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Short communication

Involvement of blockade of leukotriene B_4 action in anti-pruritic effects of emedastine in mice

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Abstract

The anti-pruritic activity of emedastine difumarate was studied in mice. Emedastine (0.03–0.3 mg/kg) inhibited scratching induced by intradermal injection of histamine (100 nmol/site). Scratching induced by substance P (100 nmol/site) and leukotriene B_4 (0.03 nmol/site), but not by serotonin (100 nmol/site), was also suppressed by emedastine (0.03–0.3 mg/kg). Intradermal injection of substance P increased the cutaneous concentration of leukotriene B_4, which was not affected by emedastine. These results suggest that the inhibition by emedastine of substance P-induced itch-associated response is mediated by the blockade of leukotriene B_4 action. Anti-leukotriene B_4 action, as well as the anti-histamine action, may contribute to the anti-pruritic effects of emedastine.

Keywords: Emedastine difumarate; Itch-associated response; Substance P; Histamine; Leukotriene B_4; 5-HT (5-hydroxytryptamine, Serotonin)

1. Introduction

Emedastine difumarate has several pharmacological actions, such as blockade of histamine H_1 receptors (Fukuda et al., 1984a), an anti-allergic effect (Fukuda et al., 1984b), inhibition of histamine release from mast cells after anaphylaxis, compound 48/80 or substance P (Fukuda et al., 1984b; Saitoh et al., 1993), and inhibition of eosinophil chemotaxis induced by platelet activating factor (El-Shazly et al., 1996). Emedastine was reported to suppress itch in atopic dermatitis, eczema, prurigo and pruritus cutaneous after its peroral administration (Furue and Yamashita, 1997; Ishibashi et al., 1994). An intradermal injection of histamine elicits itch, which is inhibited by a histamine H_1 receptor antagonist (Hägermark et al., 1978). Thus, the blockade of histamine H_1 receptors may play a role in the inhibition of histamine-mediated pruritus. However, histamine does not play a main role in the itching of many pruritic diseases, including atopic dermatitis (Berth-Jones and Graham-Brown, 1989; Wahlgren et al., 1990). Therefore, it is assumed that some pharmacological action(s), other than the blockade of histamine H_1 receptors, contributes to the anti-pruritic action of emedastine.

Substance P is one of the most potent pruritogenic peptides (Hägermark et al., 1978). This peptide is thought to elicit itching through the release of histamine from mast cells in humans (Hägermark et al., 1978; Devillier et al., 1989). In mice, substance P also elicits scratching, an itch-associated response, which is not primarily mediated by histamine and mast cells (Andoh et al., 1998). The substance P effect is at least partly mediated by leukotriene B_4 (Andoh and Kuraishi, 1998b), the latter being a potent pruritogenic in mice (Andoh and Kuraishi, 1998a). Although serotonin is a weak pruritogen in human subjects (Fjellner and Hägermark, 1979), it is more potent than histamine to elicit scratching in mice (Yamaguchi et al., 1999). Thus, to determine whether anti-pruritic effects of emedastine are exclusively due to the anti-histamine action, we examined the effects of this agent on the scratching induced by several pruritogens such as histamine, substance P, leukotriene B_4 and serotonin in mice. We also determined the effect of emedastine on the production of leukotriene B_4 in the skin after substance P injection.

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2. Materials and methods

2.1. Animals

Male ICR mice (5–6 weeks of age) were used. 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 back on the day before the injection. All experimental procedures were approved by the Institutional Animal Care and Use Committee, Toyama Medical and Pharmaceutical University.

2.2. Materials

Emedastine difumarate, a gift from Kanebo (Tokyo, Japan), was dissolved in tap water. The dosages of emedastine are given in terms of the weight of the salt. Substance P (Peptide Institute, Minoh, Japan), histamine (Wako, Osaka, Japan), serotonin (Sigma, St. Louis, MO, USA) and the sodium salt of leukotriene B_4 (a generous gift from Ono Pharmaceutical, Osaka, Japan) were dissolved in physiological saline. Zileuton (Ono Pharmaceutical) was dissolved in ethanol.

2.3. Behavioral experiments

Histamine, serotonin, substance P and leukotriene B_4 were injected intradermally in a volume of 50 μl into the hair-clipped skin. Immediately after injection, the animals were individually put into one cell of an acrylic cage ($26 \times 18 \times 30$ cm) composed of four cells. The mice were allowed to get used to the cage cells for at least 1 h prior to the experiment. Behavior was videotaped for 1 h with no one present in the observation room. The playback served for counting scratching of the injected site with the hind paws (Kuraishi et al., 1995). Emedastine difumarate was administered perorally 30 min before pruritogen injection.

2.4. Enzyme immunoassay

Five minutes after substance P injection, the treated skin (2 cm in diameter) was isolated and immediately put into ice-chilled ethanol containing the 5-lipoxygenase inhibitor zileuton (10 μM). After homogenization and centrifugation, the supernatant was diluted 10-fold with ice-chilled 0.1 M acetic acid, applied to Bond Elute C_2 column (Varian, MA, USA) and eluted with ethyl acetate. After the evaporation of the eluate, the residue was suspended in 150 μl of 0.01 M sodium bicarbonate buffer (pH 10.0), sonicated for 5 min, and then diluted with enzyme immunoassay buffer (Cayman Chemical, MI, USA) for the assay of leukotriene B_4 with an EIA kit (Cayman Chemical).

2.5. Data processing

All data are presented as means ± S.E.M. Statistical significance was analyzed using the one-way analysis of variance followed by Dunnett's multiple comparisons; $P < 0.05$ was considered significant.

3. Results

3.1. Effects on pruritogen-induced scratching

Histamine was injected intradermally at a dose of 100 nmol/site. This dosage produces a submaximal effect on scratching (Kitagawa et al., 1997). In vehicle-pretreated animals, scratching peaked during the initial 10-min period after histamine injection and had almost subsided by 30 min ($n = 8$). Pretreatment with emedastine at doses of 0.1 and 0.3 mg/kg, but not 0.03 mg/kg, significantly suppressed histamine-induced scratching (Fig. 1A).

Substance P and leukotriene B_4 were injected at a dose of 100 and 0.03 nmol/site, respectively, doses producing

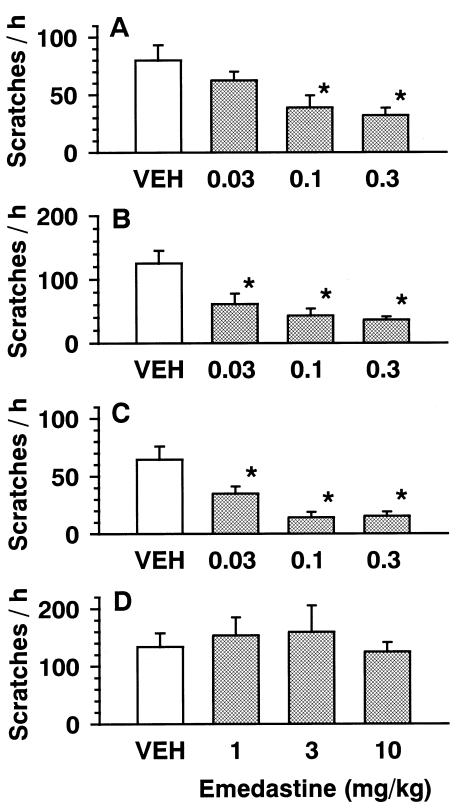

Fig. 1. Suppressive effect of emedastine difumarate on itch–scratch responses induced by histamine, substance P, leukotriene B_4 or serotonin. Histamine (100 nmol/site), substance P (100 nmol/site), leukotriene B_4 (0.03 nmol/site) or serotonin (100 nmol/site) was injected intradermally into the rostral back of the mice. Emedastine difumarate was administered perorally 30 min before pruritogen injection. Values are the means and S.E. for eight animals. $^{*}P < 0.05$ when compared with vehicle (VEH).

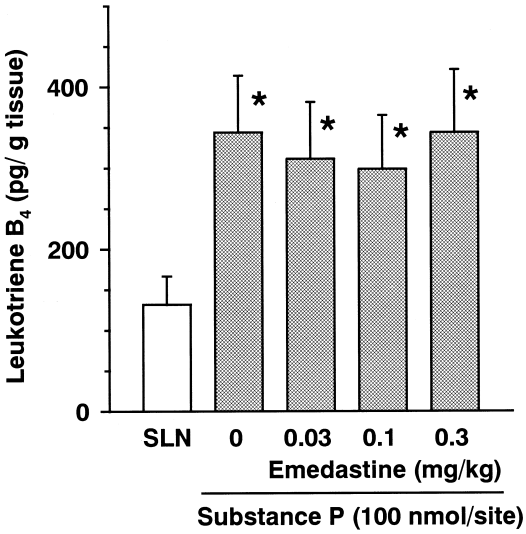

Fig. 2. The production of leukotriene B_4 after substance P injection in the mouse skin. Substance P (100 nmol/site) or saline (SLN) was injected intradermally and 5 min later, the concentration of leukotriene B_4 in the treated skin was determined. Emedastine difumarate was administered perorally 30 min before substance P injection. Values are the means and S.E. for six animals. $^{*}P < 0.05$ vs. SLN.

maximal effects (Andoh et al., 1998; Andoh and Kuraishi, 1998a). Scratching peaked during the initial 10-min period and had almost subsided by 30 min ($n = 8$ each). Pretreatment with emedastine (0.03, 0.1, 0.3 mg/kg) significantly inhibited the scratching induced by substance P and leukotriene B_4 (Fig. 1B,C).

Serotonin was injected at a dose of 100 nmol/site because this dose produces a maximal effect (Yamaguchi et al., 1999). The effect peaked during the initial 10-min period and had almost subsided by 40 min ($n = 8$). Emedastine at higher doses, 1–10 mg/kg, did not affect the scratching induced by serotonin (Fig. 1D).

3.2. Effects on substance P-induced production of leukotriene B_4

An intradermal injection of substance P elicited scratching within 5 min and the effect peaked during the initial 10-min period. The cutaneous concentration of leukotriene B_4 was determined 5 min after substance P injection. Intradermal substance P (100 nmol/site) significantly increased the concentration of leukotriene B_4 as compared to that in the saline control (Fig. 2). Peroral pretreatment with emedastine (0.03–0.3 mg/kg) did not affect the substance P-induced increase in leukotriene B_4 concentration (Fig. 2).

4. Discussion

The present study showed that emedastine difumarate inhibits itch-associated responses at relatively low peroral doses (0.03–0.3 mg/kg), which are comparable to clinical doses (Furue and Yamashita, 1997; Ishibashi et al., 1994). The inhibition of scratching induced by histamine, substance P and leukotriene B_4 may not be due to non-specific behavioral suppression, such as sedation, because the higher doses of emedastine (1–10 mg/kg) did not affect serotonin-induced scratching. As emedastine is a potent histamine H_1 receptor antagonist (Fukuda et al., 1984a), the inhibition of histamine-induced scratching may be primarily due to the blockade of histamine H_1 receptors in the skin.

One important finding in the present experiments was that emedastine was more potent to inhibit the scratching induced by substance P than that induced by histamine; the dose of 0.03 mg/kg significantly inhibited the action of substance P, but not of histamine. Emedastine inhibits histamine release from rat peritoneal mast cells induced by passive cutaneous anaphylaxis and by compound 48/80 (Fukuda et al., 1984b). However, in mice, the histamine H_1 receptor antagonist, chlorpheniramine, suppresses histamine-induced scratching, but not the substance P-induced response (Kitagawa et al., 1997). Substance P elicits scratching even in mast cell-deficient mice (Andoh et al., 1998). These findings together suggest that, at least in mice, emedastine inhibits the substance P action through mechanisms other than blockade of histamine H_1 receptors.

Another important finding in the present experiments was that emedastine was more potent to inhibit the scratching induced by leukotriene B_4 than that induced by histamine. The lowest dose tested significantly suppressed the action of leukotriene B_4 as well as of substance P, but not that of histamine. Scratching induced by substance P is inhibited by a leukotriene B_4 receptor antagonist and a 5-lipoxygenase inhibitor (Andoh and Kuraishi, 1998b), suggesting the involvement of leukotriene B_4 in the pruritogenic action of substance P. With these findings taken into account, the present results raise the possibility that the inhibition of leukotriene B_4 action by emedastine is involved in the suppression of substance P action. Leukotriene B_4 was reported to be increased in patients with psoriasis (Brain et al., 1984; Ruzicka et al., 1986) or on chronic hemodialysis (Kanai et al., 1995). Although its precise role in the itching of patients with pruritic diseases is unclear, we should consider the involvement of anti-leukotriene B_4 action in the anti-pruritic effect of emedastine.

Substance P increased the cutaneous concentration of leukotriene B_4 at an intradermal dose that elicited scratching. The 5-lipoxygenase inhibitor, zileuton, inhibits substance P-induced scratching and increases in leukotriene B_4 concentration (Andoh and Kuraishi, 1998b). In contrast, emedastine did not affect the substance P-induced increase in leukotriene B_4 concentration at the doses at which it significantly suppressed the substance P-induced scratching. Therefore, the inhibition by emedastine of sub-

stance P-induced scratching may be due to an anti-leukotriene B_4 action rather than to inhibition of leukotriene B_4 production.

In summary, emedastine showed potent anti-leukotriene B_4 action as well as anti-histamine action. Emedastine may be effective against histamine H_1 receptor antagonist-sensitive and resistant itching.

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