

## Full Paper

**Possible Involvement of 5-Lipoxygenase Metabolite in Itch-Associated Response of Mosquito Allergy in Mice**Yasushi Kuraishi<sup>1,2,\*</sup>, Eiji Ohtsuka<sup>1,#</sup>, Tasuku Nakano<sup>1</sup>, Sanae Kawai<sup>1</sup>, Tsugunobu Andoh<sup>1</sup>, Hiroshi Nojima<sup>3</sup>, and Kiyoshi Kamimura<sup>1</sup><sup>1</sup>Department of Applied Pharmacology, Graduate School of Medicine and Pharmaceutical Sciences, and <sup>2</sup>21st COE Program, University of Toyama, 2630 Sugitani, Toyama 930-0194, Japan<sup>3</sup>Department of Pharmacology, Faculty of Pharmaceutical Sciences, Ohu University, Koriyama 963-8611, Japan

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**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 B<sub>4</sub> antagonist ONO-4057, the cysteinyl leukotriene antagonist pranluceast, the leukotriene D<sub>4</sub> antagonist MK-571, the platelet-activating factor antagonist CV-3988, the nitric oxide synthase inhibitor N<sup>G</sup>-nitro-L-arginine methyl ester, the H<sub>2</sub> histamine-receptor antagonist cimetidine, the H<sub>1</sub> histamine-receptor antagonist terfenadine plus cimetidine, and cypheptadine that blocks the 5-HT<sub>1/2</sub> 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<sup>+</sup>-induced increase in intracellular Ca<sup>2+</sup> concentration in cultured dorsal root ganglion neurons. The results suggest that 5-lipoxygenase metabolite(s) other than leukotriene B<sub>4</sub> 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 wheal 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 X<sub>1</sub>, Aed a X<sub>2</sub>, 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 H<sub>1</sub> 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

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elicited by an intradermal injection of ESGM are not affected by the H<sub>1</sub> histamine-receptor antagonist terfenadine in sensitized mice, they are significantly inhibited by another H<sub>1</sub> histamine-receptor antagonist azelastine (5). Azelastine suppresses the high K<sup>+</sup>-induced increase of intracellular Ca<sup>2+</sup> 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 B<sub>4</sub>, a metabolite formed by 5-lipoxygenase, and less efficiently inhibits the production of leukotriene B<sub>4</sub> in the skin induced by an intradermal injection of substance P (6). Leukotriene B<sub>4</sub> is pruritogenic and is responsible for substance P-induced scratching in mice (7, 8). These findings raise the possibility that leukotriene B<sub>4</sub> is involved in itching of mosquito allergy. Thus, this study was carried out to determine whether 5-lipoxygenase metabolites including leukotriene B<sub>4</sub> 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%  $\gamma$ -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), N<sup>G</sup>-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 N<sup>G</sup>-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), pranlukast (8), MK-571 (12), CV-3988 (13), N<sup>G</sup>-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  $\mu$ g/site and 50  $\mu$ l, respectively.

In our preliminary experiments, ESGM was separated by sodium dodecylsulfate 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  $\alpha$ -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 $\text{Ca}^{2+}$ concentration

The preparation of dorsal root ganglion neurons and the determination of intracellular  $\text{Ca}^{2+}$  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  $\mu\text{g}/\text{ml}$  streptomycin, and 8  $\mu\text{M}$  arabinosylcytosine for one day. Primary cultures of neurons were loaded with fluo-3 acetoxymethyl ester and the concentration of intracellular free  $\text{Ca}^{2+}$  ions in the nerve cells ( $<25 \mu\text{m}$ ) 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 (Fig. 1C). Other series of experiments showed that zileuton (10 – 100 mg/kg) produced a dose-dependent inhibition of 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 activator protein inhibitor MK-886 (1, 3, and 10 mg/kg, i.v.;

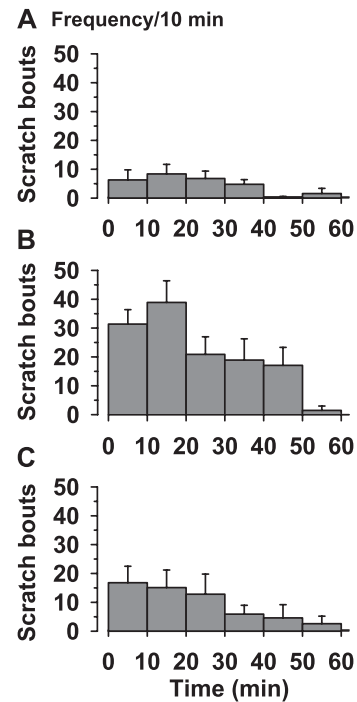


**Fig. 1.** Scratching response to an intradermal injection of an extract of mosquito salivary gland in mice and its inhibition by zileuton. A: Scratching following the extract injection in naive mice. B: Scratching following the extract injection in sensitized mice. C: Scratching following the extract injection in sensitized mice given zileuton. See the text for sensitizing procedures. The extract was injected into the rostral back for challenge and scratch bouts were counted. Zileuton (100 mg/kg) was administered orally 1 h before challenge. Values represent the means and S.E.M. of eight animals.

–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 prostanoid receptor antagonist SQ-29548 (2 mg/kg, i.v.; -15 min), the leukotriene  $\text{B}_4$  antagonist ONO-4057 (30 and 100 mg/kg, p.o.; -1 h), the cysteinyl leukotriene antagonist pranlukast (30 and 100 mg/kg, p.o.; -1 h), the leukotriene  $\text{D}_4$  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  $\text{N}^G$ -nitro-L-arginine methyl ester (10 mg/kg, i.v.; -15 min), the  $\text{H}_2$  histamine-receptor antagonist cimetidine (30 mg/kg, p.o.; -30 min), cimetidine (30 mg/kg) plus the  $\text{H}_1$  histamine-receptor antagonist terfenadine (30 mg/kg, p.o.; -30 min), and cyproheptadine (1 mg/kg, p.o.; -1 h) that blocks the  $\text{H}_1$  histamine receptor and 5-HT $_{1/2}$  serotonin receptors.

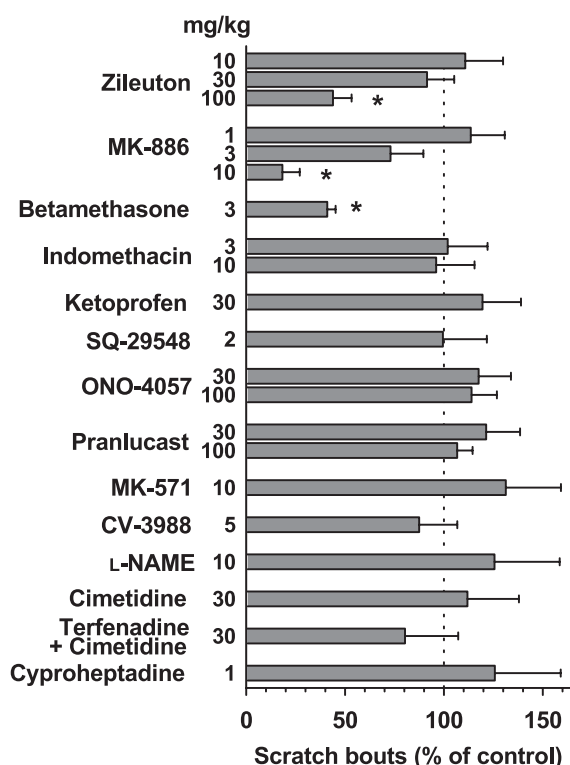


**Fig. 2.** Effects of 14 agents on scratching elicited by an extract of mosquito salivary gland in sensitized mice. See the text for sensitizing procedures. The extract was injected into the rostral back for challenge and scratch bouts were counted for 1 h. Agents (route, pretreatment time) were as follows: Indomethacin (p.o., -30 min), ketoprofen (s.c., -30 min), SQ-29548 (i.v., -15 min), betamethasone 17-valerate (p.o., -1 h), zileuton (p.o., -1 h), MK-886 (i.v., -15 min), ONO-4057 (p.o., -1 h), pranlu cast (p.o., -1 h), MK-571 (p.o., -1 h), CV-3988 (i.v., -5 min), *N*<sup>G</sup>-nitro-L-arginine methyl ester (i.v., -15 min), cimetidine (p.o., -30 min), terfenadine (p.o., -30 min) + cimetidine (p.o., -30 min), and cyproheptadine (p.o., -1 h). Data are presented as percentage of the number of scratch bouts, as compared with the value of the corresponding vehicle control. Values represent the means and S.E.M. of eight animals. \**P* < 0.05 vs corresponding control (Student's *t*-test or Dunnett's multiple comparisons).

#### 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

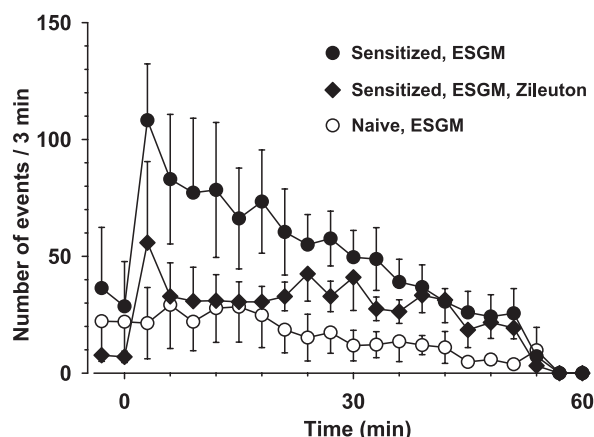


**Fig. 3.** Effects of zileuton on the increased firing of cutaneous nerve branch following an injection of an extract of the salivary gland of mosquito (ESGM) in sensitized mice. See the text for sensitizing procedures. An intradermal injection of the extract increased nerve activity in sensitized, but not naive, mice. Zileuton (100 mg/kg, -1 h) was administered orally 1 h before ESGM injection. Values are expressed as means  $\pm$  S.E.M. of eight animals. Two-way repeated measures analysis of variance (sensitized groups): main effect of zileuton,  $F(1, 9) = 5.45$ ,  $P < 0.05$ .

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. The response almost subsided by 50 min after challenge, which was similar to the duration of ESGM-elicited scratching. Therefore, the evoked nerve activity may be associated to the scratching. Zileuton markedly suppressed the evoked

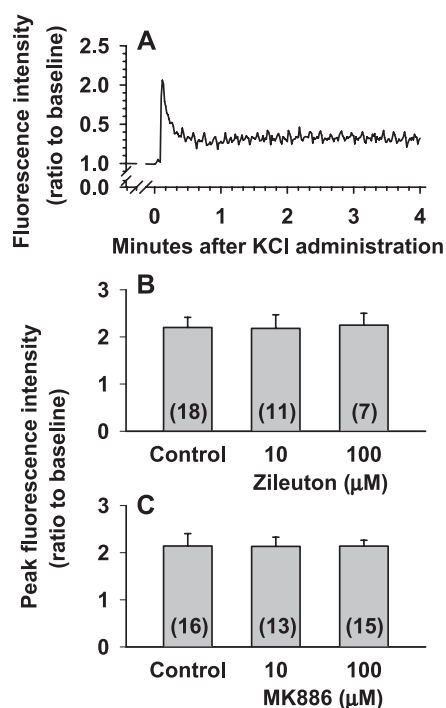


**Fig. 4.** Effects of zileuton and MK-886 on high  $K^+$ -induced increase in intracellular  $Ca^{2+}$  ion concentration in mouse dorsal root ganglion neurons. Primary cultures of the neurons pre-loaded with fluo-3 were stimulated with the increase of extracellular concentration of KCl up to 30 mM. A: Typical example of changes in intracellular  $Ca^{2+}$  ion 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.

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, although dexamethasone, another glucocorticoid, has been shown not to suppress the scratching (5). Dexamethasone inhibits the production of leukotriene  $B_4$  induced by substance P in skin in vivo and cultured keratinocytes in vitro (8). An intradermal injection of leukotriene  $B_4$  at relatively low doses elicits scratching (7), and the leukotriene  $B_4$  antagonist suppresses scratching induced by intradermal injections of substance P and nociceptin (8, 19). Azelastine suppresses scratching elicited by ESGM in sensitized mice

(5) and by leukotriene  $B_4$  in healthy mice (6). In conjunction with these findings, the above results raise the possibility that leukotriene  $B_4$  is involved in the ESGM-elicited scratching. However, the ESGM-elicited scratching was not affected by the leukotriene  $B_4$  antagonist ONO-4057 at doses that had been shown to suppress scratching induced by intradermal injections of substance P (8) and nociceptin (19). Therefore, the result suggests that leukotriene  $B_4$  is not involved in the ESGM-elicited scratching. The inhibitory action of azelastine on the leukotriene  $B_4$  receptor and leukotriene  $B_4$  production may not play a role in its suppression of the ESGM-elicited scratching.

Azelastine inhibits  $K^+$ -induced increase in the intracellular  $Ca^{2+}$  concentration in cultured dorsal root ganglion neurons, which may play a role in the inhibitory effect of azelastine on mosquito allergy-associated itching (5). On the other hand, zileuton and MK-886 did not affect the  $K^+$ -induced increase of intracellular  $Ca^{2+}$  concentration, suggesting the lack of inhibitory action of these agents on the primary sensory neurons.

MK-866 has been shown not to affect scratching elicited by an intradermal injection of compound 48/80 in BALB/c mice (20). In this strain of mice, compound 48/80 releases histamine from mast cells to induce plasma extravasation, but mast cell mediators including histamine do not elicit scratching (20). Compound 48/80 not only degranulates mast cells but also acts on primary afferents directly (21). The direct action on primary afferents may play an important role in the scratch-eliciting effect of compound 48/80 in this strain of mice. The lack of inhibitory action on the primary afferents may be a cause of the lack of suppressive effect of MK-886 on compound 48/80-elicited scratching.

The ESGM-elicited scratching was not suppressed by the cysteinyl leukotriene antagonist pranlukast and the leukotriene  $D_4$  antagonist MK-571. Intradermal injections of leukotriene  $C_4$  and  $D_4$  at doses of 0.012–0.038 nmol/site are not pruritogenic in humans (22), and leukotriene  $D_4$  at doses of 0.01–0.3 nmol/site does not elicit scratching in mice (8). Therefore, it is suggested that cysteinyl leukotrienes are not involved in the ESGM-elicited scratching. Taken together, the present results suggest that 5-lipoxygenase metabolite(s) other than leukotriene  $B_4$  and cyteinyl 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

mediated by cysteinyl 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<sub>4</sub> 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<sub>2</sub> is a weak pruritogen (28) and enhances serotonin-induced itching in healthy humans (26). In mice, intradermal injection of prostaglandin E<sub>2</sub> does not elicit scratching (7), but intradermal injection of the TP thromboxane A<sub>2</sub>-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 prostanoids 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 involvement of prostanoids 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 involvement of platelet-activating factor in mosquito allergy-associated itching.

Repeated application of the nitric oxide synthase inhibitor *N*<sup>G</sup>-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*<sup>G</sup>-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<sub>1</sub> histamine-receptor antagonist and H<sub>2</sub>-receptor antagonist and the suppression is potentiated by combina-

tion of the agents (38). Scratching elicited by passive cutaneous anaphylaxis is also suppressed by an H<sub>1</sub>-receptor antagonist and H<sub>2</sub>-receptor antagonist, but the suppression is not potentiated by combination of the agents (38). On the other hand, the ESGM-elicited scratching is not suppressed by the H<sub>1</sub>-receptor antagonist terfenadine (5). Therefore, this study examined the effects of H<sub>2</sub>-receptor antagonist alone and the combination of H<sub>1</sub>- and H<sub>2</sub>-receptor antagonists on the ESGM-elicited scratching. The H<sub>2</sub>-receptor antagonist cimetidine alone and its combination with terfenadine did not affect the ESGM-elicited scratching. The results support the idea that histamine does not play an important role in mosquito allergy-associated itching.

Serotonin is a weak pruritogen in human subjects (26) and strongly elicits scratching through 5-HT<sub>2</sub> receptors in mice and rats (16, 39). The ESGM-elicited scratching was not suppressed by cyproheptadine that blocks the 5-HT<sub>2</sub> receptor, suggesting that serotonin is not involved in mosquito allergy-associated itching.

In conclusion, a 5-lipoxygenase metabolite(s) other than leukotriene B<sub>4</sub> and cysteinyl leukotrienes may be involved in mosquito allergy-associated itching. Many itch mediators released from mast cells and keratinocytes may not be responsible for mosquito allergy-associated itching.

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