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Inhibitory effects of azelastine on substance P-induced itch-associated response in mice

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Abstract

The anti-pruritic mechanisms of azelastine were studied in mice. Scratching induced by intradermal histamine was inhibited by azelastine (30 mg/kg) and chlorpheniramine (30 mg/kg). Substance P-induced scratching was dose dependently suppressed by azelastine (3–30 mg/kg), but not by chlorpheniramine (10 and 30 mg/kg). Azelastine (30 mg/kg) inhibited the substance P-induced production of leukotriene B_4, but not prostaglandin E_2, in the skin. Azelastine (3–30 mg/kg) suppressed scratching induced by intradermal injection of leukotriene B_4. The results suggest that inhibition of the production and action of leukotriene B_4, as well as an anti-histamine action, is involved in the anti-pruritic action of azelastine.

Keywords: Azelastine; Itch; Leukotriene B_4; Antihistamine; Prostaglandin E_2; Scratching

1. Introduction

Histamine is considered an important mediator of itch, a sensation that provokes a desire to scratch, and antihistamines inhibit histamine-mediated itch, for example, the itch of urticaria. However, the itch of atopic dermatitis and hemodialysis-associated pruritus is generally resistant to antihistamines (Blagg, 1986; Wahlgren et al., 1990), suggesting the involvement of other mechanisms and mediators of itch. Azelastine, an anti-allergic agent with anti-histamine activity, is used clinically for the treatment of itch in patients with atopic dermatitis (Kawazu, 1993), chronic hemodialysis (Kanai et al., 1995) or other pruritic diseases (Nakagawa and Ishibashi, 1994). Since azelastine relieves antihistamine-resistant pruritus, it is possible that pharmacological action(s) other than blocking of histamine H_1 receptor are also responsible for the anti-pruritic effects of azelastine. However, these actions of azelastine are unclear.

Substance P is a potent pruritogenic peptide (Hägermark et al., 1978) and is speculated to be involved in some pruritic diseases (Faerber et al., 1986). Substance P degranulates mast cells to release histamine (Ebertz et al., 1987), and substance P-induced itch is thought to be mediated by histamine released from mast cells (Hägermark et al., 1978). We have shown that scratching is elicited by intradermal substance P in mice and that it shares some features of human itching (Kuraishi et al., 1995; Andoh et al., 1998). At least in mice, histamine and other mast cell mediators may not play a critical role in substance P-induced itching because the scratch-inducing activity of substance P is not apparently different in mast cell-deficient and normal mice (Andoh et al., 1998). Leukotriene B_4 is a potent pruritogen in mice (Andoh and Kuraishi, 1998). An intradermal injection of substance P increases the cutaneous concentration of leukotriene B_4 (Andoh et al., in press). 5-Lipoxygenase inhibitor suppresses leukotriene B_4 production and scratching following substance P injection, suggesting that this leukotriene is at least partly involved in substance P-induced scratching (Andoh et al., in press). Recently, we have found that the blockade of leukotriene B_4 action is involved in the inhibitory effect of emedastine, an antihistamine, on substance P-induced scratching (Andoh and Kuraishi, 2000). In addition, azelastine may suppress the production of leukotriene B_4 in humans (Kanai et al., 1995). In the present experiments, therefore, we examined in mice whether azelastine could inhibit the substance P-induced itch-associated response and whether leukotriene B_4 could be involved in the anti-pruritic action of this agent.

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2. Materials and methods

2.1. Animals

Male ICR mice were used at 5–6 weeks of age. 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. Experiments were conducted in accordance with the guidelines of the Guiding Principles for the Care and Use of Laboratory Animals approved by the Committee for Animal Experiments in Toyama Medical and Pharmaceutical University.

2.2. Materials

Substance P (Peptide Institute, Minoh, Japan), histamine (Wako, Osaka, Japan) and leukotriene B_4 (Ono Pharmaceutical, Osaka, Japan) were dissolved in physiological saline. These agents and saline were injected intradermally in a volume of 50 μl into the rostral part of the mouse back. Azelastine (Eizai, Tokyo, Japan) was suspended in 0.5% (w/v) sodium carboxymethyl cellulose (Wako), and chlorpheniramine maleate (Sigma, St. Louis, USA) was dissolved in tap water. These agents and vehicle were administered perorally (p.o.) 30 min before the injection of substance P, histamine or leukotriene B_4. Indomethacin (Sigma) and zileuton (Ono Pharmaceutical) were dissolved in ethanol.

2.3. Behavioral observation

The hair was clipped over the rostral part of the back the day before the experiment. Immediately after intradermal injection, the animals were put into an acrylic cage (26 × 18 × 30 cm) composed of four cells, to which they had been allowed to get used to for at least 1 h. The behaviors were videotaped with no one present and the recording was used to count scratching of the injected site with the hind paws (Andoh et al., 1998). The number of scratchings per 10 min is presented to show the time course of pruritogen-induced scratching. The total number of scratchings for 60 min is presented to show the effects of antihistamines.

2.4. Enzyme immunoassay

The extraction and assay of leukotriene B_4 and prostaglandin E_2 were performed as described previously (Andoh and Kuraishi, 2000). In brief, 5 min after intradermal injection, the treated skin (2 cm in diameter) was isolated and homogenized in ethanol containing 10 μM indomethacin (cyclooxygenase inhibitor) and 10 μM zileuton (5-lipoxygenase inhibitor) on ice. After centrifugation, the supernatant was applied to a Bond Elut C2 column (Varian Associates, Harbor City, CA, USA), eluted with ethyl acetate and evaporated. The residue was suspended in enzyme immunoassay buffer (Cayman Chemical, Ann Arbor, MI, USA), and leukotriene B_4 and prostaglandin E_2 were determined with an enzyme immunoassay kit (Cayman Chemical).

2.5. Measurement of intracellular Ca^{2+} concentration

Primary cultures of mouse neutrophils were washed twice with a loading medium (20 mM HEPES, 115 mM NaCl, 5.4 mM KCl, 1.8 mM $CaCl_2$, 0.8 mM $MgCl_2$, 13.8 mM glucose, pH.7.4), incubated with 10 μM fluo-3/AM (Dojindo, Kumamoto, Japan) in loading medium containing 0.05% poloxamer (Calbiochem, Dermstadt, Germany) for 45 min at room temperature, and washed with loading medium for 1 h. The intracellular Ca^{2+} concentration of neutrophils was measured using a confocal microscope system (Meridian Instrument Far East, Tokyo, Japan) at 515–545 nm emission with excitation at 488 nm.

2.6. Data processing

Data are presented as means and S.E.M. Statistical significance was analyzed using a one-way analysis of variance

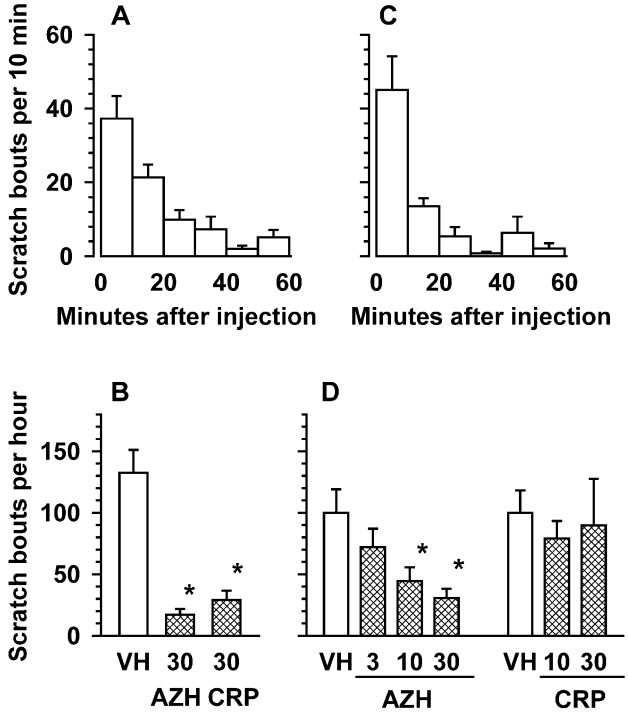

Fig. 1. Effects of azelastine (AZH) and chlorpheniramine (CRP) on scratching induced by histamine and substance P in mice. (A) The time course of scratching following histamine (100 nmol/site) injection. (B) Effects of AZH and CRP on histamine-induced scratching. (C) The time course of scratching following substance P (100 nmol/site) injection. (D) Effects of AZH and CRP on substance P-induced scratching. AZH, CRP and vehicle (VH; 0.5% CMC solution) were administered perorally 30 min before the injection of histamine (100 nmol/site) or substance P (100 nmol/site). Values are the means and S.E.M. for eight animals. $*P<0.05$ when compared with VH.

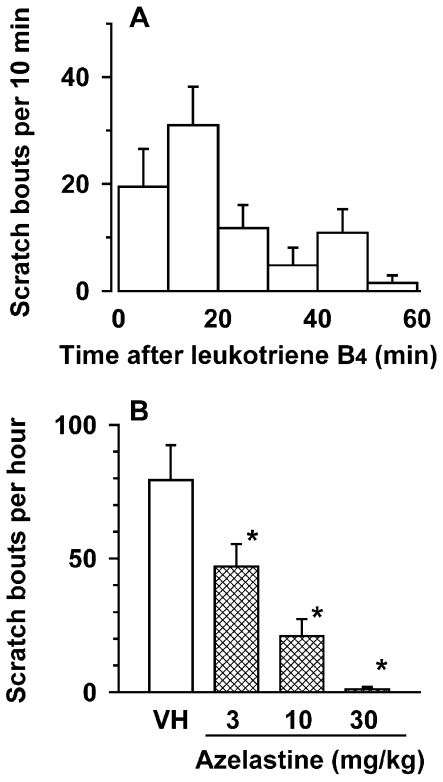

Fig. 2. Suppressive effects of azelastine on leukotriene B_4-induced scratching in mice. (A) The time course of scratching induced by leukotriene B_4 (0.03 nmol/site). (B) Effects of azelastine on leukotriene B_4-induced scratching. Azelastine and vehicle (VH) were administered perorally 30 min before the injection of leukotriene B_4 (0.03 nmol/site). Values are the means and S.E.M. for eight animals. * $P<0.05$ when compared with VH.

followed by Dunnett's multiple comparisons; $P<0.05$ was considered significant.

3. Results

3.1. Effects of antihistamines on histamine- and substance P-induced scratching

An intradermal injection of histamine (100 nmol/site) elicited scratching of the skin around the injected site by the hind paws within 5 min, and the effect peaked during the initial 10-min period (Fig. 1A). This action of histamine was markedly suppressed by pretreatment with azelastine (30 mg/kg, p.o.) or chlorpheniramine (30 mg/kg, p.o.) (Fig. 1B). An intradermal injection of substance P (100 nmol/site) also elicited scratching with a time course similar to that of histamine (Fig. 1C). This action of substance P action was dose dependently ($P<0.01$) inhibited by pretreatment with azelastine (3–30 mg/kg, p.o.) (Fig. 1D). Pretreatment with chlorpheniramine (10 and 30 mg/kg, p.o.) was without effect on the action of substance P (Fig. 1D). These antihistamines at the doses tested did not affect locomotor activity (data not shown).

3.2. Effects of azelastine on leukotriene B_4-induced scratching

When injected intradermally into the rostral back, leukotriene B_4 (0.03 nmol/site) elicited scratching (Fig. 2A, vehicle control). Peroral pretreatment with azelastine (3–30 mg/kg) dose dependently ($P<0.01$) suppressed the leukotriene B_4-induced scratching, with complete inhibition after the dose of 30 mg/kg (Fig. 2B).

3.3. Effects of azelastine on substance P-induced cutaneous leukotriene B_4 and prostaglandin E_2 production

Since an intradermal injection of substance P elicited scratching within 5 min and the effect was maximum during the initial 10-min period, the cutaneous contents of leukotriene B_4 and prostaglandin E_2 were determined 5 min after substance P injection. Although saline did not affect the cutaneous contents of leukotriene B_4 and prostaglandin E_2, an intradermal injection of substance P (100 nmol/site) significantly increased these contents (Fig. 3). When azelastine (30 mg/kg) was administered p.o. 30 min before substance P injection, the increase in leukotriene B_4 content was partially but significantly suppressed (Fig. 3A). The increase in prostaglandin E_2 content in the same cutaneous samples was not significantly affected by azelastine (Fig. 3B).

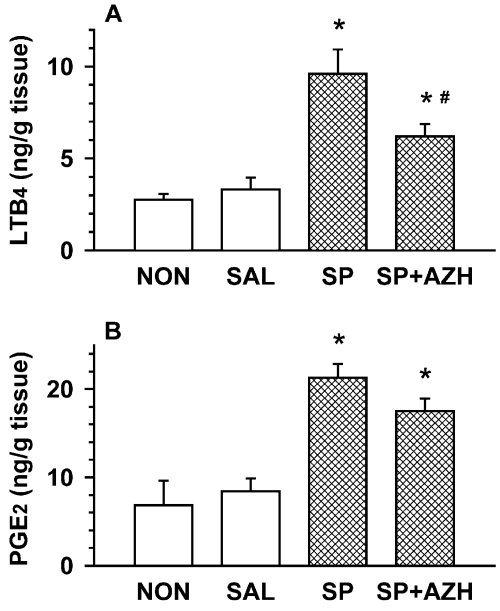

Fig. 3. Effects of azelastine (AZH) on the cutaneous level of leukotriene B_4 (LTB_4) and prostaglandin E_2 (PGE_2) produced by intradermal injection of substance P (SP). SP (100 nmol/site) was injected intradermally, and the concentrations of cutaneous LTB_4 and PGE_2 were measured 5 min later. AZH (30 mg/kg) and vehicle were administered perorally 30 min before the injection of substance P. NON and SAL indicate the results from non- and saline-treated skin, respectively. Values are the means and S.E.M. for six animals. * $P<0.05$ when compared with SAL. # $P<0.05$ when compared with SP alone.

3.4. Effects of azelastine on the increase in the intracellular Ca^{2+} level induced by leukotriene B4 in neutrophils

The application of leukotriene B_4 (3 μM) apparently increased the intracellular Ca^{2+} concentration in mouse neutrophils. Simultaneous application of azelastine (0.1–3 μM) with leukotriene B_4 dose dependently inhibited the increase in Ca^{2+} concentration; the levels of Ca^{2+} (expressed as a percentage of non-stimulated levels) following leukotriene B_4 application in the presence of azelastine (0, 0.1, 0.3, 1, and 3 μM) were 202 ± 7.3%, 203 ± 7.9%, 158 ± 3.7%, 121 ± 2.8%, and 108 ± 1.4% (n = 39–50), respectively.

4. Discussion

The histamine H_1 receptor antagonist chlorpheniramine (30 mg/kg) produced apparent inhibition of histamine-induced scratching. An intradermal injection of histamine (100 nmol/site) elicits the firing of cutaneous afferent nerves, an effect which is almost abolished by subcutaneous pretreatment with chlorpheniramine at a dose of 3 mg/kg in mice (Maekawa et al., 2000). In addition, peroral pretreatment with chlorpheniramine (0.3–10 mg/kg) inhibits scratching induced by a subcutaneous injection of compound 48/80, an agent that induces the degranulation of mast cells, in mice (Sugimoto et al., 1998). Thus, chlorpheniramine at peroral doses of 10 and 30 mg/kg may inhibit the action of endogenous histamine in the skin. However, such doses of chlorpheniramine did not suppress scratching induced by substance P, thereby supporting the idea that cutaneous histamine does not play an important role in substance P-induced itching in mice (Andoh et al., 1998). Unlike chlorpheniramine, another antihistamine, azelastine, produced a dose-dependent inhibition of substance P-induced scratching, suggesting that pharmacological action(s) other than the blockade of histamine H_1 receptors are involved in the inhibitory effect of azelastine on substance P-induced scratching.

Intradermal substance P increased the cutaneous concentrations of leukotriene B_4 in mice, and azelastine significantly suppressed the increase of leukotriene B_4. The results are consistent with other reports showing that azelastine inhibits the production of leukotrienes such as leukotriene B_4, leukotriene C_4 and 5-hydroxyeicosatetraenoic acid (Nishihira et al., 1989). Azelastine suppresses leukotriene production, probably by inhibiting 5-lipoxygenase activity and the translocation of this enzyme from cytosol to the nuclear envelope (Nishihira et al., 1989; Chand and Sofia, 1995). It exerts little or no inhibitory effects on leukotriene C_4 synthetase, leukotriene A_4 hydrolase, phospholipase A_2 and 12-lipoxygenase (Nishihira et al., 1989). Azelastine was reported to inhibit interleukin-1-induced production of prostaglandin E_2 in cultured cutaneous fibroblasts (Inoue et al., 1997). In the present experiments, however, azelastine did not significantly suppress the substance P-induced production of prostaglandin E_2 in the skin. Thus, azelastine may selectively inhibit leukotriene production in vivo. Substance P acts on cultured keratinocytes to produce leukotriene B_4 through NK_1 tachykinin receptors (Andoh et al., in press). Considering that itch is a sensation arising from the superficial layers of skin and that keratinocytes are the largest cell group in the epidermis, epidermal keratinocytes may be the primary site of leukotriene B_4-producing action of intradermal substance P. In mast cell-deficient mice, substance P elicits scratching (Andoh et al., 1998) and this substance P action is inhibited by leukotriene B_4 antagonist (Andoh et al., in press). Thus, mast cells may not play an important role in the substance P-induced production of pruritogenic leukotriene B_4 in mice.

One important finding in the present experiments is that azelastine inhibited intradermal leukotriene B_4-induced scratching. This scratching was abolished by the 30 mg/kg dose of azelastine, a dose which produced only a 54% decrease in the substance P-induced leukotriene B_4 production. Thus, azelastine may be more active in inhibiting the action of leukotriene B_4 than it is in inhibiting the production of leukotriene B_4. It is unclear whether azelastine acts directly on leukotriene B_4 receptors. However, since azelastine suppressed the leukotriene B_4-induced increase in intracellular Ca^{2+} concentration in cultured neutrophils, it may block the leukotriene B_4 receptor and/or intracelluar signaling systems of this receptor.

Another important finding is that azelastine inhibited substance P-induced scratching at doses of 10 and 30 mg/kg, doses at which it suppressed both the action and production of leukotriene B_4. Considering that histamine does not play an important role in substance P-induced scratching (present experiment, Andoh et al., 1998), it is suggested that azelastine inhibited substance P-induced scratching at least partly by the suppression of the action and production of leukotriene B_4. The present results do not rule out the possibility that endogenous mediator(s) other than leukotriene B_4 are also involved in substance P-induced scratching because azelastine (30 mg/kg) abolished leukotriene B_4-induced scratching but only partially inhibited substance P-induced scratching.

In summary, azelastine inhibited the substance P-induced cutaneous production of leukotriene B_4 and leukotriene B_4-induced scratching in mice. These actions may be at least partly responsible for the inhibition of substance P-induced scratching. It would be interesting to examine whether these actions are involved in the anti-pruritic actions of azelastine in human subjects.

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