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RESEARCH PAPER

Involvement of the BLT_2 receptor in the itch-associated scratching induced by 12-(*S*)-lipoxygenase products in ICR mice

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Background and purpose: Recently, we reported that 12(*S*)-HPETE (12(*S*)-hydroperoxyeicosa-5Z,8Z,10E,14Z-tetraenoic acid) induces scratching in ICR mice. We hypothesized that 12(*S*)-HPETE might act as an agonist of the low-affinity leukotriene B_4 receptor BLT_2. To confirm the involvement of the BLT_2 receptor in 12(*S*)-HPETE-induced scratching, we studied the scratch response using the BLT_2 receptor agonists compound A (4′-{[pentanoyl (phenyl) amino]methyl}-1,1′-biphenyl-2-carboxylic acid) and 12(*S*)-HETE (12(*S*)-hydroxyeicosa-5Z,8Z,10E,14Z-tetraenoic acid).
Experimental approach: A video recording was used to determine whether the BLT_2 receptor agonists caused itch-associated scratching in ICR mice. Selective antagonists and several chemicals were used.
Key results: Both 12(*S*)-HETE and compound A dose dependently induced scratching in the ICR mice. The dose–response curve for compound A showed peaks at around 0.005–0.015 nmol per site. Compound A- and 12(*S*)-HETE-induced scratching was suppressed by capsaicin and naltrexon. We examined the suppressive effects of U75302 (6-[6-(3-hydroxy-1E,5Z-undecadienyl)-2-pyridinyl]-1,5-hexanediol, the BLT_1 receptor antagonist) and LY255283 (1-[5-ethyl-2-hydroxy-4-[[6-methyl-6-(1*H*-tetrazol-5-yl)heptyl]oxy]phenyl]-ethanone, the BLT_2 receptor antagonist) on the BLT_2 agonist-induced scratching. LY255283 suppressed compound A- and 12(S)-HETE-induced scratching, but U75302 did not. LY255283 required a higher dose to suppress the compound A-induced scratching than it did to suppress the 12(*S*)-HETE-induced scratching. One of the BLT_2 receptor agonists, 12(*R*)-HETE (12(*R*)-hydroxyeicosa-5Z,8Z,10E,14Z-tetraenoic acid), also induced scratching in the ICR mice.
Conclusions and implications: Our present results corroborate the hypothesis that the BLT_2 receptor is involved in 12(*S*)-lipoxygenase-product-induced scratching in ICR mice. We also confirmed that this animal model could be a valuable means of evaluating the effects of BLT_2 receptor antagonists.
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Keywords: 12(*S*,*R*)-HETE; compound A; 12(*R*)-HETE; scratching; BLT_2 receptor; LY255283

Abbreviations: Compound A, 4′-{[pentanoyl (phenyl) amino]methyl}-1,1′-biphenyl-2-carboxylic acid; 12(*S*) or (*R*)-HETE, 12(*S*) or (*R*)-hydroxyeicosa-5Z,8Z,10E,14Z-tetraenoic acid; 12(*S*)-HPETE, 12(*S*)-hydroperoxyeicosa-5Z,8Z,10E,14Z-tetraenoic acid; LY255283, 1-[5-ethyl-2-hydroxy-4-[[6-methyl-6-(1*H*-tetrazol-5-yl)heptyl]oxy]phenyl]-ethanone; TRPV1, transient receptor potential vanilloid type-1; U75302, 6-[6-(3-hydroxy-1E,5Z-undecadienyl)-2-pyridinyl]-1,5-hexanediol

Introduction

Pruritus can be defined as a poorly localized, non-adapting, usually unpleasant sensation (itch) that provokes a desire to scratch (Weisshaar *et al*., 2003). This itch accompanies various skin diseases (for example, atopic dermatitis, contact dermatitis and urticaria) and several systemic disorders (for example, chronic renal failure and cholestasis) (Wahlgren, 1991; Andoh *et al*., 2001). Details on the mechanisms and endogenous mediators of this itch remain both incomplete and unclear. Intradermal injection of various endogeneous chemicals, including biogenic amines, neuropeptides,

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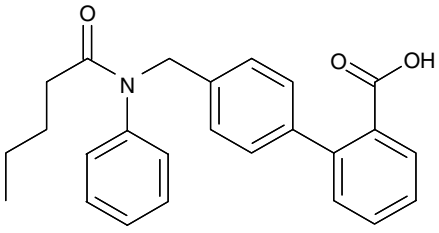
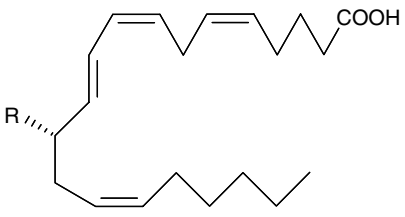
autacoids, proteases, growth factors and cytokines, has been shown to induce local pruritogenic responses in the skin (Stander and Steinhoff, 2002).

As to the involvement of the arachidonic acid cascade in pruritus, intradermal injection of prostaglandin E_2 in humans is pruritogenic (Hagermark and Strandberg, 1977), prolonging experimentally induced itching (Hagermark and Strandberg, 1977; Fjellner and Hagermark, 1979). Lipoxygenase products have also been shown to be involved; the concentration of leukotriene B_4 increases in the skin of pruritus patients (Brain *et al*., 1984; Ruzicka *et al*., 1986). In fact, intradermal injection of leukotriene B_4 elicits an apparent itch-associated response in both mice (Andoh and Kuraishi, 1998) and humans (Camp *et al*., 1983). In contrast, intradermal injections of leukotriene D_4 and leukotriene C_4 are not pruritogenic in humans (Camp *et al*., 1983). Kuraishi has recently demonstrated that thromboxane A_2 induces itch-associated responses through thromboxane A_2 receptors in the skin of mice (Andoh *et al*., 2007).

In a recent study, we established that 12(*S*)-HPETE (12(*S*)-hydroperoxyeicosa-5Z,8Z,10E,14Z-tetraenoic acid; its structure is shown in Figure 1), one of the products of 12-lipoxygenase, induces itch-associated-scratching in ICR mice (Kim *et al*., 2007). In fact, 12(*S*)-HPETE was almost as potent as leukotriene B_4 in inducing itch-associated scratch responses. As leukotriene B_4 acts through BLT_1 receptors (Andoh and Kuraishi, 2005) and hydroxyeicosanoids bind to and activate the low-affinity leukotriene B_4 receptor BLT_2, we hypothesized that BLT_2 receptors are involved in 12(*S*)-HPETE-induced scratching. 12(*S*)-HPETE is a TRPV1 (transient receptor potential vanilloid type-1) agonist (Hwang *et al*., 2000; Alexander *et al*., 2007) and also mediates the inhibitory synaptic response to the neuropeptide FMRF-amide (Phe-Met-Arg-Phe-NH_2) in *Aplysia* sensory neurons (Piomelli *et al*., 1987). In addition, hydroxyeicosanoids have been shown to bind to and activate the low-affinity leukotriene B_4 receptor BLT_2 (Yokomizo *et al*., 2000; Alexander *et al*., 2007), indicating that 12(*S*)-HPETE can function as an agonist of the BLT_2 receptor (Yokomizo *et al*., 2001).



Compound A



R = OH: 12(*S*)-HETE
R = OOH: 12(*S*)-HPETE

Figure 1 The structures of the BLT_2 receptor agonists.

As, to our knowledge, there is still no *in vivo* model for evaluating the effect of BLT_2 receptor antagonists on pruritus, we studied the involvement of BLT_2 receptors in 12(*S*)-HPETE-induced scratching in ICR mice using the BLT_2 agonists 12(*S*)-HETE (12(*S*)-hydroxyeicosa-5Z,8Z,10E,14Z-tetraenoic acid) and compound A (4′-{[pentanoyl (phenyl) amino]methyl}-1,1′-biphenyl-2-carboxylic acid).

Methods

Animals

Male ICR mice, 7–8 weeks of age, were used in the behavioural experiments. To test the various compounds, we used the same method as that used by Kuraishi *et al*. (1995) with minor modifications. The experiments were conducted under the Korea Association for Laboratory Animal Science's Guidelines for the Care and Use of Experimental Animals.

Counting of scratchings

Briefly, the hairs were shaved over the rostral part of the mouse's back to facilitate the intradermal injections. Twenty-four hours later, each mouse was acclimatized for 1 h in an individual cell within a 20-cell acrylic cage ($140 \times 32 \times 12$ cm). Immediately after an intradermal injection of the BLT_2 receptor agonist (12(*S*)-HETE or compound A) or concomitant injections of the BLT_2 agonist with other testing compounds including capsaicin, naltrexon, U75302 (6-[6-(3-hydroxy-1E,5Z-undecadienyl)-2-pyridinyl]-1,5-hexanediol) and LY255283 (1-[5-ethyl-2-hydroxy-4-[[6-methyl-6-(1*H*-tetrazol-5-yl)heptyl]oxy]phenyl]-ethanone), the mice were put back into their respective cells and videotaped with no one present. Scratchings of the injected site with the hind paws were counted in formulating an itch–response index. One scratching bout generally consisted of more than 10 repetitions of the scratching movement of their hind paws. Because one movement occurs too quickly to count individually when observing videotaped replays, one scratching bout was regarded as one scratching frequency, a protocol followed in previous studies (Kuraishi *et al*., 1995).

Statistical analysis

All of the data are presented as means and s.d. Statistical significance ($P<0.05$) was determined by one-way ANOVA followed by appropriate *post hoc* tests (Newman–Keuls). $P<0.05$, relative to the control group, was considered to be significant.

Materials

12(*S*)-HETE or (*R*)-HETE ((*R*)-hydroxyeicosa-5Z,8Z,10E,14Z-tetraenoic acid), LY255283 (1-[5-ethyl-2-hydroxy-4-[[6-methyl-6-(1*H*-tetrazol-5-yl)heptyl]oxy]phenyl]-ethanone) the BLT_2 receptor antagonist, Souza *et al*., 2000; Alexander

et al., 2007) and U75302 (the BLT_1 receptor antagonist, Falcone and Aharony, 1990; Alexander *et al*., 2007) were purchased from BIOMOL. Capsaicin (the TRPV1 receptor agonist, Bevan and Szolcsányi, 1990; Alexander *et al*., 2007) and naltrexone (the μ-opioid receptor antagonist, Bienkowski, 1999; Alexander *et al*., 2007) were purchased from Sigma (St Louis, MO, USA). The ethanol stocks of 12(*S*)-HETE, 12(*R*)-HETE, compound A (4′-{[pentanoyl (phenyl) amino]methyl}-1,1′-biphenyl-2-carboxylic acid) (Iizuka *et al*., 2005), LY255283 and U75302 were dissolved in physiological saline. These reagents, in volumes of 50 μL, were administered intradermally into the rostral part of the back. Additional reagents included the following: compound A (Iizuka *et al*., 2005), from Dr Sang Hee Kim (Lipidomics National Research Laboratory, Seoul, Korea).

Results

12(S)-HETE-induced scratching in ICR mice

We found that 12(*S*)-HETE induced an itch-associated scratch response that was dose dependent (Figure 2). The maximum response peaked at around 0.05–0.15 nmol per site ($P<0.05$ when compared with the saline); the higher dose of 0.5 nmol was less effective than 0.15 nmol at inducing scratching.

Characterization of compound A-induced scratching in ICR mice

To prove that the BLT_2 receptor is involved in 12(*S*)-HPETE-induced itch-associated scratching, we tested whether compound A (a strong synthetic BLT_2 agonist; Figure 1; Iizuka *et al*., 2005) induces scratching. Figure 3a shows the 40-min time course of the scratching behaviour observed after an injection of compound A (0.015 nmol per site). Scratching first occurred within 1 min post injection in all of the mice examined, and was observed intermittently thereafter. This scratching behaviour had diminished substantially by 30 min.

The compound A-induced scratching behaviour was dose dependent (Figure 3b). The response peaked at around 0.005–0.015 nmol per site ($P<0.05$ when compared with the saline vehicle). The higher dose of 0.05 nmol was less effective than 0.015 nmol at inducing scratching. The number of episodes of scratching induced by compound A was similar to that induced by 12(*S*)-HETE (Figure 2). However, the amount of compound A needed to induce a similar response was approximately one-tenth of that of 12(*S*)-HETE, reflecting the potency of compound A as a BLT_2 receptor agonist.

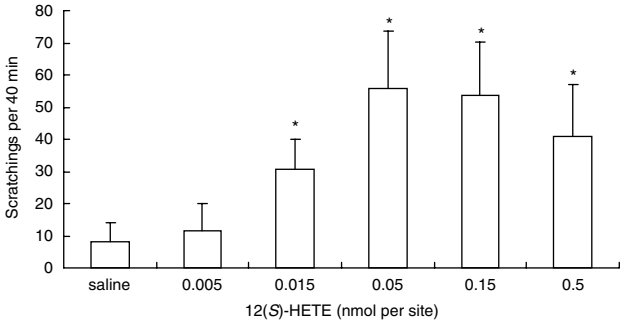


Figure 2 Dose–response curves for the scratch response induced by 12(*S*)-HETE. The mice were given an intradermal injection of 12(*S*)-HETE ($n=8$) or physiological saline (saline, $n=8$). Scratching following intradermal injection of 12(*S*)-HETE in mice. Values represent the means and s.d. *$P<0.05$ relative to saline. 12(*S*)-HETE, 12(*S*)-hydroxyeicosa-5Z,8Z,10E,14Z-tetraenoic acid.

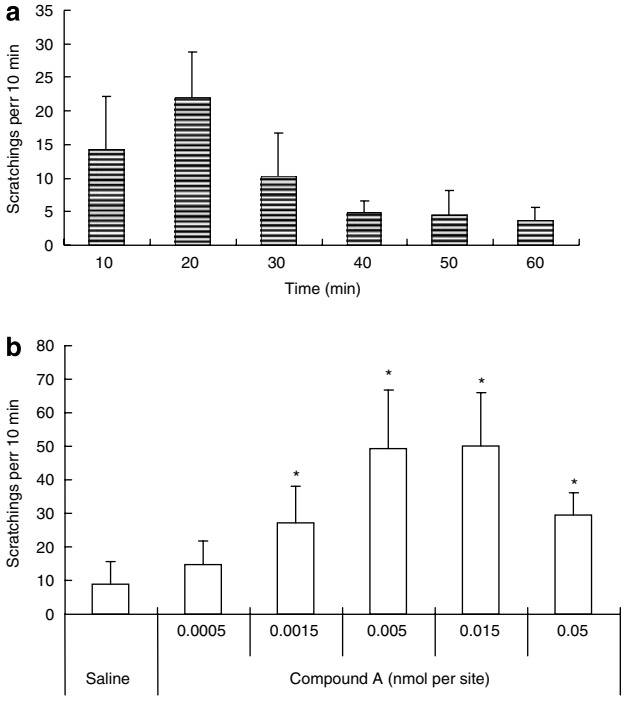


Figure 3 Time course of the scratching induced by compound A (0.005 nmol per site) (**a**) and dose–response curves for this effect of compound A (**b**). The mice were given an intradermal injection of compound A ($n=8$), or physiological saline (vehicle, $n=8$). Scratching following intradermal injection of compound A in mice. The values represent the means and s.d. *$P<0.05$ relative to the saline. Compound A, 4′-{[pentanoyl (phenyl) amino]methyl}-1, 1′-biphenyl-2-carboxylic acid.

Effects of capsaicin, naltrexon, U-75302 and LY255283 on the scratching responses to BLT_2 receptor agonists

To evaluate the characteristics of scratching induced by the BLT_2 receptor agonists 12(*S*)-HETE and compound A, we determined the effects of capsaicin and naltrexone on the scratching responses to these agonists. Capsaicin, a well-known TRPV1 receptor agonist (Bevan and Szolcsányi, 1990; Alexander *et al*., 2007), was applied topically after the administration of 12(*S*)-HETE or compound A. Naltrexone was administered orally 1 h before injection of either 12(*S*)-HETE or compound A. Capsaicin and naltrexone at doses of 0.05% (w/v) and 10 mg kg^{-1}, respectively, suppressed the scratching responses induced by 12(*S*)-HETE and compound A ($P<0.05$ when compared with the control responses to 12(*S*)-HETE and compound A; Figure 4a). Two kinds of leukotriene B_4 receptor antagonist, U75302 (the BLT_1 receptor antagonist; Falcone and Aharony, 1990) and LY255283 (the BLT_2 receptor antagonist; Souza *et al*., 2000)

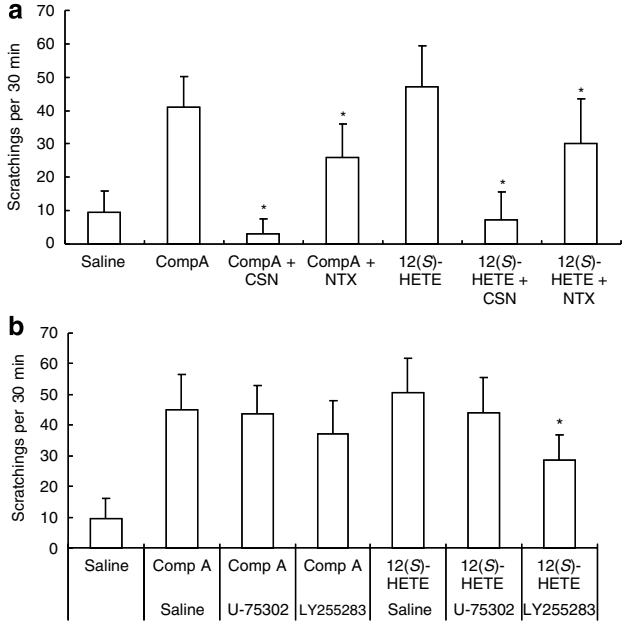


Figure 4 Suppressive effects of U75302, LY255283, capsaicin (CSN) and naltrexone (NTX) on compound A- and 12(*S*)-HETE-induced scratching. (**a**) Compound A (0.005 nmol per site) or 12(*S*)-HETE was injected intradermally alone or together with capsaicin at 0.05% (topically applied) or naltrexone (10 mg kg^{-1}, administered orally 1 h before injection). (**b**) Compound A (0.005 nmol per site) or 12(*S*)-HETE was injected intradermally alone or together with U75302 (5 mg kg^{-1}) or LY255283 (5 mg kg^{-1}). The values are the means and s.d. for eight animals. $*P<0.05$ relative to the compound A or 12(*S*)-HETE control. Compound A, 4′-{[pentanoyl (phenyl) amino]methyl}-1,1′-biphenyl-2-carboxylic acid; 12(*S*)-HETE, 12(*S*)-hydroxyeicosa-5Z,8Z,10E,14Z-tetraenoic acid; LY255283, 1-[5-ethyl-2-hydroxy-4-[[6-methyl-6-(1*H*-tetrazol-5-yl)heptyl]oxy]phenyl]-ethanone; U75302, 6-[6-(3-hydroxy-1E,5Z-undecadienyl)-2-pyridinyl]-1,5-hexanediol.

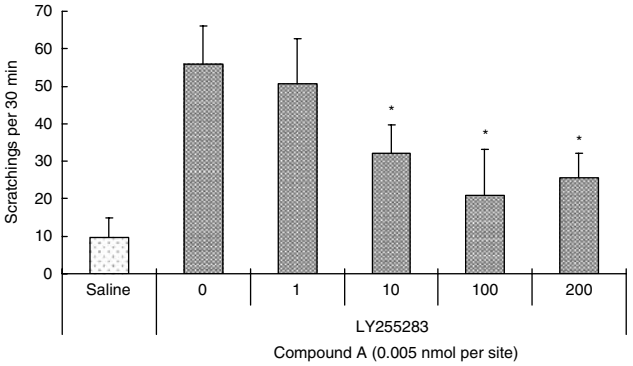


Figure 5 Suppressive effects of LY255283 on compound A-induced scratching. Compound A (0.005 nmol per site) was injected intradermally alone or together with LY255283 in the range of 1–200 nmol per site. The values are the means and s.d. for eight animals. $*P<0.05$ relative to compound A only. Compound A, 4′-{[pentanoyl (phenyl) amino]methyl}-1,1′-biphenyl-2-carboxylic acid; LY255283, 1-[5-ethyl-2-hydroxy-4-[[6-methyl-6-(1*H*-tetrazol-5-yl)heptyl]oxy]phenyl]-ethanone.

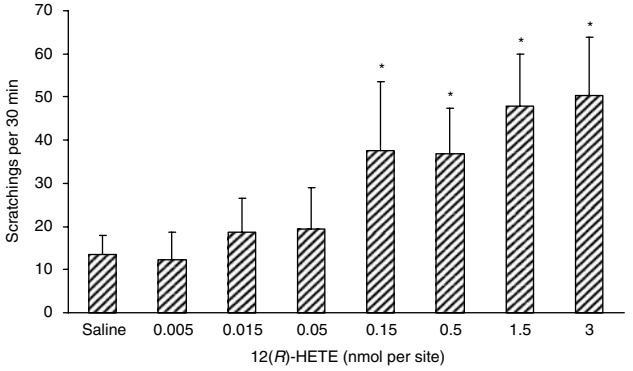


Figure 6 Dose–response curves for the scratch effect induced by 12(*R*)-HETE. The mice were given an intradermal injection of 12(*R*)-HETE or physiological saline (saline, $n=8$). Scratching followed intradermal injection of 12(*R*)-HETE in the mice. The values represent the means and s.d. $*P<0.05$ relative to saline. 12(*R*)-HETE, (*R*)-hydroxyeicosa-5Z,8Z,10E,14Z-tetraenoic acid.

were tested against 12(*S*)-HETE- and compound A-induced scratching. LY255283 (5 mg kg^{-1}) suppressed 12(*S*)-HETE-induced scratching but did not suppress compound A-induced scratching (Figure 4b). Higher doses of LY255283 (10–200 nmol per site) suppressed compound A-induced scratching (Figure 5).

*12(*R*)-HETE-induced scratching in ICR mice*

According to the results of Yokomizo *et al*. (2001), 12(*R*)-HETE might also be a BLT_2 receptor agonist. Hence, we tested whether 12(*R*)-HETE induces scratching in mice. At high doses, 12(*R*)-HETE was found to induce the itch-associated scratch response (Figure 6). However, the dose required to elicit the maximal response was higher than that of 12(*S*)-HETE (Figure 2), 12(*S*)-HPETE (Kim *et al*., 2007) or compound A (Figure 3).

Discussion

Lipoxygenase products are implicated in the physiology and pathology of skin (Ziboh *et al*., 2002). In a recent study on pruritus, leukotriene B_4, a 5-lipoxygenase metabolite of arachidonic acid, was discovered to be acting as a new pruritogen at low doses in mice (Andoh and Kuraishi, 1998), and we showed that 12(*S*)-HPETE induces itch-associated scratch responses (Kim *et al*., 2007). These results and the finding that hydroxyeicosanoids bind to and activate the low-affinity leukotriene B_4 receptor BLT_2 implicate the BLT_2 pathway in 12(*S*)-HPETE-induced itch-associated scratching in mice. To determine whether the BLT_2 receptor is involved in 12(*S*)-HPETE-induced itch-associated scratching, we used the BLT_2 receptor agonists 12(*S*)-HETE (Yokomizo *et al*., 2000) and compound A (Iizuka *et al*., 2005) instead of 12(*S*)-HPETE. As expected, 12(*S*)-HETE induced the itch-associated scratching at doses (Figure 2) similar to those of 12(*S*)-HPETE (Kim *et al*., 2007). Also as expected, compound A induced itch-associated scratching at lower doses (Figure 3b) than either 12(*S*)-HPETE (Kim *et al*., 2007) or 12(*S*)-HETE (Figure 2). The dose range (0.005–0.015 nmol per site) for maximal compound A-induced scratching was lower than that (0.05–0.15 nmol per site) for 12(*S*)-HETE (Figure 2). This might be

related to the fact that the affinity of compound A for the BLT_2 receptor is much stronger than that of 12(*S*)-HETE or 12(*S*)-HPETE (Yokomizo *et al*., 2001; Iizuka *et al*., 2005). The minimal concentration of leukotriene B_4 required to induce ERK phosphorylation is 10 nM, but that of compound A is only 1 nM. The EC_{50} of leukotriene B_4 for calcium mobilization by the BLT_2 receptor is 170 nM and that of compound A is 20 nM (Yokomizo *et al*., 2000; Iizuka *et al*., 2005).

To evaluate the characteristics of BLT_2 agonist-induced scratching, we also tested the ability of several compounds, such as the TRPV1 receptor agonist, the opioid receptor antagonist, and the BLT_1 and BLT_2 receptor antagonists, to suppress the itch-associated scratching induced by the BLT_2 receptor agonist. We observed that capsaicin and naltrexon suppressed the itch-associated scratching induced by 12(*S*)-HETE and compound A (Figure 4a). These results suggest that the BLT_2 agonist-induced scratch response might be, at least partly, mediated by capsaicin-sensitive primary afferents (Schmelz *et al*., 1997), and also that the opioid systems might be involved in the transmission of itch signalling in the central nervous system, which would be consistent with our observation that both capsaicin and naltrexone suppressed 12(*S*)-HPETE-induced scratching (Kim *et al*., 2007).

In the experiment using the BLT_2 receptor antagonist LY255283, the doses needed to suppress the compound A-induced scratching were higher than those able to suppress 12(*S*)-HETE-induced scratching (Figures 4a, b and 5). This phenomenon might be explained by the fact that compound A has a strong affinity for the BLT_2 receptor compared with the antagonizing effects of LY255283 (IC_{50} 950 nM for the human BLT_2 receptor).

As shown in Figure 6, 12(*R*)-HETE also induced the itch-associated scratch response at high doses; the dose required to elicit a maximal response was higher than those of 12(*S*)-HETE (Figure 2), 12(*S*)-HPETE (Kim *et al*., 2007) or compound A (Figure 3). This result is consistent with those of Yokomizo *et al*. (2001); they showed that 12(*S*)-HPETE, 12(*S*)-HETE and 12(*R*)-HETE exhibited dose-dependent inhibition of [^{3}H]-leukotriene B_4 binding to the BLT_2 receptor with the following rank order of potency: leukotriene B_4 > 12(*S*)-HETE > 12(*S*)-HPETE > 12(*R*)-HETE.

What is the location of the BLT_2 receptor that mediates the itch-associated scratch response? Recent studies have found that the BLT_1, the high-affinity receptor for leukotriene B_4, but not the BLT_2 receptor, is located in cultured dorsal root ganglion neurons (Andoh and Kuraishi, 2005). However, according to another study, not only the BLT_1 but also the BLT_2 receptors are expressed in mast cells (Lundeen *et al*., 2006). Hence, BLT_2 agonist-induced itch-associated scratching might involve the BLT_2 receptor from mast cells, rather than any direct action on peripheral nerve endings. Confirmation of this hypothesis requires further study.

What is the role of the BLT_2 receptor in disease pathology? The BLT_2 agonists 12(*S*)-HETE, 12(*R*)-HETE and 12(*S*)-HPETE have been shown to have a role in several pathophysiological phenomena. For example, significantly higher amounts of 12(*S*)-HETE have been found to be present in unstimulated platelets from human subjects with atopic dermatitis. Moreover, 12(*S*)-HETE production has been found to be increased in platelets stimulated with heat-killed isolates of *Staphylococcus aureus* (Neuber *et al*., 1992). Our result that 12(*R*)-HETE can induce the itch-associated scratch response indicates that BLT_2 could be involved in the pruritic symptoms of psoriasis or other proliferative dermatoses manifesting the characteristic accumulation in the skin of the unusual arachidonic acid metabolite, 12(*R*)-HETE (Boeglin *et al*., 1998).

12(*S*)-HETE is thought to be involved in cardiovascular disorders (Gonzalez-Nunez *et al*., 2001; Pock, 2003), Alzheimer's disease (Yao *et al*., 2005), diabetes (Antonipillai *et al*., 1996) and metastatic tumours (Tang and Honn, 1999) and to show that the BLT_2 receptor is involved in these clinical situations requires further study.

To our knowledge, ours is the first animal model indicating that the BLT_2 receptor involved in itching since the discovery of the BLT_2 receptor (Yokomizo *et al*., 2000). This model, incorporating the BLT_2 receptor agonist, is very valuable for evaluating the effect of antagonists in suppressing itching. The results from this model suggest that in elucidating leukotriene-related itching, it is necessary to consider not only the BLT_1 receptor but also the BLT_2 receptor.

Conclusion

Our proposal that the itch-associated scratching induced by the 12(*S*)-lipoxygenase product 12(*S*)-HPETE is attributable to the BLT_2 receptor pathway is confirmed by the involvement of the BLT_2 receptor agonists 12(*S*)-HETE, 12(*R*)-HETE and compound A. This animal model is a valuable means of evaluating the effects of BLT_2 receptor antagonists.

Acknowledgements

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Conflict of interest

The authors state no conflict of interest.

References

Alexander SPH, Mathie A, Peters JA (2007). Guide to Receptors and Channels (GRAC), 2nd edition (2007 revision). *Br J Pharmacol* **150** (Suppl 1): S1–S168.

Andoh T, Katsube N, Maruyama M, Kuraishi Y (2001). Involvement of leukotriene B_4 in substance P-induced itch-associated response in mice. *J Invest Dermatol* **117**: 1621–1626.

Andoh T, Kuraishi Y (1998). Intradermal leukotriene B_4, but not prostaglandin E_2 induces itch-associated responses in mice. *Eur J Pharmacol* **353**: 93–96.

Andoh T, Kuraishi Y (2005). Expression of BLT_1 leukotriene B_4 receptor on the dorsal root ganglion neurons in mice. *Brain Res Mol Brain Res* **137**: 263–266.

Andoh T, Nishikawa Y, Yamaguchi-Miyamoto T, Nojima H, Narumiya S, Kuraishi Y (2007). Thromboxane A_2 induces itch-associated responses through TP receptors in the skin in mice. *J Invest Dermatol* **127**: 2042–2047.

Antonipillai I, Nadler J, Vu EJ, Bughi S, Natarajan R, Horton R (1996). A 12-lipoxygenase product, 12-hydroxyeicosatetraenoic acid, is increased in diabetics with incipient and early renal disease. *J Clin Endocrinol Metab* **81**: 1940–1945.

Bevan S, Szolcsányi J (1990). Sensory neuron-specific actions of capsaicin: mechanisms and applications. *Trends Pharmacol Sci* **11**: 330–333.

Bienkowski P, Kostowski W, Koros E (1999). Ethanol-reinforced behaviour in the rat: effects of naltrexone. *Eur J Pharmacol* **374**: 321–327.

Boeglin WE, Kim RB, Brash AR (1998). A 12R-lipoxygenase in human skin: mechanistic evidence, molecular cloning, and expression. *Proc Natl Acad Sci USA* **95**: 6744–6799.

Brain S, Camp R, Dowd P, Black AK, Greaves M (1984). The release of leukotriene B_4-like material in biologically active amounts from the lesional skin of patients with psoriasis. *J Invest Dermatol* **83**: 70–73.

Camp RD, Coutts AA, Greaves MW, Kay AB, Walport MJ (1983). Responses of human skin to intradermal injection of leukotrienes C_4, D_4, and B_4. *Br J Pharmacol* **80**: 497–502.

Falcone RC, Aharony D (1990). Modulation of ligand binding to leukotriene B_4 receptors on guinea pig lung membranes by sulfhydryl modifying reagents. *J Pharmacol Exp Ther* **255**: 565–571.

Fjellner B, Hagermark Q (1979). Pruritus in polycythemia vera: treatment with aspirin and possibility of platelet involvement. *Acta Deratovener* **59**: 505–512.

Gonzalez-Nunez D, Claria J, Rivera F, Poch E (2001). Increased levels of 12(*S*)-HETE in patients with essential hypertension. *Hypertension* **37**: 334–338.

Hagermark O, Strandberg K (1977). Pruritogenic activity of prostaglandin E_2. *Acta Dermatovener* **57**: 37–43.

Hwang SW, Cho H, Kwak J, Lee SY, Kang CJ, Jung J *et al.* (2000). Direct activation of capsaicin receptors by products of lipoxygenases: endogenous capsaicin-like substances. *Proc Natl Acad Sci USA* **97**: 6155–6160.

Iizuka Y, Yokomizo T, Terawaki K, Komine M, Tamaki K, Shimizu T (2005). Characterization of a mouse second leukotriene B_4 receptor, $mBLT_2$: BLT_2-dependent Erk activation and cell migration of primary mouse keratinocytes. *J Biol Chem* **280**: 24816–24823.

Kim DK, Kim HJ, Sung KS, Kim H, Cho SA, Kim KM *et al.* (2007). 12(*S*)-HPETE induces itch-associated scratchings in mice. *Eur J Pharmacol* **554**: 30–33.

Kuraishi Y, Nagasawa T, Hayashi K, Satoh M (1995). Scratching behavior induced by pruritogenic but not algesiogenic agents in mice. *Eur J Pharmacol* **275**: 229–233.

Lundeen KA, Sun B, Karlsson L, Fourie A (2006). Leukotriene B_4 receptors BLT_1 and BLT_2: expression and function in human and murine mast cells. *J Immunol* **177**: 3439–3447.

Neuber K, Hilger RA, Konig W (1992). Differential increase in 12-HETE release and CD29/CD49f expression of platelets from normal donors and from patients with atopic dermatitis. *Int Arch Allergy Immunol* **98**: 339–342.

Piomelli D, Volterra A, Dale N, Siegelbaum SA, Kandel ER, Schwartz JH *et al.* (1987). Lipoxygenase metabolites of arachidonic acid as second messengers for presynaptic inhibition of Aplysia sensory cells. *Nature* **328**: 38–43.

Pock E (2003). Role of lipoxygenase metabolites in cardiovascular disease. *Curr Hypertens Rep* **5**: 1–2.

Ruzicka T, Simmet T, Peskar BA, Ring J (1986). Skin levels of arachidonic acid-derived inflammatory mediators and histamine in atopic dermatitis and psoriasis. *J Invest Dermatol* **86**: 105–108.

Schmelz M, Schmidt R, Bickel A, Handwerker HO, Torebjork HE (1997). Specific C-receptors for itch in human skin. *J Neurosci* **17**: 8003–8008.

Souza DG, Coutinho SF, Silveira MR, Cara DC, Teixeira MM (2000). Effects of a BLT receptor antagonist on local and remote reperfusion injuries after transient ischemia of the superior mesenteric artery in rats. *Eur J Pharmacol* **403**: 121–128.

Stander S, Steinhoff M (2002). Pathophysiology of pruritus in atopic dermatitis: overview. *Exp Dermatol* **11**: 12–24.

Tang K, Honn KV (1999). *12(S)-HETE in Cancer Metastasis. Lipoxygenases and Their Metabolites*. Pienum Press: New York.

Wahlgren CF (1991). Itch and atopic dermatitis: clinical and experimental studies. *Acta Derm Venereol Suppl (Stockh)* **165**: 1–53.

Weisshaar E, Kucenic MJ, Fleischer Jr AB (2003). Pruritus: a review. *Acta Derm Venereol Suppl* **213**: 5–32.

Yao Y, Clark CM, Trojanowski JQ, Lee VMY, Pratico D (2005). Elevation of 12/15 lipoxygenase products in AD and mild cognitive impairment. *Annal Neurol* **58**: 623–626.

Yokomizo T, Kato K, Hagiya H, Izumi T, Shimizu T (2001). Hydroxyeicosanoids bind to and activate the low affinity leukotriene B_4 receptor, BLT_2. *J Biol Chem* **276**: 12454–12459.

Yokomizo T, Kato K, Terawaki K, Izumi T, Shimizu T (2000). A second leukotriene B(4) receptor, BLT_2. A new therapeutic target in inflammation and immunological disorders. *J Exp Med* **192**: 421–432.

Ziboh V, Cho Y, Mani I, Xi S (2002). Biological significance of essential fatty acids/prostanoids/lipoxygenase-derived monohydroxy fatty acids in the skin. *Arch Pharm Res* **25**: 747–758.