Possible Involvement of 5-Lipoxygenase Metabolite in Itch-Associated Response of Mosquito Allergy in Mice

Yasushi Kuraishi1,2,* Eiji Ohtsuka1,# Tasuku Nakano1, Sanae Kawai1, Tsugunobu Andoh1, Hiroshi Nojima3, and Kiyoshi Kamimura1

1Department of Applied Pharmacology, Graduate School of Medicine and Pharmaceutical Sciences, and 21st COE Program, University of Toyama, 2630 Sugitani, Toyama 930-0194, Japan
3Department of Pharmacology, Faculty of Pharmaceutical Sciences, Ohu University, Koriyama 963-8611, Japan

Received February 19, 2007; Accepted June 29, 2007

Abstract. This study investigated endogenous mediators involved in mosquito allergy-associated itching in mice. An intradermal injection of an extract of mosquito salivary gland elicited marked scratching in sensitized mice. The 5-lipoxygenase inhibitor zileuton (100 mg/kg), the 5-lipoxygenase activating peptide inhibitor MK-886 (10 mg/kg), and the glucocorticoid betamethasone 17-valerate (3 mg/kg) inhibited the scratching. The scratching was not affected by the cyclooxygenase inhibitors indomethacin and ketoprofen, the TP prostanoid receptor antagonist SQ-29548, the leukotriene B4 antagonist ONO-4057, the cysteinyl leukotriene antagonist pranlucast, the leukotriene D4 antagonist MK-571, the platelet-activating factor antagonist CV-3988, the nitric oxide synthase inhibitor N(G)-nitro-L-arginine methyl ester, the H2 histamine-receptor antagonist cimetidine, the H1 histamine-receptor antagonist terfenadine plus cimetidine, and cypohedatine that blocks the 5-HT1A serotonin receptors. Zileuton (100 mg/kg) inhibited the increased activity of the cutaneous nerve branch induced by an intradermal injection of the extract, suggesting the peripheral action. Zileuton and MK-886 (10 and 100 µM) did not affect high K+ induced increase in intracellular Ca2+ concentration in cultured dorsal root ganglion neurons. The results suggest that 5-lipoxygenase metabolite(s) other than leukotriene B4 and cysteinyl leukotrienes are involved in mosquito allergy-associated itching.

Keywords: itch of mosquito allergy, 5-lipoxygenase metabolite, zileuton, cutaneous nerve branch, dorsal root ganglion neuron

Introduction

Mosquito bites cause itching as well as cutaneous reactions such as weal in humans. When volunteers are bitten by a mosquito for the first time, there is neither itch nor immediate cutaneous reactions, and itch develops after recurring mosquito bites (1). Similarly, the first bite of a mosquito does not cause an itch-related response, hind-paw scratching, in mice, and recurring bites induce it (2). Mosquito bite induces the scratching and plasma extravasation in mice that have been repeatedly given injections of an extract of the salivary gland of mosquitoes (ESGM), suggesting that the scratching is due to immediate allergy to components of mosquito saliva, such as α-amylase I, anticoagulant-factor Xa, Aed a X1, Aed a X2, female-specific protein (D7), which showed the same molecular weight as results obtained by using ESGM and IgE of serum in human sensitized with mosquito bite (3, 4) (unpublished observation of Nakano, Andoh, Kosaka, and Kuraishi). The H1 histamine-receptor antagonist terfenadine does not affect the mosquito bite-elicited scratching in sensitized mice at a dose that inhibits the mosquito bite-elicited plasma extravasation and histamine-elicited scratching (2), suggesting that histamine released from mast cells does not play an important role in itching of mosquito allergy.

Although scratching and the firing of primary afferents...
elicted by an intradermal injection of ESGM are not affected by the H1 histamine-receptor antagonist terfenadine in sensitized mice, they are significantly inhibited by another H1 histamine-receptor antagonist azelastine (5). Azelastine suppresses the high K+-induced increase of intracellular Ca2+ concentration in primary cultures of mouse dorsal root ganglion neurons, raising the possibility that the inhibitory action on primary sensory neurons is at least partly involved in the inhibition of the scratching (5). Azelastine inhibits scratching elicited by intradermal injections of substance P and leukotriene B4, a metabolite formed by 5-lipoxygenase, and less efficiently inhibits the production of leukotriene B4 in the skin induced by an intradermal injection of substance P (6). Leukotriene B4 is pruritogenic and is responsible for substance P-induced scratching in mice (7, 8). These findings raise the possibility that leukotriene B4 is involved in itching of mosquito allergy. Thus, this study was carried out to determine whether 5-lipoxygenase metabolites including leukotriene B4 and other potential itch mediators would be involved in itching of mosquito allergy.

**Materials and Methods**

**Animals**

Male ICR mice (Japan SLC, Shizuoka) 5-week-old at the start of experiments were used. They were housed in a room under controlled temperature (22 ± 2°C), humidity (55 ± 10%), and light (lights on 07:00–19:00 h). Food and water were freely available. The study was approved by the Committee for Animal Experiments at University of Toyama.

**Agents**

5-[2-(2-Carboxyethyl)-3-\{6-(4-methoxyphenyl)-5E-hexenyl\}oxyphenoxy] valeric acid (ONO-4057; Ono Pharmaceutical Co., Osaka), zileuton (Ono Pharmaceutical Co.), pranlukast (Ono Pharmaceutical Co.), betamethasone 17-valerate (Sigma, St. Louis, MO, USA), indomethacin (Sigma), cimetidine (Research Biochemicals International, Natick, MA, USA), and terfenadine (Sigma) were suspended in 0.5% sodium carboxymethylcellulose. MK-886 (BIOMOL Research Laboratories, Plymouth Meeting, PA, USA) was suspended 0.25% γ-cyclodextrin (Sigma). MK-571 (Cayman Chemical Co., Ann Arbor, MI, USA) and cyproheptadine (Research Biochemicals International) were dissolved in tap water. Ketoprofen (Chugai Pharmaceutical Co., Ltd., Tokyo), N5-nitro-L-arginine methyl ester (Research Biochemicals International), CV-3988 (Wako Pure Chemical Industries Ltd., Osaka), and SQ-29548 (Cayman Chemical Co.) were dissolved in physiological saline. ONO-4057, zileuton, pranlukast, betamethasone 17-valerate, MK-571, and indomethacin were administered orally (p.o.). MK-886 and N5-nitro-L-arginine methyl ester were administered intravenously (i.v.) and ketoprofen was administered subcutaneously (s.c.). The doses and administration routes of agents used were selected on the basis of the following reports: zileuton (8), MK-886 (9), betamethasone (8), indomethacin (8), ketoprofen (10), SQ-29548 (11), ONO-4057 (8), pranlucast (8), MK-571 (12), CV-3988 (13), N5-nitro-L-arginine methyl ester (14), cimetidine (15), terfenadine (2), and cyproheptadine (16).

**Sensitization and challenge**

ESGM was prepared from the thorax including the salivary gland of two hundred female mosquitoes (Aedes albopictus), lyophilized, and kept at −80°C until use, as previously described (2). ESGM was dissolved in saline before use and was injected intradermally into the caudal back twice a week for four weeks for sensitization and into the rostral back for challenge; the dose and volume of injection were 10 µg/site and 50 µl, respectively.

In our preliminary experiments, ESGM was separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis, transferred to a polyvinylidene difluoride membrane, and then immunoblotted by sequential incubation with serum prepared from sensitized mice and horseradish peroxidase-conjugated anti-mouse IgE antibody. It revealed the presence of antigenic molecules of 81.5 (probably α-amylase 1), 54 (probably anticoagulant factor Xa), 50, 44 (probably Aed a X1), 37 (probably Aed a X2 and/or female-specific protein), 35.5, 33, and 20.5 kDa (Nakano et al., preliminary experiments). In human subjects, 17 antigens were observed in ESGM and serum levels of IgE specific for the antigens varied between individuals (3). These findings suggest that sensitization with ESGM is more efficient than that with single antigen. This let us use ESGM rather than specific antigen for sensitization and challenge in this study.

**Behavioral observation**

Before behavioral observation, the mice were put into an acrylic cage composed of four cells (13 × 9 × 35 cm) for at least 1 h for acclimation. Immediately after intradermal injection, the animals were put back into the same cells and the behaviors were videotaped for 1 h with personnel kept out of the observation room. Playing back of the video served for counting scratching behavior. Mice stretch either hind paw toward the injection site, lean the head toward the hind paw, and rapidly scratch several times for about 1 s. A series of these movements was counted as one bout of scratching (17).
Recording of the activity of cutaneous nerve branch

The activity of cutaneous nerve branch was recorded as described (5, 18). Briefly, under urethane (1.5 g/kg) anesthesia, the skin of the rostral back was turned inside out and nerve activity was recorded extracellularly using bipolar electrodes of silver wire and an AC amplifier (AB651; Nihon Kohden, Tokyo) with a band-pass filter of 1 kHz. ESGM was injected intradermally into the receptive field of the rostral back.

Determination of intracellular Ca\textsuperscript{2+} concentration

The preparation of dorsal root ganglion neurons and the determination of intracellular Ca\textsuperscript{2+} concentration were done as described (5). Briefly, dorsal root ganglia were removed from healthy mice and then mechanically dispersed by passage through micropipettes after 45-min incubation with 2 mg/ml collagenase. Isolated cells were cultured in Dulbecco’s modified Eagle medium containing 10% fetal bovine serum, 100 U/ml penicillin, 100 µg/ml streptomycin, and 8 µM arabinosylcytosine for one day. Primary cultures of neurons were loaded with fluo-3 acetoxyethyl ester and the concentration of intracellular free Ca\textsuperscript{2+} ions in the nerve cells (<25 µm) was determined at 488-nm excitation and 530-nm emission with a laser scanning microscope (Meridian Instrument Far East, Tokyo). The neurons were excited with 30 mM KCl, and zileuton and MK-886 were added to the medium 30 min before KCl administration.

Statistical analysis

Values given are means and S.E.M. Statistical significance was analyzed using Student’s t-test, Dunnett’s multiple comparisons, or two-way repeated measures analysis of variance; P<0.05 was considered significant.

Results

Effects of various agents on itch-associated behavior

An intradermal injection of ESGM elicited marked scratching in sensitized, but not unimmunized, mice (Fig. 1: A and B). Scratching was most frequent within 20 min after challenge and almost subsided by 50 min (Fig. 1B).

Pretreatment with the 5-lipoxygenase inhibitor zileuton (100 mg/kg, p.o.; −1 h) markedly suppressed the ESGM-elicited scratching: the inhibition was significant at a dose of 100 mg/kg, but not at 30 mg/kg or less (Fig. 2). Figure 2 also shows the effects of pretreatment with various agents on the ESGM-elicited scratching. The 5-lipoxygenase activating protein inhibitor MK-886 (1, 3, and 10 mg/kg, i.v.; −15 min) produced a dose-dependent inhibition of the scratching. The glucocorticoid betamethasone 17-valerate (3 mg/kg, p.o.; −1 h) produced a significant inhibition of the scratching. The scratching was not affected by the cyclooxygenase inhibitors indomethacin (3 and 10 mg/kg, p.o.; −30 min) and ketoprofen (30 mg/kg, s.c.; −30 min), the TP prostanooid receptor antagonist SQ-29548 (2 mg/kg, i.v.; −15 min), the leukotriene B\textsubscript{4} antagonist ONO-4057 (30 and 100 mg/kg, p.o.; −1 h), the cysteinyl leukotriene antagonist pranlucast (30 and 100 mg/kg, p.o.; −1 h), the leukotriene D\textsubscript{4} antagonist MK-571 (10 mg/kg, p.o.; −1 h), the platelet-activating factor antagonist CV-3988 (5 mg/kg, i.v.; −5 min), the nitric oxide synthase inhibitor N\textsuperscript{G}-nitro-L-arginine methyl ester (10 mg/kg, i.v.; −15 min), the H\textsubscript{2} histamine-receptor antagonist cimetidine (30 mg/kg, p.o.; −30 min), cimetidine (30 mg/kg) plus the H\textsubscript{1} histamine-receptor antagonist terfenadine (30 mg/kg, p.o.; −30 min), and cyproheptadine (1 mg/kg, p.o.; −1 h) that blocks the H\textsubscript{1} histamine receptor and 5-HT\textsubscript{1A} serotonin receptors.
Effects of zileuton on itch-associated nerve activity

The activity of the cutaneous nerve branch was not affected by an intradermal injection of ESGM into the receptive field, the rostral back, in naive mice, but it was markedly increased in sensitized mice; the effect peaked 3 min after injection and almost subsided by 50 min (Fig. 3). One-hour pretreatment with zileuton (100 mg/kg) produced a significant and marked inhibition of the increased activity following ESGM injection (Fig. 3).

Effects of zileuton and MK-886 on cultured dorsal root ganglion neurons

The intracellular Ca\(^{2+}\) concentration of cultured dorsal root ganglion neurons rapidly increased during 10 – 20 s following administration of 30 mM KCl, then partially returned, and the increase lasted at least until 4 min after administration (Fig. 4). Zileuton and MK-886 at final concentrations of 10 and 100 \(\mu\)M did not exert an influence on the K\(^+\)-induced increase of the intracellular Ca\(^{2+}\) concentration (Fig. 4).

Discussion

An intradermal injection of ESGM elicited marked scratching in sensitized mice, which was suppressed by the 5-lipoxygenase inhibitor zileuton at a dose of 100, but not 30, mg/kg. Zileuton at a dose of 30 mg/kg suppressed scratching elicited by an intradermal injection of substance P in mice, which may be due to the inhibition of substance P-induced production of leukotriene B\(_4\) in the skin, especially in the epidermal keratinocytes (8). Zileuton could inhibit allergy-associated scratching at a dose higher than that needed for the inhibition of substance P-induced scratching. Therefore, to determine whether zileuton would inhibit itch signaling in the skin, we studied the effect of zileuton on the response of cutaneous nerve branch to an intradermal injection of ESGM in sensitized mice. ESGM markedly increased the nerve activity in sensitized, but not naive, mice. Zileuton markedly suppressed the evoked
nerve activity, suggesting that the peripheral action of zileuton is at least partly involved in mosquito allergy-associated itching.

In addition to zileuton, the 5-lipoxygenase activating protein inhibitor MK-886 produced a dose-dependent suppression of the ESGM-elicited scratching. These results raise the possibility that 5-lipoxygenase metabolite(s) are involved in the allergy-associated scratching. The idea is consistent with the result that the glucocorticoid betamethasone suppressed the ESGM-elicited scratching. Taken together, the present results suggest that 5-lipoxygenase metabolite(s) other than leukotriene B\(_4\) and cysteinyl leukotrienes are involved in allergy-associated itch. It should be investigated which metabolite(s) are involved in the ESGM-elicited scratching, including metabolic intermediates, 5-hydroperoxyeicosatetraenoic acid and leukotriene A\(_4\), and lipoxins (23).

Zileuton was reported to inhibit aversive behavior and plasma extravasation induced by an intraperitoneal injection of acetic acid, which were concluded to be

Fig. 4. Effects of zileuton and MK-886 on high K\(^+\)-induced increase in intracellular Ca\(^{2+}\) ion concentration in mouse dorsal root ganglion neurons. Primary cultures of the neurons pre-loaded with fluo-3 were stimulated with the increase of extracellular concentration of KCl up to 30 mM. A: Typical example of changes in intracellular Ca\(^{2+}\) concentration after KCl administration. Effects of zileuton (B) and MK-886 (C) on high K\(^+\)-induced increase in intracellular Ca\(^{2+}\) concentration. Zileuton and MK-886 at final concentrations of 10 and 100 \(\mu\)M was added to the medium from 30 min before K\(^+\) stimulation. Values represent the means and S.E.M.: figures in parentheses indicate the number of neurons tested.
mediated by cysteiny1 leukotriene (24). Zileuton was also shown to produce a dose-dependent suppression of carrageenan-induced hyperalgesia; the effects became apparent 2 and 4 h, but not 30 min, after carrageenan treatment, and the effects were claimed to be due to the indirect action caused by the suppression of leukotriene B\textsubscript{4} production (24). Thus, the mechanisms of anti-pruritic action of zileuton may be different from those of the anti-nociceptive action.

In human subjects, the cyclooxygenase inhibitor aspirin does not affect itch of some pruritic diseases such as eczema, psoriasis, and biliary cirrhosis (25). However, it alleviates itch of patients with polycythemia vera (26) and topical aspirin reduces itch of lichen simplex chronicus (27). Prostaglandin E\textsubscript{2} is a weak pruritogen (28) and enhances serotonin-induced itching in healthy humans (26). In mice, intradermal injection of prostaglandin E\textsubscript{2} does not elicit scratching (7), but intradermal injection of the TP thromboxane A\textsubscript{2}-receptor agonist U-46619 elicits marked scratching, which is inhibited by a TP-receptor antagonist and is abolished by deficiency in TP receptor (29). These findings suggest that prostanooids are involved in itch of some pruritic symptoms. In the present study, cyclooxygenase inhibitors, indomethacin and ketoprofen, and the TP-receptor antagonist SQ-29548 were without effects on the ESGM-elicited scratching. Thus, the results do not provide evidence for the involvment of prostanooids in mosquito allergy-associated itching.

Intradermal injection of platelet-activating factor elicits itch in normal skin of humans (30, 31) and elicits scratching in mice (32) but not in rats (33). It has been claimed to be an important mediator of ocular itching in guinea pigs (34). However, the platelet-activating factor antagonist CV-3988 did not affect the ESGM-elicited scratching. Thus, the result does not provide evidence for the involvment of prostanooids in mosquito allergy-associated itching.

Repeated application of the nitric oxide synthase inhibitor N\textsuperscript{\textalpha}-nitro-L-arginine methyl ester relieves itch of patients with atopic dermatitis (35). In mice, nitric oxide may be as an itch enhancer and increases scratching induced by substance P (36) and medicine that decreases nitric oxide in the skin inhibits substance P-elicited scratching (37). However, the ESGM-elicited scratching was not suppressed by N\textsuperscript{\textalpha}-nitro-L-arginine methyl ester at a dose that had been shown to suppress scratching induced by substance P (36), suggesting that nitric oxide is not involved in mosquito allergy-associated itching.

Scratching elicited by histamine is suppressed by an H\textsubscript{1} histamine-receptor antagonist and H\textsubscript{2}-receptor antagonist and the suppression is potentiated by combina-

References

5 Ohtsuka E, Kawai S, Nojima H, Andoh T, Kamimura K, Kuraishi Y. Inhibitory effect of zileutone on allergic itch-associated response in mice sensitized with mosquito salivary
Mosquito Itch and 5-Lipoxygenase


