodium laurate to the keratin layer in a concentration-dependent manner; this effect was significant after application of 1% sodium laurate (Fig. 7A). We did not examine the effects of the long-duration application and the 10% sodium laurate because they disrupted the structure of the keratinocyte culture. Treatment with 0.1–1% sodium laurate did not affect the cell viability of the keratinocyte culture (unpublished data).

The levels of 53- and 74-kDa HDC in the human keratinocyte culture were low compared with those in the mouse epidermis, and there were no clear effects of sodium laurate treatment (Fig. 7B) except that the ratio of 53-kDa HDC to 74-kDa HDC was increased by sodium laurate treatment (0.1–1%) in a concentration-dependent manner; this increase was significant after application of 1% sodium laurate (Fig. 7C).

**Discussion**

A single topical application of sodium laurate increased scratching, an itch-related response, in mice; the scratching responses had two phases (an early and a delayed phase). Although the early-phase response was observed after both
sodium laurate and N-lauroylsarcosine sodium salt applications, the delayed-phase response was observed only after sodium laurate application. In the present study, we investigated the mechanisms of sodium laurate–induced delayed scratching. Delayed scratching was significantly increased by 1% and 10%, but not 0.1%, sodium laurate. Sodium laurate significantly increased the pH of the skin surface at concentrations of 1% and 10%, but not 0.1%. In contrast, 10% N-lauroylsarcosine sodium salt did not increase the skin surface pH. These results, taken together, suggest that alkalinity is an important factor in the pruritogenic activity of topically applied surfactants.

Sodium laurate–induced delayed-phase scratching was almost completely suppressed by terfenadine at a dose of 30 mg/kg. Terfenadine at this dose has been shown to inhibit scratching and plasma extravasation induced by histamine injection and mast cell degranulation (Inagaki et al., 1999; Ohtsuka et al., 2001; Ui et al., 2006) but not scratching induced by other pruritogens, such as nociceptin and sphingo- sylphosphorylcholine, in ICR mice (Andoh et al., 2004, 2009), suggesting that terfenadine inhibits scratching through a peripheral action but not sedation. Thus, it is suggested that the effect of terfenadine on sodium laurate–induced delayed-phase scratching is due to the blockade of H1 histamine receptors in the skin. The main cutaneous cells that contain histamine are the mast cells in the dermis. However, the topical application of sodium laurate increased scratching to a similar degree in mast cell–deficient mice and their normal littermates. In addition, sodium laurate did not increase the numbers of total and degranulated mast cells, induce plasma extravasation, or affect the content of histamine in the dermis. These results, taken together, suggest that the histamine contained in the mast cells is not a causal mediator of topical sodium laurate–induced delayed scratching. Macrophages have been shown to have HDC activity and contain histamine in vitro (Kawaguchi-Nagata et al., 1988; Shiraishi et al., 2000). However, sodium laurate–induced delayed scratching might not involve macrophage-contained histamine because sodium laurate application did not induce the infiltration of inflammatory cells into the skin or increase histamine in the dermis of mast cell–deficient mice and ICR mice.

Since PB cells (a mouse keratinocyte–derived cell line) have been reported to contain histamine (Fitzsimons et al., 2001), we investigated whether sodium laurate would increase histamine in keratinocytes. Although the level was low, there was a detectable amount of histamine in the epidermis of normal mice. Therefore, using a three-dimensional human keratinocyte culture, we examined whether the topical application of sodium laurate increased histamine in the keratinocytes. A brief (1-minute duration) application of sodium laurate increased histamine in the culture without affecting keratinocyte viability. Therefore, the increased production of histamine in the keratinocytes may be a cause of the delayed phase of sodium laurate–induced scratching.

Topical application of sodium laurate did not decrease, but instead increased, the content of histamine in the epidermis. This increase was obvious 2 hours after application, which corresponds to the delayed-phase scratching induced by the topical application of sodium laurate. Therefore, the increase

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**Fig. 5.** The effects of topical application of sodium laurate on the histamine content and HDC level in the skin of ICR mice. Sodium laurate or vehicle was applied topically to the rostral part of the back. (A) The histamine content of the epidermis and dermis. Open column: nontreated (NT); hatched columns: vehicle; closed columns: 10% sodium laurate. *P < 0.05, Tukey’s test after one-way ANOVA ($F_{4,10} = 6.705, P = 0.007$). (B) Western blotting of HDC and β-actin in the epidermis. (C) The ratio of 53-kDa HDC to 74-kDa HDC in the epidermis. The value was normalized to the average of the ratio in the nontreated vehicle group. Open columns: vehicle; closed columns: 10% sodium laurate. *P < 0.05, Tukey’s test after two-way ANOVA (main effect of treatment: $F_{1,12} = 12.24, P = 0.004$; treatment × time interaction: $F_{1,12} = 15.12, P < 0.001$). The values represent the mean and S.D. ($n = 3$).
in the epidermal histamine content may be a key cause of sodium laurate–induced delayed scratching. Histamine was also increased in the epidermis of mast cell–deficient mice, and it was unchanged in the dermis of ICR mice and mast cell–deficient mice. Histamine was detectable in the human keratinocyte culture treated with 1% sodium laurate, whereas it was below the detection limit in the untreated keratinocyte culture. Therefore, the increase in epidermal histamine may be due to an increase in histamine production in keratinocytes.

HDC is a key enzyme in the biosynthesis of histamine (Ichikawa et al., 2010). We observed expression of HDC in mouse epidermis and the human keratinocyte culture. Mouse epidermis has been previously shown to have low HDC activity, approximately 1/10 that in the dermis (Taguchi et al., 1982), and the PB cells have been shown to express HDC mRNA (Fitzsimons et al., 2001). We found that 74- and 53-kDa HDCs were present in murine epidermis and human keratinocytes. In mast cells, HDC is translated as a 74-kDa precursor protein, and is post-translationally cleaved to a 53–55-kDa species (Ichikawa et al., 2010). The 74-kDa HDC exhibits low enzyme activity, and thus histamine is synthesized mainly by the 53-kDa HDC and then stored in granules (Ichikawa et al., 2010). In macrophages, only the 74-kDa form of HDC is present (Hirasawa et al., 2001), and histamine is spontaneously released without being stored intracellularly because of a deficiency of histamine-storing granules (Hirasawa et al., 2001; Ichikawa et al., 2010). In these respects, epidermal keratinocytes are different from mast cells and macrophages; keratinocytes contain both 74- and 53-kDa HDCs but no histamine-storing granules. An increase in the ratio of 53-kDa HDC to 74-kDa HDC and histamine synthesis might result in an increase in the spontaneous release of histamine.

In this study, the mechanisms of sodium laurate–induced HDC modulation remain unclear. We did not examine the effect of sodium laurate application on the HDC mRNA level in the human keratinocyte culture. This was because the HDC mRNA level was so low that we could not reproducibly determine HDC mRNA with real-time reverse-transcriptase polymerase chain reaction. However, an increase in de novo

![Fig. 6.](image-url)

**Fig. 6.** The effects of topical application of sodium laurate on the histamine content in the skin of WBB6F1-W/Wv mice. Sodium laurate (10%) or vehicle was applied topically to the rostral part of the back. Histamine content was determined separately in the epidermis and dermis of the treated skin. Open column: nontreated (NT); hatched columns: vehicle; closed columns: 10% sodium laurate. *P < 0.05, Tukey’s test after one-way ANOVA (F4,10 = 5.054, P = 0.017). The values represent the mean and S.D. (n = 3).

![Fig. 7.](image-url)

**Fig. 7.** Histamine and HDC in a 3-dimensional human epidermal keratinocyte culture. Sodium laurate and vehicle (VH) were applied topically to a keratinocyte culture for 1 minute; afterward, the culture was washed, left to stand for 3 hours, and then used for the assays. (A) The concentration of histamine in the culture and medium. *P < 0.05 versus VH, Dunnett’s test after one-way ANOVA (F4,20 = 30.297, P = 0.031). The values represent the geometric mean ± 95% confidence limit (n = 4 or 5). (B) A typical example of Western blotting of HDC and β-actin. (C) The ratio of 53-kDa HDC to 74-kDa HDC. The value was normalized to the average of the ratio of the nontreated (NT) group. *P < 0.05 versus VH, Dunnett’s test after one-way ANOVA (F4,15 = 7.532, P = 0.002). The values represent the mean and S.D. (n = 4).
HDC protein synthesis through an increase in gene transcription requires more than 4 hours after cell stimulation (Jeong et al., 2009). Therefore, mRNA expression may not play an important role in the increase of 53-kDa HDC. An increase in the ratio of 53-kDa HDC to 74-kDa HDC suggests an increase in post-translational processing from 74- to 53-kDa HDC.

Although details of the mechanisms of HDC processing remain unclear, benzamidine-sensitive proteinase has been reported to be involved in HDC processing (Ichikawa et al., 1998). Benzamidine-sensitive proteinase is activated at an alkaline pH (pH 8–9) (Ichikawa et al., 1998; Demartini et al., 2007). In the present study, the pH values of pruritogenic 1% and 10% sodium laurate were 9.8 and 10.1, respectively, whereas those of nonpruritogenic 0.1% sodium laurate and 10% N-lauroylsarcosine sodium salt were 7.6 and 7.7, respectively. An increase in skin surface pH results in the destruction of the barrier function of the stratum corneum (Hachem et al., 2003), and in rats, the topical application (1-minute duration) of sodium hydroxide solution increases the subcutaneous pH from 7 to higher than 10 (Yano et al., 1993; Andrews et al., 2003). In this study, scratching bouts were not increased 2 hours after the application of sodium hydroxide solution at pH 10.2, which is similar to the pH of 10% sodium laurate. On the basis of these findings, it is suggested that HDC processing is not increased by the elevation of extracellular pH; sodium laurate might increase HDC processing through an elevation of intracellular pH due to both its anionic and surface activities. In the experiments using the keratinocyte culture, sodium laurate was applied for 1 minute. In in vitro experiments using cell cultures, [14C]laurate uptake increases within 5 minutes after soap administration (Spector et al., 1972), and intracellular Ca2+ concentration increases within 2 minutes after sodium laurate administration (Okuda et al., 2006). Thus, it is possible that 1-minute application of sodium laurate altered the intracellular conditions of cultured keratinocytes to induce cellular events, although we did not determine how long intracellular pH increased.

Histamine is synthesized from histidine by HDC. To our knowledge, there have been no reports on the content of histidine in the keratinocytes. However, keratohyalin granules contain histidine (Matolsy and Matolsy, 1970), and keratinocytes contain the histidine-rich protein filaggrin, which is degraded into amino acids, including histidine, and there is a substantial amount of histidine in the stratum corneum (Scott et al., 1982). Therefore, there may be a sufficient concentration of histidine in the keratinocytes.

There was a wide difference in the average pH of the untreated skin surface between ICR (pH 5.1) and WBB6F1 mice (pH 6.3), which is similar to individual differences (pH 4.1–6.5) in the pH of normal human skin that is out of contact with water and agents for 24 hours (Lambers et al., 2006). Skin surface pH might markedly increase immediately after topical application of 10% sodium laurate (pH 10.1) and then gradually decrease. There was a wide difference in the average pH of the skin surface between ICR (pH 6.0) and WBB6F1 mice (pH 7.1) 2 hours after sodium laurate application. This strain difference might be due to a difference in the acidifying function of the skin. Sodium laurate–induced increase in histamine in the epidermis was more marked in ICR mice than in WBB6F1/W/W mice. Although we did not determine pH in the epidermis, a difference in the increase in histamine content might be at least partly due to a difference in the acidifying function. With regard to the physiologic role of histamine in the keratinocytes, the expression level of HDC mRNA and histamine content are markedly lower in differentiated PB cells than in undifferentiated PB cells (Fitzsimons et al., 2002), and histamine inhibits the proliferation of cultured keratinocyte (Harper and Flaxman, 1975). Histamine increases and decreases the expression of semaphorin 3A and nerve growth factor, respectively, in cultured keratinocytes, which may result in the suppression of nerve fiber elongation in the epidermis (Fukamachi et al., 2011). In addition, histamine increases the proinflammatory cytokines interleukin-6 and interleukin-8 in HaCaT cells, a human skin keratinocyte cell line (Kohda et al., 2002). Thus, histamine may play diverse roles in keratinocyte proliferation and keratinocyte-mediated intraepidermal nerve growth and inflammation.

In conclusion, the present study showed that sodium laurate elicited delayed scratching at an alkaline pH through the increased production of histamine in keratinocytes, which may be at least partly due to increased processing of 74-kDa HDC to 53-kDa HDC. Histamine contained in the mast cells in the dermis may not play a role in sodium laurate–induced delayed scratching. Neutral surfactants may be better for the skin than alkaline surfactants in that the former do not cause delayed pruritus. Although pruritogenic, sodium laurate is an important component of toiletries. The combination of sodium laurate with the H1 histamine receptor antagonist may be better than sodium laurate alone for the prevention of delayed-phase itching.

Authorship Contributions

**Participated in research design:** Inami, Andoh, Sasaki, Kuraishi.

**Conducted experiments:** Inami.

**Performed data analysis:** Inami, Andoh, Sasaki, Kuraishi.

**Wrote or contributed to the writing of the manuscript:** Inami, Andoh, Kuraishi.

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