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Short communication

12(*S*)-HPETE induces itch-associated scratchings in mice

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Abstract

The itch-associated responses evoked by intradermal injection of 12(*S*)-HPETE and leukotriene B_4 were compared in ICR-mice. 12(*S*)-HPETE and leukotriene B_4 (0.01–0.2 nmol/site) induced scratching of the injected site, respectively; the dose-responses were a peak at 0.05 nmol/site (12(*S*)-HPETE) or 0.03 nmol/site (leukotriene B_4). The scratching response by 12(*S*)-HPETE (0.05 nmol/site) started within 1 min, peaked in the first 10 min period, had almost subsided by 25 min whereas the effect of leukotriene B_4 peaked in the second 10 min. The effect of leukotriene B_4 is slightly stronger than that of 12(*S*)-HPETE in 40 min of count. The scratching induced by 12(*S*)-HPETE was inhibited by capsaicin, naltrexon, and LY255283. These results suggest the possibility that 12-lipoxygenase product can be added to a new member of an endogenous itch mediator in the skin.
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Keywords: 12(*S*)-HPETE; Leukotriene B_4; Itch; Scratching; Intradermal injection; 12-lipoxygenase

1. Introduction

Pruritis (itching) can be defined subjectively as a poorly localized, non-adapting, usually unpleasant sensation that provokes a desire to scratch (Weisshaar et al., 2003). This sensation accompanies various skin diseases (e.g. atopic dermatitis, contact dermatitis, and urticaria) and several systemic disorders (e.g. chronic renal failure and cholestasis) (Wahlgren, 1991; Andoh et al., 2001). The details of mechanisms and endogenous mediators of itching are still unclear. Many endogenous chemicals, such as amines, proteases, growth factors, neuropeptides, opioids, cytokines and eicosanoids, are locally pruritogenic when injected into the skin. With regard to lipoxygenase products, the level of leukotriene B_4 was reported to increase in the skin of pruritus patients (Brain et al., 1984; Ruzicka et al., 1986). Intradermal injection of leukotriene B_4 elicits apparent itching-associated response in mice (Andoh and Kuraishi, 1998) and humans (Camp et al., 1983). Leukotriene D_4 and leukotriene C_4 are not pruritogenic after intradermal injection in human subjects (Camp et al., 1983).

In an effort to find a new pruritogen in animal models, we tested the possibility that products of other lipoxygenases (12-, or 15-), not 5-lipoxygenase, could be pruritogens.

Here, we report the case of 12(*S*)-HPETE as a possible pruritogen to evoke an itching in animal models (Andoh and Kuraishi, 1998).

2. Materials and methods

2.1. Materials

Leukotriene B_4, 12(*S*)-HPETE (12(*S*)-hydroperoxyeicosa-5*Z*,8*Z*,10*E*,14*Z*-tetraenoic acid), one of products of 12-lipoxygenase, LY255283 (1-[5-ethyl-2-hydroxy-4-[[6-methyl-6-(1*H*-tetrazol-5-yl)heptyl]oxy]phenyl]-ethanone, Souza et al., 2000) and U75302 (6-[6-(3-hydroxy-1*E*,5*Z*-undecadienyl)-2-pyridinyl]-1,5-hexanediol, Falcone and Aharony, 1990) were purchased from BIOMOL. The ethanol stocks of leukotriene B_4, 12(*S*)-HPETE, LY255283, and U75302 were dissolved in physiological saline. These reagents were administered intradermally in a volume of 50 μl into the rostral part of the back.

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2.2. Behavioral observation

Male ICR mice 7–8 weeks of age were used in the experiments. They were housed under controlled temperature (23–25 °C) and light (lights on from 08:00 to 20:00). Food and water were freely available. The hair was clipped over the rostral part of the mouse back. Before the experiments, the mice were put into an acrylic cage (140 × 32 × 12 cm) composed of 20 cells for at least 1 h acclimation. Immediately after intradermal injection, they were put back into the same cell and videotaped with no one present. Scratching of the injected site with the hind paws was counted as an index of itch response (Kuraishi et al., 1995).

2.3. Data processing

All data are presented as means and S.D. Statistical significance was analyzed using two sample *t* test and the two-way ANOVA test; $P<0.05$ was considered significant.

3. Results

3.1. Time-course of scratching behavior by 12(S)-HPETE and leukotriene B_4

Fig. 1A and B show the time-course of scratching behavior for 40 min after the injection of 12(*S*)-HPETE (0.03 nmol/site) and leukotriene B_4 (0.03 nmol/site), respectively. Scratching was first observed within 1 min after injection in all mice examined and then appeared intermittently. The scratching behavior diminished substantially by 25 min in our experiments.

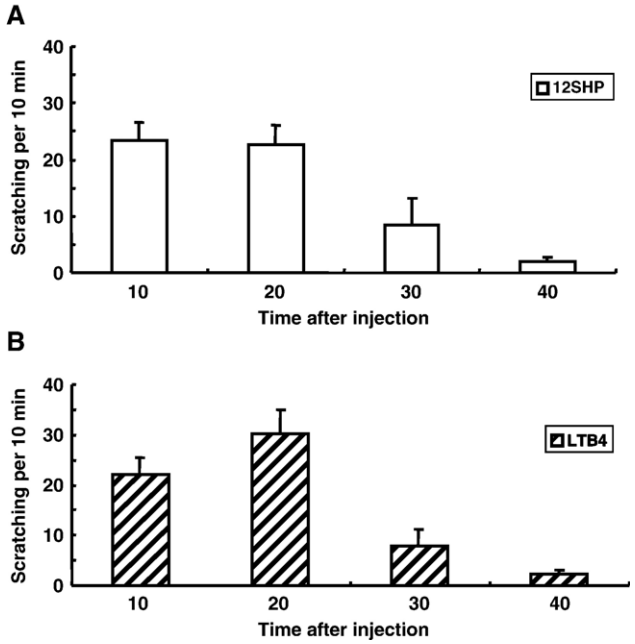

Fig. 1. Scratching following intradermal injection of 12(*S*)-HPETE (A) and leukotriene B_4 (B) in mice. Time-course of scratching induced by 12(*S*)-HPETE (0.05 nmol/site) and leukotriene B_4 (0.03 nmol/site). 12(*S*)-HPETE ($n=8$) or leukotriene B_4 ($n=8$) was injected intradermally as 50 μl. Values represent the means and S.D.

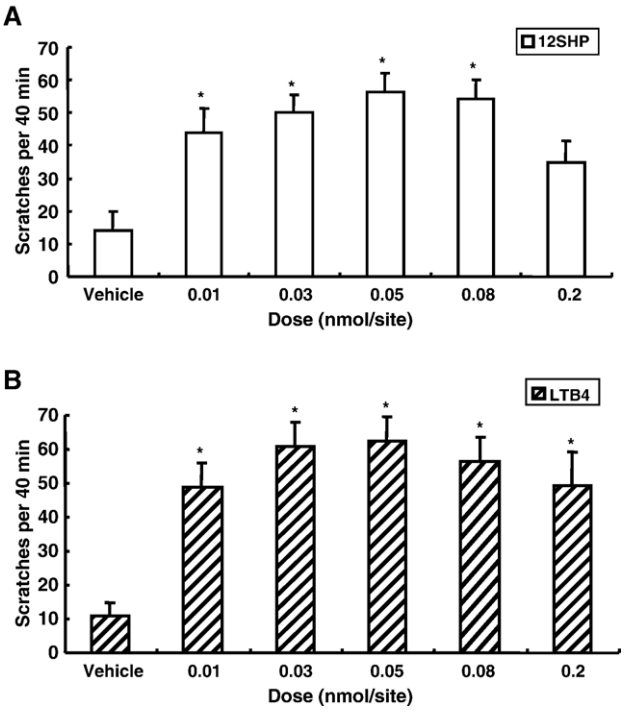

Fig. 2. Dose–response curves for the scratch-inducing effect of 12(*S*)-HPETE (A) and leukotriene B_4 (B). The mice were given an intradermal injection of 12(*S*)-HPETE ($n=8$–12), leukotriene B_4 ($n=8$) or physiological saline (vehicle, $n=8$). Scratching following intradermal injection of 12(*S*)-HPETE and leukotriene B_4 in mice. Values represent the means and S.D. $*P<0.05$ when compared with vehicle.

3.2. Dose–response curve of 12(S)-HPETE and leukotriene B_4

Fig. 2A and B show that both 12(*S*)-HPETE and leukotriene B_4 elicited significant scratching at intradermal doses of 0.01–0.2 nmol/site, respectively. The dose–response curve had a peak around 0.03–0.08 nmol/site ($*P<0.05$ when compared with vehicle). Intradermal injection of vehicle solution (saline) at a volume of 50 μl did not significantly elicited scratching when compared with the reactions in untreated mice (data not shown). Higher dose of 3 and 10 nmol/site were not effective in inducing scratching from ICR mice. Scratching induced by leukotriene B_4 is slightly stronger than that of 12(*S*)-HPETE. No changes in gross behaviors other than scratching were observed after these doses of leukotriene B_4 and 12(*S*)-HPETE.

3.3. Effect of capsaicin, naltrexone and leukotriene B_4 receptor antagonists on 12(S)-HPETE induced scratching

To evaluate the characteristics of scratching induced by 12(*S*)-HPETE, we tested whether capsaicin and naltrexone inhibited scratching by 12(*S*)-HPETE, respectively. Capsaicin, a well-known vanilloid receptor agonist (Bevan and Szolcsanyi, 1990), was applied topically after the injection of 12(*S*)-HPETE. Naltrexone was applied orally 1 h before the injection of 12(*S*)-HPETE. Capsaicin at dose of 0.05% (w/v) and naltrexone at 10 mg/kg suppressed the 12(*S*)-HPETE-induced scratching ($*P<0.05$ when compared with 12(*S*)-HPETE control), respectively (Fig. 3). Two kinds of leukotriene B_4 receptor antagonist

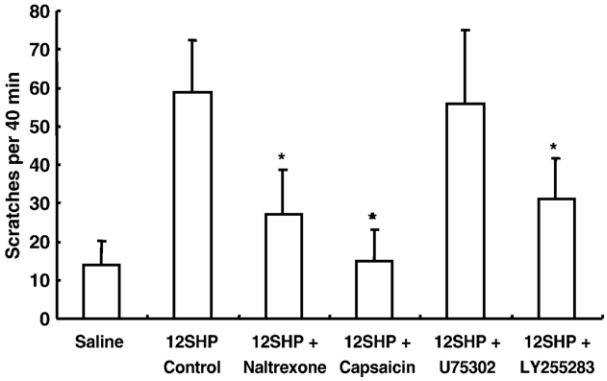

Fig. 3. Suppressive effects of the capsaicin, naltrexone, U75302 and LY255283 on 12(*S*)-HPETE induced scratching, respectively. 12(*S*)-HPETE (0.03 nmol/site) was injected intradermally alone (12SHP) or together with capsaicin at 0.05% (topically applied), U75302 (5 mg/kg), LY255283 (5 mg/kg), and naltrexone (10 mg/kg, orally 1 h before injection), respectively. Values are the means and S.D. for eight animals. $*P<0.05$ when compared with 12(*S*)-HPETE control.

U75302 (BLT1 receptor antagonist, Falcone and Aharony, 1990) and LY255283 (BLT2 receptor antagonist, Souza et al., 2000) were tested in 12(*S*)-HPETE-induced scratching.

Interestingly not U75302 but LY255283 suppressed the scratching induced by 12(*S*)-HPETE.

4. Discussion

Products of lipoxygenases are implicated in skin physiology and pathology (Ziboh et al., 2002). In a recent study of pruritus, leukotriene B_4, a 5-lipoxygenase metabolite of arachidonic acid, was discovered as a new pruritogen in mice acting at low dose (Andoh and Kuraishi, 1998). However, other lipoxygenase metabolites of arachidoic acid were not fully studied for their pruritogenic activities. We tested the products of other lipoxygenases, 5-lipoxygenase, as possible pruritogens. We found that 12(*S*)-HPETE, one of the products of 12-lipoxygenase, could induce itching-associated scratching in ICR mice. The potency of 12(*S*)-HPETE as a pruritogen is approximately similar to that of leukotriene B_4. Our result also confirms that intradermal injection of leukotriene B_4 induces scratching at relatively low dose (Andoh and Kuraishi, 1998).

The scratch-inducing mechanisms of 12(*S*)-HPETE and leukotriene B_4 could not be clearly explained. 12(*S*)-HPETE-induced scratching response may be mediated by C fibers (Schmelz et al., 1997), which is consistent with our observation that capsaicin and naltrexone suppressed scratching induced by 12(*S*)-HPETE (Fig. 3).

It has been expected that leukotriene B_4 and 12(*S*)-HPETE mediate their function through the G protein-coupled receptors. Two receptors for leukotriene B_4 have been identified: BLT1, a high-affinity receptor specific for leukotriene B_4 and BLT2, a low-affinity receptor that also binds other eicosanoids (Tager and Luster, 2003; Yokomizo et al., 2000). We could not find any literature report for the expression of BLT1 and BLT2 in nerve ending extruding the skin. But recently it was reported that BLT1, high affinity receptor for leukotriene B_4, is found at dorsal root ganglion neurons (Andoh and Kuraishi, 2005). And BLT1 and BLT2 are believed to exist in the skin (Andoh and Kuraishi, 2005; Iizuka et al., 2005). If we assume that 12(*S*)-HPETE induces itch through leukotriene B_4 receptor, it should be through BLT2, not BLT1, because 12(*S*)-HPETE binds and activate BLT2 and binding of 12(*S*)-HPETE to BLT1 is not quite strong (Yokomizo et al., 2001). Moreover, U75302 (BLT1 antagonist) did not suppress the scratching by 12(*S*)-HPETE, while LY255283 (BLT2 antagonist) did. One could argue that 12(*S*)-HPETE is converted to leukotriene B_4 in injected skin site of ICR mouse. But this is not plausible because there is no literature report on such conversion and the onset of the scratching after the injection of 12(*S*)-HPETE is very rapid. Another explanation might be that 12(*S*)-HPETE can also act as agonist of VR-1 (Shin et al., 2002). One will need data on the specific inhibition by VR-1 antagonist, such as capsazepine, to prove this proposition. Further studies are still ongoing to understand the detailed mechanism of 12(*S*)-HPETE-induced itching in our laboratory.

In conclusion, intradermal injection of 12(*S*)-HPETE (product of 12-lipoxygenase) induced scratching at relatively low doses similar to leukotriene B_4, suggesting that it is also a candidate of endogenous itch mediator.

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