

Preliminary report

Effect of loratadine on mouse models of atopic dermatitis associated pruritus

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Abstract

To confirm the effectiveness of loratadine for relieving pruritus in atopic dermatitis, we examined the effect of this drug using animal models of atopic dermatitis associated pruritus in ICR and hairless mice. As for the results, in ICR mice, single oral administration of loratadine at a dose of 5 or 10 mg/kg significantly inhibited the dorsal scratching behavior induced by histamine or an antigen, and the effect of loratadine was more potent than that of fexofenadine and chlorpheniramine. In hairless mice, oral administration of loratadine at a dose of 10 mg/kg for 6 days significantly inhibited the facial scratching behavior induced by the feeding of a low magnesium diet. Furthermore, oral administration of loratadine at a dose of 10 mg/kg for 7 days also significantly inhibited the histamine-induced scratching behavior in the same animals. These results indicate that loratadine may be effective in preventing pruritus associated with atopic dermatitis.

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1. Introduction

Atopic dermatitis is a condition manifesting eczema, serous papules, scaling and crust. In severe cases, erosion and infiltration were also observed [1]. It is known that atopic dermatitis generally produces

severe itching of the skin, and repeated scratching further aggravates the skin symptoms [1]. Histamine has been identified as one of the substances causing itching [2]. Therefore, histamine H₁-antagonists are used widely in the treatment of atopic dermatitis [1,3]. Loratadine is a drug with selective histamine H₁ blocking action, and is effective by once daily administration. It has been also reported that loratadine has superior long-acting and non-sedative properties [4]. Currently, the clinical application of

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loratadine is limited to allergic rhinitis and itching associated with skin diseases (eczema, dermatitis) and urticaria. It is generally recognized that H_1 -antagonists are effective toward pruritus associated with skin diseases, though these effects are not always sufficient. As for loratadine, however, no data are available to show whether the drug is effective in the atopic dermatitis model in animals. With the objective of confirming the effectiveness of loratadine for relieving pruritus in atopic dermatitis, the present study examined the efficacy of loratadine in various mouse models of atopic dermatitis associated pruritus.

2. Materials and methods

2.1. Experimental animals

Female ICR mice (6–8-weeks-old; Japan SLC, Shizuoka) and female Hos:HR-1 strain hairless mice (4-weeks-old; Hoshino Laboratory Animals, Saitama, Japan) were used in this study. The animals were kept under conventional animal housing conditions at room temperature ($24 \pm 2^\circ\text{C}$), a humidity of $55 \pm 15\%$, and a 12-h light and darkness cycle. The ICR mice were fed a commercial MF diet (Oriental Yeast, Tokyo, Japan) while the hairless mice were fed a modified AIN-93G diet with low magnesium content (Oriental Yeast, Table 1). Drinking water was supplied ad libitum.

2.2. Drugs and reagents

Loratadine (provided by Schering-Plough K.K.), fexofenadine hydrochloride (Aventis Pharma, Tokyo, Japan), and D-chlorpheniramine maleate (Schering-Plough K.K.) were suspended in a 5% Arabic gum solution (Kokusan Chemical, Tokyo, Japan). Histamine dihydrochloride (Sigma-Aldrich, St Louis, MO, USA), mouse anti-dinitrophenyl immunoglobulin E (DNP-IgE) monoclonal antibody (Sigma-Aldrich) and dinitrophenyl-ovalbumin (DNP-OVA; LSL, Tokyo, Japan) were used.

2.3. Histamine-induced dorsal scratching behavior in ICR mice

ICR mice were injected intradermally in the back with histamine (100 nmol/site), and scratching behavior

Table 1

Composition of low magnesium diet

Composition of diet	Corn starch	397.486 g
	Casein	200.000 g
	α Corn starch	132.000 g
	Sucrose	100.000 g
	Soybean oil	70.000 g
	Mineral	35.000 g
	Vitamin	10.000 g
	L-Cystine	3.000 g
	Choline bitartrate	2.500 g
	Tert-butylhydroquinone	0.014 g
	Fiber	50.000 g
	Sum	1000.000 g
	Mineral (/100 g)	
	CaCO_3	35 700.000 mg
Mineral (/100 g)	KH_2PO_4	19 600.000 mg
	$\text{K}_3\text{C}_6\text{H}_5\text{O}_7 \cdot \text{H}_2\text{O}$	7078.000 mg
	NaCl	7400.000 mg
	K_2SO_4	4660.000 mg
	$\text{FeC}_6\text{H}_5\text{O}_7 \cdot x\text{H}_2\text{O}$	606.000 mg
	ZnCO_3	165.000 mg
	MnCO_3	63.000 mg
	$\text{CuCO}_3, \text{Cu}(\text{OH})_2 \cdot \text{H}_2\text{O}$	32.400 mg
	KIO_3	1.000 mg
	Na_2SeO_4	1.025 mg
	$(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$	0.795 mg
	$\text{Na}_2\text{SiO}_3 \cdot 9\text{H}_2\text{O}$	145.000 mg
	$\text{CrK}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$	27.500 mg
	LiCl	1.740 mg
	H_3BO_3	8.150 mg
	NaF	6.350 mg
	$\text{NiCO}_3, 2\text{Ni}(\text{OH})_2 \cdot 4\text{H}_2\text{O}$	3.060 mg
	NH_4VO_3	0.660 mg
	Powdered sucrose	24 500.320 mg
	Sum	100 000.000 mg
Vitamin (/100 g)	Vitamin A (palmitate) (500,000 IU/g)	80.00 mg
	Vitamin D ₃ (400,000 IU/g)	25.00 mg
	Vitamin E (50%)	1500.00 mg
	Vitamin B ₁ (hydrochloride)	60.00 mg
	Vitamin B ₂	60.00 mg
	Vitamin B ₆ (hydrochloride)	70.00 mg
	Vitamin B ₁₂ (0.1%)	250.00 mg
	Vitamin K ₁	7.50 mg
	D-Biotin	2.00 mg
	Folic acid	20.00 mg
	Ca pantothenate	160.00 mg
	Nicotinic acid	300.00 mg
	Powdered sucrose	97 465.50 mg
	Sum	100 000.00 mg

was observed for 60 min using a Micro Act (automatic itch measuring device, Neuroscience, Tokyo, Japan). Drugs were administered orally 2 h before the histamine injection.

2.4. Antigen-induced dorsal scratching behavior in ICR mice

ICR mice were sensitized with an intradermal injection of 0.05 mg/ml of mouse anti-DNP IgE monoclonal antibody dissolved in physiological saline, in the back at 30 μ l/site. After 24 h, 0.5 mg/ml of DNP-OVA was injected intravenously as the antigen at 10 ml/kg, and the elicited scratching behavior was measured for 60 min using the Micro Act. Drugs were administered orally, 2 hr before the antigen injection.

2.5. Facial scratching and histamine-induced dorsal scratching behaviors in hairless mice

The hairless mice were fed a low magnesium diet (Table 1) for 50 days. From the following day (Day 1), the drugs were administered orally once daily for 7 days. Two hours after the Day 6 drug administration, facial scratching behavior was observed visually for 60 min. Two hours after drug administration on Day 7, histamine at 100 nmol/site was injected intradermally in the back, and the dorsal scratching behavior was observed visually for 60 min.

2.6. Statistical analysis

All experimental data are shown as the mean $\pm$ S.E.M. The data were analyzed by Student's *t* test or Dunnett's test. A *p*-value less than 0.05 was considered significant.

3. Results

3.1. Effect on histamine-induced dorsal scratching behavior in ICR mice

As shown in Fig. 1, in the control group, following the intradermal injection of histamine at 100 nmol/site, the number of scratches observed during the 60 min period was 66.8 ± 18.4 ($n=10$). Loratadine reduced the number of scratches in a dose-dependent manner, and a significant effect was observed at doses of 5 and 10 mg/kg. On the other hand, fexofenadine and chlorpheniramine showed no significant inhibitory effects, even at 10 and 20 mg/kg, respectively.

3.2. Effect on antigen-induced dorsal scratching behavior in ICR mice

As shown in Fig. 2, loratadine at doses of 2, 5 and 10 mg/kg inhibited the antigen-induced dorsal scratching behavior in a dose dependent manner, and a significant reduction was observed at a dose of 10 mg/kg. Fexofenadine showed no significant inhibitory effect, even at a dose of 10 mg/kg. Chlorpheniramine apparently inhibited scratching at doses of 10 and 20 mg/kg, but no significant differences were observed, even at a dose of 20 mg/kg.

3.3. Effect on facial scratching and histamine-induced scratching behavior in hairless mice

In the hairless mice, the 56-day feeding of a low magnesium diet significantly increased the number of

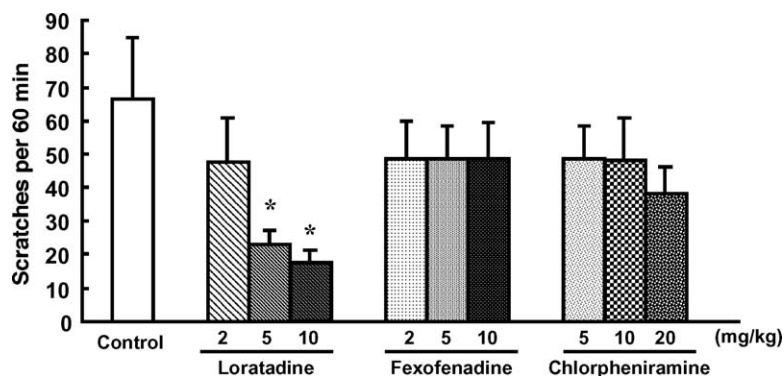


Fig. 1. Effects of H_1 -antagonists on dorsal scratching behavior induced by histamine in ICR mice. Each column and vertical bar represents the mean $\pm$ S.E.M. ($n=8-10$). *Significantly different from the sensitized control group with $P<0.05$ (Dunnett's test).

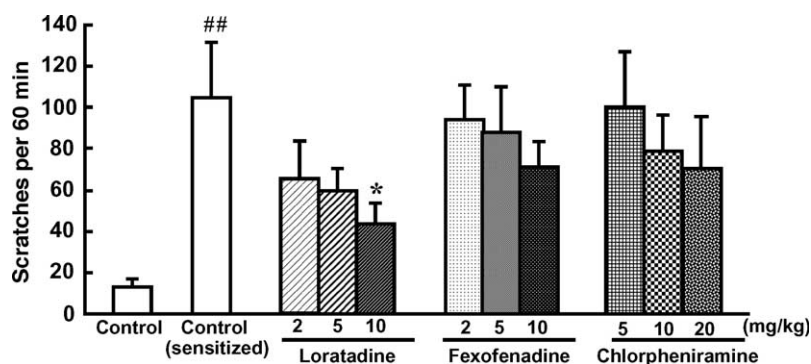


Fig. 2. Effects of H₁-antagonists on dorsal scratching behavior induced by an antigen in passive sensitized ICR mice. Each column and vertical bar represents the mean $\pm$ S.E.M. ($n=7-10$). ##Significantly different from the control group with $P<0.01$ (Student's t test). *Significantly different from the control (sensitized) group with $P<0.05$ (Dunnett's test).

facial scratches (30.6 ± 4.8 , $n=8$) compared with the group fed a standard diet (14.0 ± 3.9 , $n=6$). On the other hand, following the administration of 5 and 10 mg/kg of loratadine once daily for 6 days, a dose-dependent inhibition of facial scratching was observed, and a significant effect was observed at a dose of 10 mg/kg (Fig. 3). Furthermore, loratadine administration (the same dose as above) on Day 7 resulted in an inhibition of histamine-induced scratching behavior in a dose-dependent manner, and a significant inhibition was observed at a dose of 10 mg/kg (Fig. 4).

4. Discussion

The present study demonstrated that in ICR mice, loratadine at doses of 5 and 10 mg/kg significantly

inhibited histamine-induced dorsal scratching behavior in a dose-dependent manner, and the drug also significantly inhibited the antigen-induced scratching behavior at a dose of 10 mg/kg. In contrast, fexofenadine and chlorpheniramine showed no significant inhibition on both histamine- and antigen-induced scratching behavior even at doses of 10 and 20 mg/kg, respectively. ICR mice are considered to be high responders to pruritogens such as histamine, serotonin and substance P. They exhibit substantial skin responses when compared to other strains [5]. In mice, histamine-induced scratching behavior is thought to be associated with histamine H₁ and H₂ receptors and chemical mediators other than histamine [6–8]. Loratadine is a selective H₁-antagonist, and thus the inhibition of scratching behavior in the ICR mice observed in this study was considered to be due to histamine H₁-antagonistic activity. Human studies

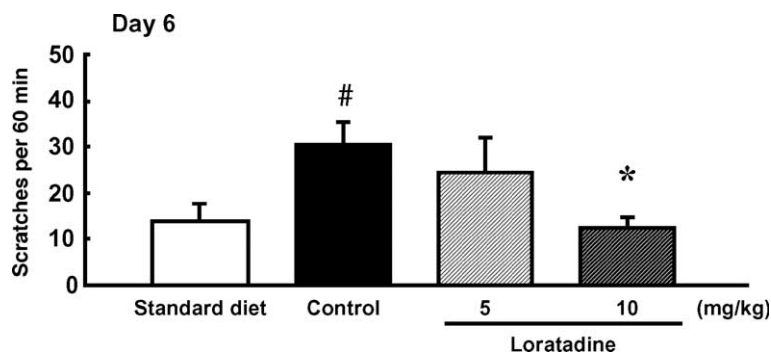


Fig. 3. Effect of loratadine on low magnesium diet-induced facial scratching behavior in hairless mice. Each column and vertical bar represents the mean $\pm$ S.E.M. ($n=6-8$). #Significantly different from the standard diet control group with $P<0.05$ (Student's t test) *Significantly different from the control group with $P<0.05$ (Dunnett's test).

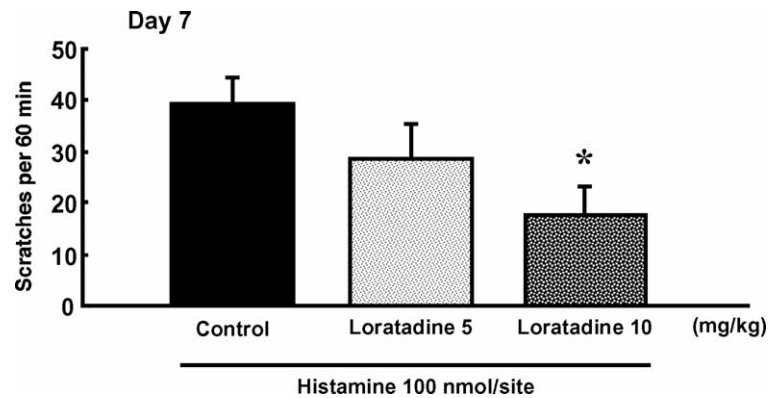


Fig. 4. Effect of loratadine on histamine-induced dorsal scratching behavior in low magnesium diet-fed hairless mice. Each column and vertical bar represents the mean $\pm$ S.E.M. ($n=6-7$). *Significantly different from the control group with $P<0.05$ (Dunnett's test).

indicated that loratadine showed a rapid onset of action in suppressing a histamine-induced wheal [9]. In addition, a histamine-induced bronchial response was inhibited by consecutive administrations of loratadine and cetirizine [10].

The present study demonstrated a significant increase in facial scratching behavior after the feeding of a low magnesium diet to hairless mice for 50 days. Similarly, erosion and excessive scratching behavior of weak areas of the body, such as the face, neck and chest, have also been observed by Hikita et al. [11] and Watanabe et al. [12] in DS-Nh hairless mice. In this mouse model, the oral administration of loratadine for 6 days significantly inhibited facial scratching behavior at a dose of 10 mg/kg. In addition, when histamine was injected to induce dorsal scratching behavior in these animals, oral administration of loratadine at a dose of 10 mg/kg for 7 days significantly inhibited this response.

Itching is a symptom observed in all patients with atopic dermatitis, and is a major characteristic of the disease [1]. In humans, histamine is known to be a provoker of the itch, and intradermal injection induces pruritus [13]. As shown in the present study, the intradermal injection of histamine to mice induces scratching behavior through the H_1 receptor [8]. On the other hand, substances other than histamine such as serotonin, proteases, kinin, neuropeptides and opioids [13] have been reported to be associated with the induction of pruritus. Klein and Clark [14] reported that although H_1 -antagonists are often used in the treatment of atopic dermatitis, little objective

evidence exists to demonstrate the relief of pruritus. However, a new selective H_1 -antagonist, fexofenadine, has been shown to inhibit pruritus associated with atopic dermatitis in a large-scale double-blind placebo-controlled comparative study, providing evidence that histamine is involved, at least in part, in causing pruritus in atopic dermatitis [15]. As shown in the text, only weak inhibition was observed with fexofenadine, even at a dose of 10 mg/kg, in antigen-induced scratching behavior. On the other hand, loratadine is effective in some animal models of atopic dermatitis at a dose of 5 or 10 mg/kg. Furthermore, Inoue et al. [16] have reported that the primary structure of the mouse histamine H_1 receptor exhibits common properties with those of the bovine, rat, guinea pig and human receptors; being the homology with the human receptor 76%. From these findings, therefore, loratadine may be effective in relieving pruritus associated with atopic dermatitis.

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