ELSEVIER

European Journal of Pharmacology 351 (1998) 1–5

ejp

Effects of histamine H_1 receptor antagonists on compound 48/80-induced scratching behavior in mice

Yukio Sugimoto, Keiko Umakoshi, Nao Nojiri, Chiaki Kamei *

Department of Pharmacology, Faculty of Pharmaceutical Sciences, Okayama University, Okayama 700-8530, Japan

Received 2 February 1998; accepted 7 April 1998

Abstract

The effects of histamine H_1 receptor antagonists on compound 48/80-induced scratching behavior were studied in mice. Classical histamine H_1 receptor antagonists such as diphenhydramine and chlorpheniramine caused a potent depressant effect on compound 48/80-induced scratching behavior. Histamine H_1 receptor antagonists having antiallergic activity (an inhibition of mast cell degranulation), such as azelastine and oxatomide and nonsedative histamine H_1 receptor antagonists such as terfenadine, epinastine and astemizole, also showed a relatively potent effect. On the other hand, the effects of tranilast and cromolyn sodium—antiallergic drugs without histamine H_1 receptor antagonistic activity—were extremely weak. Diazepam had weak or no depressant effects on compound 48/80-induced scratching behavior. These results suggest that inhibition of compound 48/80-induced scratching behavior is mainly due to histamine H_1 receptor antagonistic activity and not to the sedative action of the drugs.

Keywords: Compound 48/80; Scratching behavior; Histamine H_1 receptor antagonist; Antiallergic drug

1. Introduction

It is widely accepted that atopic dermatitis is a skin disease of itching, that is, a sensation causing the urge to scratch. Although scratching behavior can be objectively measured, itchiness has previously been assessed by recording scratch marks or scratching movement. It has been reported that a movement-sensitive radar system was developed clinically for the measurement of pruritus. This method has the advantage of requiring no physical contact with the patients (Mustakallio, 1991). However, no useful method for the measurement of itching in animals has been reported. Recently, Kuraishi et al. (1995) demonstrated that a subcutaneous (s.c.) injection of some pruritogenic agents into the rostral back of the mouse produced scratching of the injected site by the hind paws, which is an itch-associated behavior. However, it is generally known that an intradermal (i.d.) injection is widely used in experiments on passive cutaneous reaction. Therefore, at first, we compared the scratching behavior induced by an s.c. or an i.d. injection of compound 48/80—a typical histamine releaser from mast cells.

Although histamine H_1 receptor antagonists and antiallergic drugs are often used for the clinical treatment of itching caused by atopic dermatitis, the efficacy of these drugs in animal models remains obscure. Recently, many histamine H_1 receptor antagonists and antiallergic drugs have been developed and widely used for the treatment of allergic diseases. For instance, considerable evidence has accumulated that terfenadine and astemizole, which are classified among nonsedative histamine H_1 receptor antagonists, are also applicable to the itching of atopic dermatitis (Cainelli et al., 1986; Doherty et al., 1989).

Therefore, although it is generally accepted that histamine is important for the pathogenesis of itching in atopic dermatitis, Krause and Shuster (1983) reported that the antipruritic effect of antihistamines is due to a central, sedative-related effect and not to peripheral histamine H_1 receptor antagonism. The present study was undertaken to clarify the role of histamine in generating the scratching behavior induced by compound 48/80.

* Corresponding author. Tel.: +81-86-251-7939; fax: +81-86-251-7939; e-mail: kamei@pheasant.pharm.okayama-u.ac.jp

0014-2999/98/$19.00

2. Materials and methods

2.1. Animals

The animals used were female BALB/c strain mice (Shimizu Laboratory Supplies, Kyoto, Japan), weighing 16–26 g and housed in an air-conditioned room maintained at 22–26°C with humidity of 30–70%. The animals were given food and water ad libitum.

2.2. Drugs

The following drugs were kindly provided by the companies indicated: D-chlorpheniramine maleate (Yoshitomi Pharmaceutical, Osaka, Japan); azelastine hydrochloride (Eisai, Tokyo, Japan); oxatomide (Kyowa Hakkou Kogyo, Tokyo, Japan); terfenadine (Marion Merrell Dow, Kansas City, MO, USA); epinastine hydrochloride (Böehringer Ingelheim, Ingelheim/Rhein); astemizole (Mochida pharmaceutical, Tokyo, Japan); tranilast (Schering Plough, Osaka, Japan); cromolyn sodium (Fujisawa pharmaceutical, Osaka, Japan); diazepam (Takede chemical industries, Osaka, Japan). Diphenhydramine hydrochloride was purchased commercially from Sigma (St. Louis, MO, USA). Test drugs were suspended in 5% gum arabic and were orally administered. The chemicals used were compound 48/80 (Sigma) and Evans blue (Wako, Tokyo, Japan). Compound 48/80 and Evans blue were dissolved in physiological saline. The doses of all the drugs used are expressed in terms of the free base.

2.3. Compound 48/80-induced scratching behavior

Before the experiment, the animals were put into an observation cage ($32 \times 22 \times 10$ cm) for about 10 min for acclimatization. With the use of a 27 gauge needle, 0.05 ml of compound 48/80 was injected subcutaneously or intradermally into the rostral part of the back. Immediately after injection, the animals were put into the observation cage (1 animal/cage) and scratching behavior was measured for 60 min. Scratching behavior was observed in accordance with the method of Kuraishi et al. (1995), i.e., scratching of the injected site with the hind paws was counted. Oxatomide was administered orally 2 h before, and the other drugs were administered orally 1 h before compound 48/80 injection.

2.4. Compound 48/80-induced vasal permeability of the skin

After s.c. or i.d. injection of 0.05 ml of compound 48/80 into the rostral part of the back, a 1% saline solution of Evans blue was injected intravenously into each animal. The animals were killed 60 min later, and the diameters of the 'Bluing' reaction at the compound 48/80 injection site were measured.

2.5. Statistical analysis

Statistical significance was tested with an analysis of variance using Dunnett's test. A probability value less than 0.05 was considered as significant. The data represent the mean ± SEM. The ED_{50} value (95% confidence limits) was calculated by the probit method.

3. Results

3.1. Scratching behavior and vasal permeability of the skin induced by compound 48/80

As shown in Fig. 1A, both s.c. and i.d. injections of compound 48/80 elicited scratching behavior in mice. No

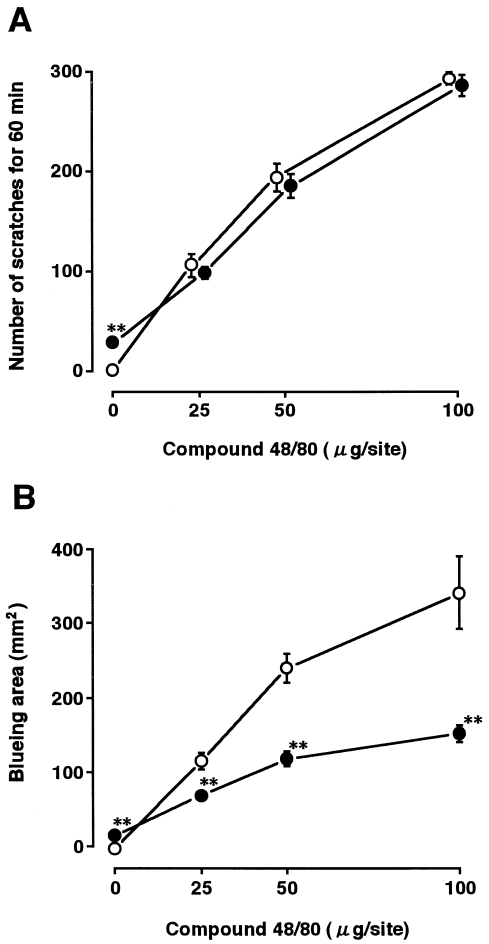

Fig. 1. (A) Dose-response curves for compound 48/80-induced scratching behavior. Mice were given a subcutaneous (○) or intradermal (●) injection of compound 48/80. The number of scratches was counted for 60 min after injection of compound 48/80. Each point represents the mean ± SEM for 5 animals. $**P < 0.01$ when compared with subcutaneous injection (Dunnett's test). (B) Dose-response curves for compound 48/80-induced vasal permeability of the skin in mice. Mice were given a subcutaneous (○) or intradermal (●) injection of compound 48/80. The bluing area of vasal permeability was measured for 60 min after injection of compound 48/80. Each point represents the mean ± SEM for 5 animals. $**P < 0.01$ when compared with subcutaneous injection (Dunnett's test).

measurable change was found after s.c. injection of saline. However, when saline was injected intradermally, a slight but significant scratching behavior was observed. The same was observed with regard to the skin reaction, i.e., the skin vasal permeability induced by i.d. injection of saline was significantly increased compared with that induced by an s.c. injection (Fig. 1B). When compound 48/80 was injected subcutaneously, a dose-dependent response was observed for the scratching behavior up to 50 μg of compound 48/80. However, no linear response was noted from 50 to 100 μg. Therefore, s.c. injection of compound 48/80 (50 μg) was used for the present study.

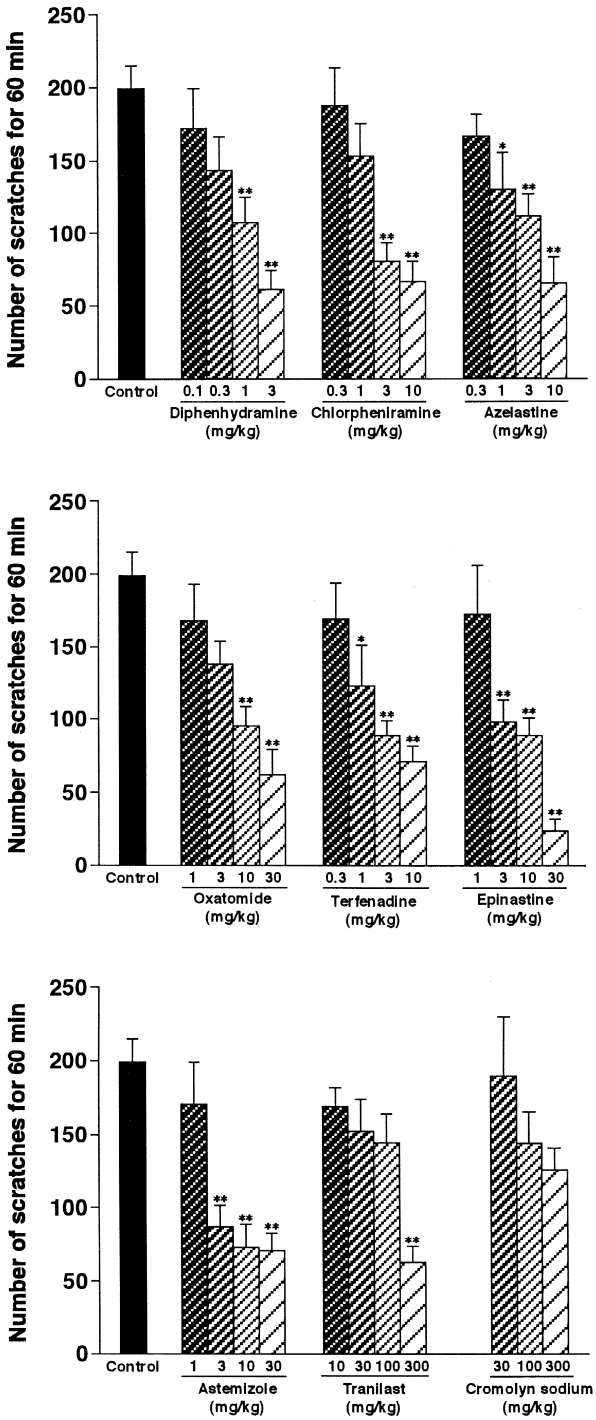

Fig. 2. Effects of drugs on compound 48/80-induced scratching behavior. Mice were given a subcutaneous injection of compound 48/80 (50 μg). Scratching behavior was observed for 60 min. Each column and vertical bar represents the mean ± SEM for 8 to 16 animals. $*P < 0.05$ and $**P < 0.01$ when compared with the control group ($n = 26$) (Dunnett's test).

3.2. Effects of histamine H_1 receptor antagonists

Histamine H_1 receptor antagonist effects on compound 48/80-induced scratching behavior are shown in Fig. 2 and Table 1. Diphenhydramine caused a dose-related inhibition of this response and at doses of 1 and 3 mg/kg, it significantly decreased the number of scratches. Chlorpheniramine inhibited compound 48/80-induced scratching similarly to diphenhydramine; a significant effect was observed at doses of 3 and 10 mg/kg. Azelastine and oxatomide also inhibited the response, but with potencies less than those of diphenhydramine and chlorpheniramine. The ED_{50} values of azelastine and oxatomide were 3.50 (1.79–11.4) and 8.68 (4.73–20.2) mg/kg, respectively.

As shown in Fig. 2, terfenadine, at doses of 1, 3 and 10 mg/kg, significantly inhibited compound 48/80-induced scratching behavior. Both epinastine and astemizole, at doses of 3, 10 and 30 mg/kg, significantly inhibited this response. The ED_{50} values of terfenadine, epinastine and astemizole were 2.56 (1.17–6.98), 4.51 (2.71–6.94) and 5.59 (2.09–13.4) mg/kg, respectively. The effects of tranilast and cromolyn sodium were relatively weak and their ED_{50} values were 208 (94.2–1642) and more than 300 mg/kg, respectively. To test whether a central depressant drug inhibits the scratching behavior induced by compound 48/80 or not, diazepam was given p.o. at 0.3 mg/kg. It caused no significant inhibition of the scratching behavior. At a dose of 1 mg/kg p.o., however, diazepam caused a significant inhibition of scratching, but showed significant muscle relaxant activity.

Table 1
ED_{50} values of histamine H_1 receptor antagonists for the scratching behavior induced by compound 48/80 in mice

Drug	ED_{50} values (95% confidence limits) (mg/kg p.o.)
Diphenhydramine	1.09 (0.58–3.06)
Chlorpheniramine	2.43 (1.37–4.47)
Azelastine	3.50 (1.79–11.4)
Oxatomide	8.68 (4.73–20.2)
Terfenadine	2.56 (1.17–6.98)
Epinastine	4.51 (2.71–6.94)
Astemizole	5.59 (2.09–13.4)
Tranilast	208 (94.2–1642)
Cromolyn sodium	> 300

4. Discussion

In the present study, we found that even when saline was injected intradermally, a slight but significant scratching behavior was observed. At the same time, skin vasal permeability was also increased by i.d. injection of saline. These findings suggest that mechanical stimulation may be responsible for the occurrence of scratching behavior as well as an increase in skin vasal permeability when saline is injected intradermally. Therefore, in the present study, s.c. injection was used for measuring scratching behavior induced by compound 48/80. This compound has been reported to cause histamine release from human cutaneous mast cells (Lowman et al., 1988). The same findings have also been reported for rats (Barrett et al., 1985) and mice (He et al., 1990). Therefore, it seems likely that the scratching behavior induced by compound 48/80 is attributable to the release of histamine from the skin mast cells. Histamine is well known to be present in mast cells and has been thought to be an important mediator of itchiness. For instance, i.d. injection of histamine (3.3–33 μg/ml, 0.02 ml/site) produced an itching sensation in humans (Hägermark et al., 1978). It has been reported that invasion of mast cells into the cutaneous nerve fiber bundles resulted in the skin lesions of atopic dermatitis (Sugiura et al., 1992). The authors suggested that the mast cell's invasion of dermal nerves with edematous changes of the nerve fiber bundles might be related to provocation or aggravation of the itchiness of atopic dermatitis. This suggestion indicates the importance of the skin mast cells in the pruritus of atopic dermatitis.

In the present study, it was also found that classical histamine H_1 receptor antagonists, diphenhydramine and chlorpheniramine, inhibited compound 48/80-induced scratching behavior. It is well known that these drugs show a sedative effect as well as a histamine H_1 receptor antagonistic action. Therefore, it was thought that the inhibition of scratching behavior induced by compound 48/80 occurred via a histamine H_1 receptor antagonistic action or sedative effect. However, as shown in the text, nonsedative histamine H_1 receptor antagonists such as terfenadine, epinastine and astemizole also inhibited this response to the same extent as diphenhydramine and chlorpheniramine. In association with this, diazepam, a sedative with no histamine H_1 receptor antagonistic activity, had no or little effect on experimental pruritus induced by histamine (Lorette and Vaillant, 1990). Therefore, it may be concluded that inhibition of compound 48/80-induced scratching behavior cannot be ascribed to the sedative action of the drugs. Azelastine and oxatomide were also found to cause inhibition of compound 48/80-induced scratching behavior. Azelastine (Katayama et al., 1981), oxatomide (Church and Gradidge, 1980) and nonsedative histamine H_1 receptor antagonists, terfenadine (Nabe et al., 1989), epinastine (Kamei et al., 1992) and astemizole (Awouters et al., 1983) that show both histamine H_1 receptor antagonistic activity and antiallergic activity that defined the inhibition of chemical mediator release from mast cells or basophils, caused almost the same effect as diphenhydramine and chlorpheniramine. In addition, both tranilast and cromolyn sodium, antiallergic drugs without histamine H_1 receptor antagonistic activity, also possessed a relatively weak inhibitory effect on this response. Azuma et al. (1976) reported that tranilast inhibited the histamine release from rat peritoneal mast cells induced by antigens as well as antigen-induced passive cutaneous anaphylaxis in rats at doses of 100 mg/kg or 200 mg/kg, while no marked inhibition was observed for histamine-induced vascular permeability at a dose of 150 mg/kg. These results were mostly consistent with the present ones, i.e., tranilast showed a weak inhibitory effect on compound 48/80-induced scratching behavior in mice. As for cromolyn sodium, no inhibitory effect on the cutaneous reaction induced by compound 48/80 was observed, as also reported by Cox (1967). From these findings, it can be assumed that the contribution of an antiallergic effect was relatively small for compound 48/80-induced scratching behavior. In conclusion, inhibition of compound 48/80-induced scratching behavior occurred mainly via H_1 antagonistic activity.

References

Awouters, F.H.L., Niemegeers, C.J.E., Janssen, P.A.J., 1983. Pharmacology of the specific histamine H_1 antagonist astemizole. Arzneim-Forsch/Drug Res. 33, 381–388.

Azuma, H., Banno, K., Yoshimura, T., 1976. Pharmacological properties of *N*-(3′,4′-dimetoxycinnamoyl) anthranilic acid (*N*-5′), a new anti-atopic agent. Br. J. Pharmacol. 58, 483–488.

Barrett, K.E., Ali, H., Pearce, F.L., 1985. Studies on histamine secretion from enzymatically dispersed cutaneous mast cells of the rat. J. Invest. Dermatol. 84, 22–26.

Cainelli, T., Seidenari, S., Valsecchi, R., Mosca, M., 1986. Double-blind comparison of astemizole and terfenadine in the treatment of chronic urticaria. Pharmatherapeutica 4, 679–686.

Church, M.K., Gradidge, C.F., 1980. Oxatomide: inhibition and stimulation of histamine release from human lung and leukocytes in vitro. Agents Actions 10, 4–7.

Cox, J.S.G., 1967. Disodium cromoglycate (FPL 670) (Intal): a specific inhibitor of reaginic antibody–antigen mechanisms. Nature 216, 1328–1329.

Doherty, V., Sylvester, D.G.H., Kennedy, C.T.C., Harvey, S.G., Calthrop, J.G., Gibson, J.R., 1989. Treatment of itching in atopic eczema with antihistamines with a low sedative profile. Br. Med. J. 298, 96.

Hägermark, Ö., Hökfelt, T., Pernow, B., 1978. Flare and itch induced by substance P in human skin. J. Invest. Dermatol. 71, 233–235.

He, D., Esquenazi-Behar, S., Sorter, N.A., Lim, H.W., 1990. Mast-cell heterogeneity: functional comparison of purified mouse cutaneous and peritoneal mast cells. J. Invest. Dermatol. 94, 178–185.

Kamei, C., Akagi, M., Mio, M., Kitazumi, K., Izushi, K., Masaki, S., Tasaka, K., 1992. Antiallergic effect of epinastine (WAL 801 CL) on immediate hypersensitivity reactions: I. Elucidation of the mechanism for histamine release inhibition. Immunopharmacol. Immunotoxicol. 14, 191–205.

Katayama, S., Akimoto, N., Shionoya, H., Morimoto, T., Katoh, Y., 1981. Anti-allergic effect of azelastine hydrochloride on immediate type hypersensitivity. Arzneim-Forsch/Drug Res. 31, 1196–1203.

Krause, L., Shuster, S., 1983. Mechanism of action of antipruritic drugs. Br. Med. J. 287, 1199–1200.

Kuraishi, Y., Nagasawa, T., Hayashi, K., Satoh, M., 1995. Scratching behavior induced by pruritogenic but not algesiogenic agents in mice. Eur. J. Pharmacol. 275, 229–233.

Lorette, G., Vaillant, L., 1990. Pruritus. Current concepts in pathogenesis and treatment. Drugs 39, 218–223.

Lowman, M.A., Rees, P.H., Benyon, R.C., Church, M.K., 1988. Human mast cell heterogeneity: histamine release from mast cells dispersed from skin, lung, adenoids, tonsils, and colon in response to IgE-dependent and nonimmunologic stimuli. J. Allergy Clin. Immunol. 81, 590–597.

Mustakallio, K.K., 1991. Scratch radar for the measurement of pruritus. Acta Derm. Venereol. (Stockholm) Suppl. 156, 44.

Nabe, M., Agrawal, D.K., Sarmiento, E.U., Townley, R.G., 1989. Inhibitory effect of terfenadine on mediator release from human blood basophils and eosinophils. Clin. Exp. Allergy 19, 515–520.

Sugiura, H., Maeda, T., Uehara, M., 1992. Mast cell invasion of peripheral nerve in skin lesions of atopic dermatitis. Acta Derm. Venereol. (Stockholm) 176, 74–76.