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European Journal of Pharmacology 470 (2003) 113–116
www.elsevier.com/locate/ejphar

Short communication

Evaluation of the effects of anti-pruritic drugs on scratch responses using histamine H_1 receptor-deficient mice

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Received 24 April 2003; accepted 29 April 2003

Abstract

The effects of anti-pruritic drugs on scratching behavior associated with passive cutaneous anaphylaxis in histamine H_1 receptor-deficient and wild-type mice were studied. Passive sensitization with mouse monoclonal anti-dinitrophenyl-immunoglobulin E (IgE) resulted in an increase in the incidence of scratching behavior induced by intravenous injection of dinitrophenyl-ovalbumin in both wild-type and histamine H_1 receptor-deficient mice. The histamine H_1 receptor antagonist diphenhydramine inhibited scratching behavior induced by antigen in passively sensitized wild-type mice, whereas no effect was observed in histamine H_1 receptor-deficient mice. On the other hand, oxatomide inhibited scratching behavior in both mice, although the effect in wild-type mice was more potent than that in histamine H_1 receptor-deficient mice. Tranilast inhibited scratching behavior with the same potency in both mice. We concluded that the scratching behavior associated with passive cutaneous anaphylaxis involves not only histamine H_1 receptors but also other chemical mediators. Furthermore, the results of the present study indicated that oxatomide has an antagonistic effect on histamine H_1 receptors as well as anti-pruritic effect in vivo.

Keywords: Scratching behavior; Histamine H_1 receptor; Histamine H_1 receptor antagonist; Anti-allergic drug

1. Introduction

It is widely suggested that atopic dermatitis is a skin disease associated with itching, i.e. a sensation causing the urge to scratch. Although pruritus is the main symptom of atopic dermatitis, its underlying mechanisms remain unknown. It is well known that atopic dermatitis is the disturbances against the immune system characterized by increases in the immunoglobulin E (IgE) synthesis and in the mediators release from mast cells and basophils. Therefore, it seems likely that the IgE-dependent allergic cutaneous reactions are suitable animal model for investigating the mechanism of allergic dermatitis. Histamine H_1 receptor antagonists and anti-allergic drugs are often used for treatment of the itching caused by atopic dermatitis. However, the efficacy of these drugs also remains obscure. Previously, we reported that the scratching behavior induced by compound 48/80, a typical inducer of histamine release from mast cells, was inhibited by anti-allergic drugs including histamine H_1 receptor antagonists in mice (Sugimoto et al., 1998). These drugs can be divided into three groups; that is, classical histamine H_1 receptor antagonists (diphenhydramine and chlorpheniramine), histamine H_1 receptor antagonists that inhibit mast cell degranulation (oxatomide and azelastine), and anti-allergic drugs having anti-allergic activity defined as the inhibition of chemical mediators release from mast cells and basophils without histamine H_1 receptor antagonistic activity (tranilast and cromolyn sodium). In vitro, the drugs classified into the second group show inhibitory effects on release of chemical mediators from mast cells. Therefore, these drugs are generally accepted to have anti-allergic activity. However, it is very difficult to demonstrate the anti-allergic activity of these drugs in vivo using normal animals, because anti-allergic drugs have potent histamine H_1 receptor antagonistic activity.

The recently developed histamine H_1 receptor-deficient mouse has been used in a number of studies (Inoue et al.,

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0014-2999/03/$ - see front matter © 2003 Elsevier Science B.V. All rights reserved.
doi:10.1016/S0014-2999(03)01786-2

1996; Nakahara et al., 2000; Kayasuga et al., 2002). Using this mouse model, the involvement of the histamine H_1 receptors in several allergic reactions has been clarified (Nakahara et al., 2000; Kayasuga et al., 2002). Therefore, the present study was performed to clarify the role of histamine H_1 receptors in scratching behavior associated with passive cutaneous anaphylaxis, and the anti-pruritic effects of three types of drugs on this behavior were also studied using histamine H_1 receptor-deficient mice.

2. Materials and methods

2.1. Animals

Histamine H_1 receptor-deficient mice were generated by homologous recombination as previously described (Inoue et al., 1996). Mutant mice have been back-crossed to C57BL/6 mice. Female mutant and wild-type mice weighing 25–30 g were used for experiments. Animals were housed in an air-conditioned room maintained at 22–26 °C with humidity of 40–70%. Mice were given standard laboratory rodent chow and water ad libitum. All procedures involving animals were conducted in accordance with the guidelines of the Animal Care and Use Committee, Faculty of Pharmaceutical Sciences, Okayama University.

2.2. Chemicals

Mouse monoclonal anti-dinitrophenyl-IgE, dinitrophenyl-ovalbumin and diphenhydramine were purchased from Sigma, St. Louis, MO, USA. Oxatomide was provided from Kyowa Hakko Kogyo, Shizuoka, Japan. Tranilast was obtained from Kissei Pharmaceuticals, Nagano, Japan.

2.3. Scratching behavior associated with passive cutaneous anaphylaxis

The histamine H_1 receptor-deficient and wild-type mice were given an intradermal injection of 20 μl of saline with or without 1 μg of mouse monoclonal anti-dinitrophenyl-IgE into the rostral part of the back. Twenty-four hours later, the animals were put into an observation cage (32 × 22 × 10 cm) for about 10 min for acclimatization. Passive cutaneous anaphylaxis was elicited by intravenous injection of 100 μl of dinitrophenyl-ovalbumin saline solution. Immediately after injection, the animals were put into the observation cage (one animal/cage) and scratching behavior at the site of antibody injection was measured for 60 min. Scratching behavior was observed in accordance with the method of Kuraishi et al. (1995); the number of incidences of scratching behavior by the hind paws was counted. Diphenhydramine, oxatomide and tranilast were administered orally 1 h before antigen injection.

2.4. Statistical analysis

All data are presented as means ± S.E.M. Statistical analysis was performed using one-way analysis of variance with Dunnett's test. A probability value of less than 0.05 was considered statistically significant.

3. Results

3.1. Scratching behavior associated with passive cutaneous anaphylaxis in wild-type and histamine H_1 receptor-deficient mice

As shown in Fig. 1, the intravenous injection of dinitrophenyl-ovalbumin solution dose-dependently elicited scratching behavior in wild-type and histamine H_1 receptor-deficient mice. No measurable changes were found after intravenous injection of saline alone in either group of mice. Dinitrophenyl-ovalbumin at doses of 3 and 10 μg/mouse caused a significant increase in the incidence of scratching behavior in both groups. The incidence of scratching behavior in wild-type mice was higher than that in histamine H_1 receptor-deficient mice. A significant difference was observed between wild-type and histamine H_1 receptor-deficient mice when dinitrophenyl-ovalbumin was injected at a dose of 10 μg/mouse.

3.2. Effects of drugs on scratching behavior associated with passive cutaneous anaphylaxis in wild-type and histamine H_1 receptor-deficient mice

The effects of drugs on scratching behavior associated with passive cutaneous anaphylaxis are shown in Table 1. Diphenhydramine showed dose-related inhibition of the

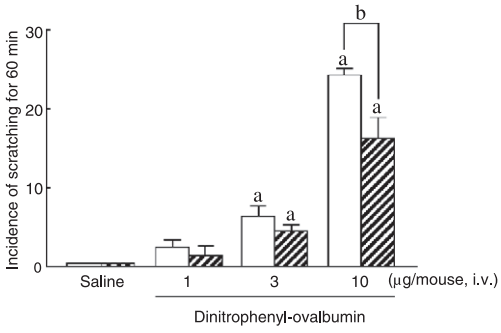

Fig. 1. Scratching behavior associated with passive cutaneous anaphylaxis in wild-type and histamine H_1 receptor-deficient mice. Wild-type mice (open columns) and histamine H_1 receptor-deficient mice (hatched columns) were sensitized by intradermal injection of monoclonal anti-dinitrophenyl-immunoglobulin E (1 μg) into the rostral part of the back, and 24 h later dinitrophenyl-ovalbumin saline solution was injected intravenously. After antigen injection, scratching behavior was monitored for 60 min. Each column and vertical bar represents the mean ± S.E.M. of 10 mice. [a]: Significantly different from saline-treated mice at $P<0.01$. [b]: Significantly different from wild-type mice at $P<0.05$.

Table 1
Effects of drugs on scratching behavior associated with passive cutaneous anaphylaxis in wild-type and histamine H_1 receptor-deficient mice

Drugs	Dose (mg/kg, p.o.)	Wild-type mice		Histamine H_1 receptor-deficient mice	
		Scratching	(% Control)	Scratching	(% Control)
Vehicle	–	24.9 ± 1.1	(100.0)	18.8 ± 1.8	(100.0)
Diphenhydramine	1	20.4 ± 2.0	(81.9)	18.3 ± 2.9	(97.3)
	3	17.9 ± 2.2[a]	(71.9)	18.0 ± 1.8	(95.7)
	10	15.5 ± 1.8[a]	(62.2)	18.7 ± 2.6	(99.5)
Oxatomide	3	23.6 ± 1.2	(94.8)	18.3 ± 2.2	(97.3)
	10	18.3 ± 2.2[a]	(73.5)	15.2 ± 2.1	(80.9)
	30	15.9 ± 2.2[b]	(63.9)	10.7 ± 2.0[a]	(56.9)
Tranilast	30	23.5 ± 2.0	(94.4)	18.5 ± 1.8	(98.4)
	100	20.7 ± 2.3	(83.1)	16.9 ± 1.8	(89.9)
	300	14.5 ± 1.4[b]	(58.2)	10.1 ± 1.5[b]	(53.7)

Mice were sensitized by injection of monoclonal anti-dinitrophenyl-immunoglobulin E (1 μg), and 24 h later dinitrophenyl-ovalbumin (10 μg/mouse) was injected intravenously. Scratching behavior was monitored for 60 min. Mice were given drugs (diphenhydramine, oxatomide and tranilast) orally 1 h before antigen injection.

Each value represents the mean ± S.E.M. of 10 mice.

[a] Significantly different from vehicle-treated mice at $P < 0.05$.

[b] Significantly different from vehicle-treated mice at $P < 0.01$.

scratching behavior and a significant effect was observed at doses higher than 3 mg/kg in wild-type mice, whereas no significant difference was observed in histamine H_1 receptor-deficient mice even at a dose of 10 mg/kg. Oxatomide inhibited antigen-induced scratching behavior similarly to diphenhydramine; a significant effect was observed at doses of 10 and 30 mg/kg in wild-type mice. In histamine H_1 receptor-deficient mice, oxatomide caused an inhibitory effect on this behavior, but the potency was less than that in wild-type mice. Tranilast at a dose of 300 mg/kg showed an inhibitory effect on the scratching behavior in both wild-type and histamine H_1 receptor-deficient mice. The potency in both mice groups was almost the same.

4. Discussion

In the present study, we found that scratching behavior was induced by IgE-mediated passive cutaneous anaphylaxis in not only wild-type but also in histamine H_1 receptor-deficient mice. The incidence of scratching behavior in histamine H_1 receptor-deficient mice was less than that in wild-type mice, and a significant difference was observed when dinitrophenyl-ovalbumin was injected intravenously at 10 μg/mouse. Previously, we reported that when histamine was injected into the back of histamine H_1 receptor-deficient mice, no scratching behavior was observed (Sugimoto et al., 1999). However, significant scratching behavior associated with passive cutaneous anaphylaxis was observed in histamine H_1 receptor-deficient mice. Similar findings were observed when compound 48/80 was injected into the back of the histamine H_1 receptor-deficient mice (Sugimoto et al., 1999). These results suggested that some chemical mediators other than histamine were released by dinitrophenyl-ovalbumin challenge in sensitized histamine H_1 receptor-deficient mice, and chemical mediators such as substance P and leukotriene B_4 (LTB_4) may cause scratching behavior. Andoh and Kuraishi (1998) and Andoh et al. (1998) reported that both substance P and LTB_4 caused frequent scratching behavior in mice. Such scratching behavior associated with passive cutaneous anaphylaxis in histamine H_1 receptor-deficient mice may be useful as an animal model for evaluation of the effect of anti-pruritic drugs with histamine H_1 receptor antagonistic activity.

Diphenhydramine has potent histamine H_1 receptor antagonistic activity, but the drug showed no inhibitory effect on chemical mediator release from mast cells. On the other hand, oxatomide showed both histamine H_1 receptor antagonistic and anti-allergic activities that defined the inhibition of chemical mediator release from mast cells in vitro (Awouters et al., 1977; Church and Gradidge, 1980; Manabe et al., 1998). We found in the present study that antigen–antibody reaction-induced scratching behavior was inhibited by oxatomide in not only wild-type but also histamine H_1 receptor-deficient mice. These findings can be accounted for by the anti-allergic activity of oxatomide in vivo. The inhibitory effect of tranilast on scratching behavior in histamine H_1 receptor-deficient mice was almost the same as that in wild-type mice. Azuma et al. (1976) reported that tranilast inhibited chemical mediator release from rat peritoneal mast cells induced by antigen–antibody reaction but showed no histamine H_1 receptor blocking activity. These observations explain why tranilast is effective against scratching behavior induced by antigen–antibody reaction in both wild-type and histamine H_1 receptor-deficient mice at the same dose levels.

In conclusion, both histamine H_1 receptor and other chemical mediators are responsible for scratching behavior associated with passive cutaneous anaphylaxis. In addition, the results of the present in vivo study indicated that oxatomide shows anti-pruritic effect. Therefore, oxatomide was suggested to be useful for the treatment of itch in humans than classical histamine H_1 receptor antagonists.

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