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Evaluation of anti-scratch properties of oxatomide and epinastine in mice

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Abstract

Anti-scratch effects of oxatomide and epinastine were examined in mice. Scratching behavior and cutaneous reactions were induced in BALB/c, ICR and ddY mice by dinitrofluorobenzene painting, passive cutaneous anaphylaxis and substance P injection, respectively. Although oxatomide and epinastine failed to inhibit scratching behavior in BALB/c mice, they inhibited the cutaneous reaction significantly. The drugs potently inhibited both scratching behavior and cutaneous reaction in ICR mice. They also inhibited scratching behavior and cutaneous reaction in ddY mice, although cetirizine and terfenadine failed to affect them. Histamine did not induce frequent scratching behavior in BALB/c and ddY mice. These results indicate that oxatomide and epinastine inhibit the scratching behavior in ICR mice associated with passive cutaneous anaphylaxis mainly through an antagonistic action on histamine H_1 receptors. The results also indicate that these drugs inhibit substance P-induced scratching behavior in ddY mice through an action independent of the antagonistic action on histamine H_1 receptors.

Keywords: Scratching behavior; Immunoglobulin E; Anaphylaxis, passive cutaneous; Substance P; Histamine; Histamine H_1 receptor; Oxatomide; Epinastine; Cetirizine; Terfenadine; (Mouse)

1. Introduction

Itching is a sensation, which causes a strong desire to scratch, and is one of the most important symptoms of allergic skin diseases. It is well known that scratching causes destruction of skin barriers, resulting in worsening of the skin lesion and more itching. It is fully expected, therefore, that the regulation of itching and scratching could prevent the worsening of skin diseases, and be a useful treatment for itchy skin diseases. At present, however, the mediators and mechanisms of itching remain to be elucidated (Bernhard, 1987; Greaves, 1992, 1993).

Kuraishi et al. (1995) reported that scratching behavior in ddY mice caused by a rostral back injection with pruritogenic agents, compound 48/80 or substance P might be due to itch, but not to pain. In agreement with their report, we observed mouse scratching behavior associated with immunoglobulin E-dependent allergic cutaneous reactions, and found that the immunoglobulin E-mediated allergic cutaneous reactions actually induce scratching behavior in BALB/c and ICR mice (Musoh et al., 1997; Inagaki et al., 1999). Furthermore, we demonstrated that histamine is the most important mediator for causing the scratching behavior induced in ICR mice by immunoglobulin E-mediated passive cutaneous anaphylaxis, and that histamine induces scratching behavior in ICR mice through not only histamine H_1 receptors but also histamine H_2 receptors (Inagaki et al., 1999).

Histamine is the only mediator characterized for inducing itch in humans, and drugs with a histamine H_1 receptor antagonistic action have been applied for the treatment of itch. Oxatomide, an anti-allergic agent with histamine H_1 receptor antagonistic property (Awouters et al., 1977), has been applied to the treatment of itch, and its efficacy is reported for patients with chronic urticaria (Peremans et al., 1981; Demaubeuge et al., 1982; Locci and Del Giacco, 1991), atopic dermatitis (Bergonizi and Mangili, 1997; Duse et al., 1998), conjunctivitis (Blockeel and Leuridan, 1980), pruritus senilis (Dupont et al., 1984) and pruritus vulvae (Origoni et al., 1990). Some of these reports indicated that the effect of oxatomide was superior to those of other histamine H_1 receptor antagonists (Peremans et al., 1981; Demaubeuge et al., 1982). Epinastine, a newer anti-allergic agent with histamine H_1 receptor antagonistic

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property (Adamus et al., 1987a,b), exhibits mediator release inhibition (Kamei et al., 1992a) and antagonism against platelet activating factor, leukotrienes and serotonin (Kamei et al., 1992b; Tasaka et al., 1994), similarly to oxatomide (Ohmori et al., 1985; Kosaka et al., 1987; Manabe et al., 1988; Nijkamp et al., 1989), and is expected to be effective for the treatment of itch.

In the present study, therefore, we examined the effects of oxatomide and epinastine on scratching behavior in BALB/c, ICR and ddY mice caused by distinct stimuli as reported previously (Kuraishi et al., 1995; Musoh et al., 1997; Inagaki et al., 1999).

2. Materials and methods

2.1. Animals

Female BALB/c, ICR and ddY mice, 8 weeks of age, obtained from Japan SLC, (Hamamatsu, Japan) were used. Experiments were undertaken following the guidelines for the care and use of experimental animals from the Japanese Association for Laboratory Animal Science (1987).

2.2. Monoclonal immunoglobulin E

Mouse monoclonal immunoglobulin E against dinitrophenyl residue was prepared by culturing a cell line, EC1, as reported previously (Sakurai et al., 1994). The culture supernatant of EC1 was stored at −80°C and used as a source of immunoglobulin E. The immunoglobulin E content of the preparation, as estimated by enzyme-linked immunosorbent assay, was 1.5 μg/ml.

2.3. Antigens

Dinitrofluorobenzene (2,4-dinitrofluorobenzene) dissolved in a mixture of acetone and olive oil (3:1) was used for eliciting a cutaneous reaction in BALB/c mice. Dinitrophenylated bovine serum albumin prepared according to the method described by Eisen et al. (1953) was used as an eliciting antigen for passive cutaneous anaphylaxis. The average number of dinitrophenyl residues introduced to a bovine serum albumin molecule was 8.7.

2.4. Agents and reagents

Oxatomide (Kyowa Hakko Kogyo, Tokyo, Japan), epinastine (Kyowa Hakko Kogyo), cetirizine (Hokuriku Seiyaku, Fukui, Japan) and terfenadine (Sigma, St. Louis, MO, USA) were used. These agents were prepared in water and administered orally 1 h before eliciting of a reaction.

Substance P (Sigma) and histamine (dihydrochloride, Nacarai Tesque, Kyoto, Japan) were dissolved in saline and used for inducing a cutaneous reaction.

2.5. Dinitrofluorobenzene-induced cutaneous reaction in BALB/c mice

A dinitrofluorobenzene-induced cutaneous reaction was obtained according to a method reported previously (Ray et al., 1983; Musoh et al., 1997). Mice were sensitized with an intravenous injection of 1-ml immunoglobulin E preparation. Twenty-four hours after the sensitization, 5 μl of 0.75% dinitrofluorobenzene solution was applied onto both sides of the ears. Immediately after the antigen challenge, the mice were placed in a chamber for observation of their behavior. When ear edema was to be examined, 25 μl of 0.15% dinitrofluorobenzene solution was applied onto the ears. Ear edema was assessed by measuring ear thickness 1 h after the antigen challenge, using a micrometer (Peacock Upright Dial Gauge, Ozaki, Tokyo, Japan).

2.6. Passive cutaneous anaphylaxis in ICR mice

Passive cutaneous anaphylaxis was induced in ICR mice as reported previously (Inagaki et al., 1999). The rostral part of the back skin of mice was clipped, and 20 μl of fivefold diluted immunoglobulin E preparation was injected intradermally. Twenty-four hours after sensitization, passive cutaneous anaphylaxis was elicited by injecting 0.25 ml of saline containing 0.25 mg (protein) of dinitrophenylated bovine serum albumin intravenously. Immediately after the antigen challenge, mice were placed in a chamber for observation of their behavior. When the vascular permeability increase was to be examined, 1.25 mg of Evans blue dye was injected simultaneously with the antigen. The vascular permeability increase was assessed by measuring the amount of extravasated dye 30 min after the antigen challenge. Control mice received the antigen challenge without sensitization with immunoglobulin E, and the results are indicated as passive cutaneous anaphylaxis (−).

2.7. Mediator-induced cutaneous reaction in mice

The rostral part of the back skin of mice was clipped, and 20 μl of substance P or histamine solution was injected intradermally (Kuraishi et al., 1995; Inagaki et al., 1999). Control mice received a saline injection instead. Immediately after the injection, the mice were placed in a chamber for observation of their behavior. When the vascular permeability increase was to be evaluated, 1.25 mg of Evans blue dye was injected intravenously at the same time. The amount of dye extravasated for 30 min after the injection was measured.

2.8. Observation of scratching behavior

Scratching behavior was assessed according to the method described previously (Kuraishi et al., 1995; Musoh

et al., 1997; Inagaki et al., 1999). In brief, immediately after elicitation of the cutaneous reactions, the mice were placed in an observation chamber. The behavior was recorded, in the absence of an observer but with a video camera, for up to 120 min. Scratching of the reaction site with the hindpaws was counted at 10-min intervals. Mice generally scratched several times for about 1 s and a series of scratchings was counted as one incidence. The number of scratchings is indicated cumulatively.

2.9. Measurement of vascular permeability increase

The increase in vascular permeability was assessed as described previously (Inagaki et al., 1986a,b). In brief, the mice were killed 30 min after elicitation of the cutaneous reactions, and the reaction site was excised. The skin specimen was dissolved in 0.7 ml of 1 N KOH solution, and 9.3 ml of a mixture of 0.6 N H_3PO_4 solution and acetone (5:13) was added. After vigorous shaking, the precipitates were filtered off and the amount of dye extracted was measured colorimetrically at 620 nm.

2.10. Statistics

The data were expressed as the mean values with standard error. In experiments containing two experimental groups, either Student's or Aspin–Welch's *t*-test was employed to evaluate the statistical significance of differences after the variances of the data were evaluated with the *F*-test ($P < 0.05$). For data including three or more experimental groups, either a parametric or a non-parametric Tukey's multiple range test was used after Bartlett's analysis ($P < 0.05$). When the P value was smaller than 0.05, the difference was considered to be significant.

3. Results

3.1. Scratching behavior and ear edema caused by dinitrofluorobenzene in BALB/c mice

Scratching behavior and ear edema were induced by dinitrofluorobenzene application in sensitized BALB/c mice, and effects of oxatomide and epinastine on the responses were observed.

As shown in Fig. 1A, application of dinitrofluorobenzene caused significant numbers of scratchings in both sensitized and non-sensitized mice, although the incidence of scratching in sensitized mice was higher than that in non-sensitized mice ($p < 0.05$). Furthermore, application of vehicle also caused scratching in both sensitized and non-sensitized mice. In sensitized mice, ear edema was

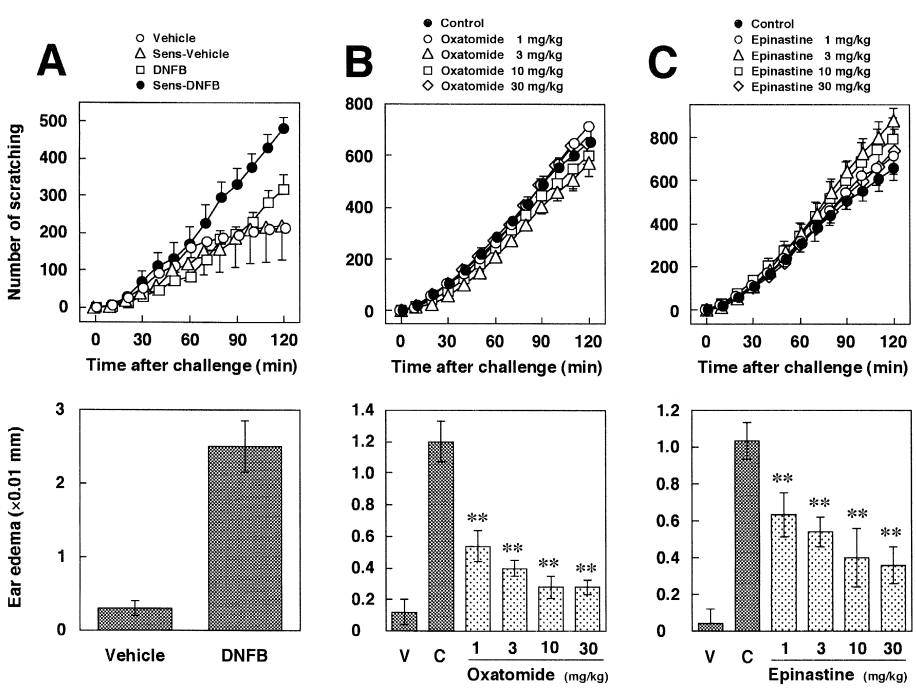

Fig. 1. Scratching behavior and ear edema induced by dinitrofluorobenzene application in sensitized BALB/c mice, and effects of oxatomide and epinastine on the responses. Mice were systemically sensitized with immunoglobulin E and challenged by dinitrofluorobenzene application to their ears 24 h later. Scratching was counted for 120 min, and ear edema was measured at 60 min after the challenge. Oxatomide and epinastine were administered orally 60 min before challenge. Each value represents the mean ± S.E.M. for six mice. Number of scratching is indicated cumulatively and was statistically evaluated based on the total number for 2 h. (A) Basic data, (B) oxatomide, (C) epinastine, $^{**}P < 0.01$.

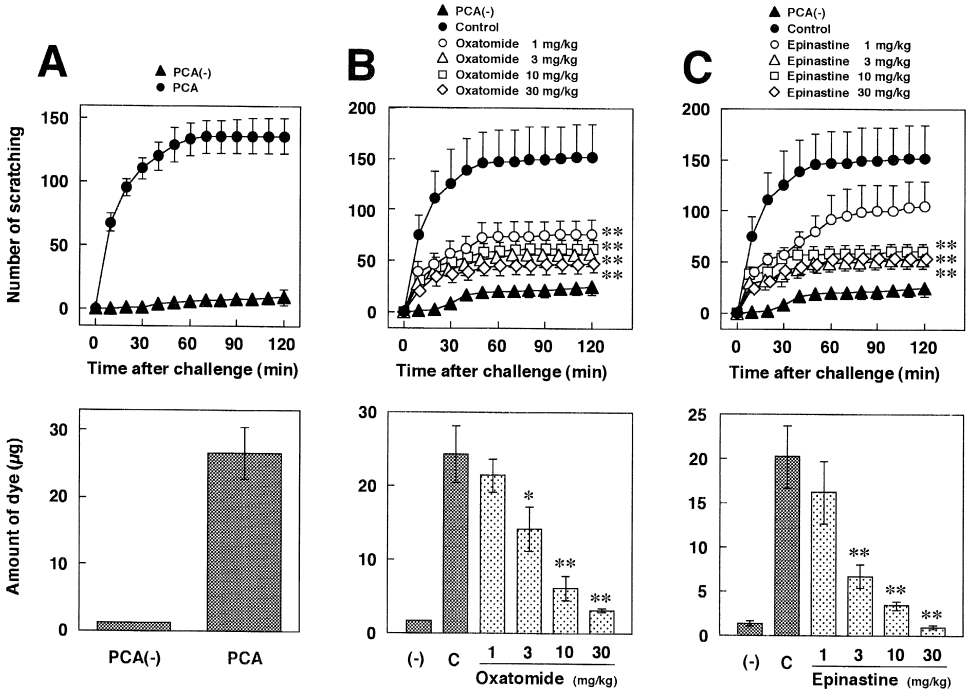

Fig. 2. Scratching behavior and vascular permeability increase induced by passive cutaneous anaphylaxis in ICR mice, and effects of oxatomide and epinastine on the responses. Mouse back skin was locally sensitized with immunoglobulin E and challenged by an intravenous injection of dinitrophenylated bovine serum albumin 24 h later. Scratching was counted for 120 min, and vascular permeability increase was measured at 30 min after the challenge. Oxatomide and epinastine were administered orally 60 min before challenge. Each value represents the mean ± S.E.M. for six mice. Number of scratching is indicated cumulatively and was statistically evaluated based on the total number for 2 h. (A) Basic data, (B) oxatomide, (C) epinastine, PCA: passive cutaneous anaphylaxis, PCA (−): PCA negative control, $^{*}P < 0.05$, $^{**}P < 0.01$.

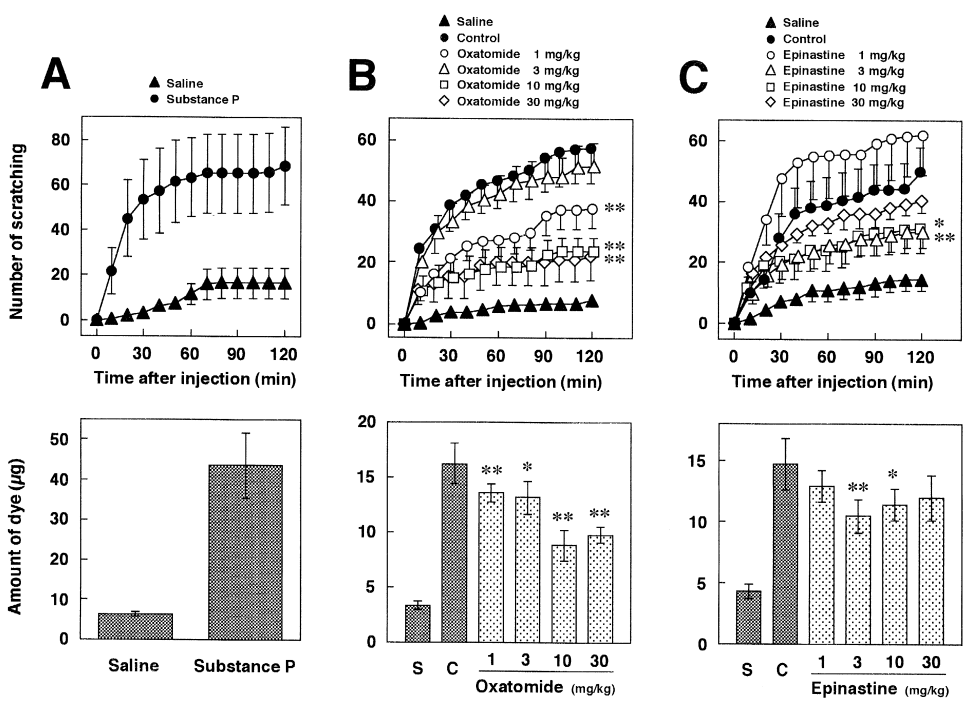

Fig. 3. Scratching behavior and vascular permeability increase induced by substance P injection in ddY mice, and effects of oxatomide and epinastine on the responses. Mice received an intradermal injection of substance P. Sratching was counted for 120 min, and vascular permeability increase was measured at 30 min after substance P injection. Oxatomide and epinastine were administered orally 60 min before. Each value represents the mean ± S.E.M. for four to six mice. Number of scratching is indicated cumulatively and was statistically evaluated based on the total number for 2 h. (A) Basic data, (B) oxatomide, (C) epinastine, $^{*}P < 0.05$, $^{**}P < 0.01$.

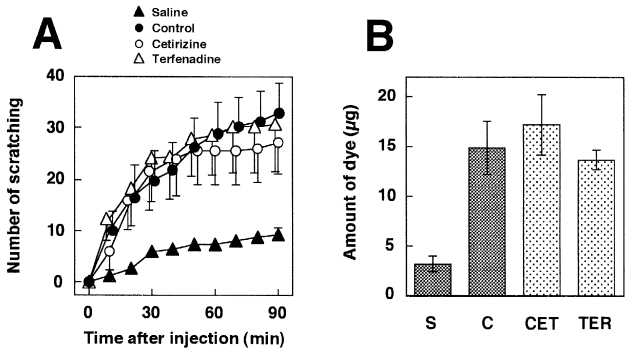

Fig. 4. Efects of cetirizine and terfenadine on the scratching behavior and vascular permeability increase induced by substance P injection in ddY mice. Mice received an intradermal injection of substance P. Scratching was counted for 90 min, and vascular permeability increase was measured at 30 min after substance P injection. Cetirizine and terfenadine at a dose of 10 mg/kg were administered orally 60 min before. Each value represents the mean ± S.E.M. for four to six mice. Number of scratching is indicated cumulatively and was statistically evaluated based on the total number for 90 min. CET: cetirizine, TER: terfenadine. (A) Scratching, (B) dye leakage.

induced at 1 h after dinitrofluorobenzene application. The irritating effect of dinitrofluorobenzene was negligible under the present experimental conditions (data not shown).

The results of oxatomide and epinastine are shown in Fig. 1B and C. Oxatomide and epinastine at doses of 1–30 mg/kg were administered orally 1 h before dinitrofluorobenzene application. Although neither drug ever affected the scratching behavior caused by dinitrofluorobenzene application in sensitized mice, the drugs inhibited the ear edema significantly. Oxatomide seemed to be slightly more effective than epinastine.

3.2. Scratching behavior and vascular permeability increase caused by passive cutaneous anaphylaxis in ICR mice

As shown in Fig. 2A, intradermal sensitization with immunoglobulin E and subsequent intravenous challenge with antigen caused scratching behavior and dye leakage in ICR mice. The scratching and dye leakage caused by passive cutaneous anaphylaxis in ICR mice were clearly inhibited by oxatomide and epinastine administered orally (Fig. 2B and C). Epinastine at a dose of 30 mg/kg completely inhibited the dye leakage, whereas the inhibition of the scratch was much less. A similar tendency was observed in the case of oxatomide.

3.3. Scratching behavior and vascular permeability increase caused by substance P in ddY mice

As shown in Fig. 3A, intradermal injection of 50 nmol substance P caused scratching behavior and dye leakage in ddY mice. The results for oxatomide and epinastine are given in Fig. 3B and C. Both oxatomide and epinastine inhibited the scratching behavior in ddY mice significantly, whereas they inhibited dye leakage only partially. In contrast, as shown in Fig. 4, cetirizine and terfenadine at a dose of 10 mg/kg administered orally 1 h before substance P injection affected neither scratching behavior nor dye leakage.

3.4. Scratching behavior and vascular permeability increase caused by histamine

As shown in Fig. 5, histamine (2 μg) induced substantial dye leakage in all three strains of mice. However,

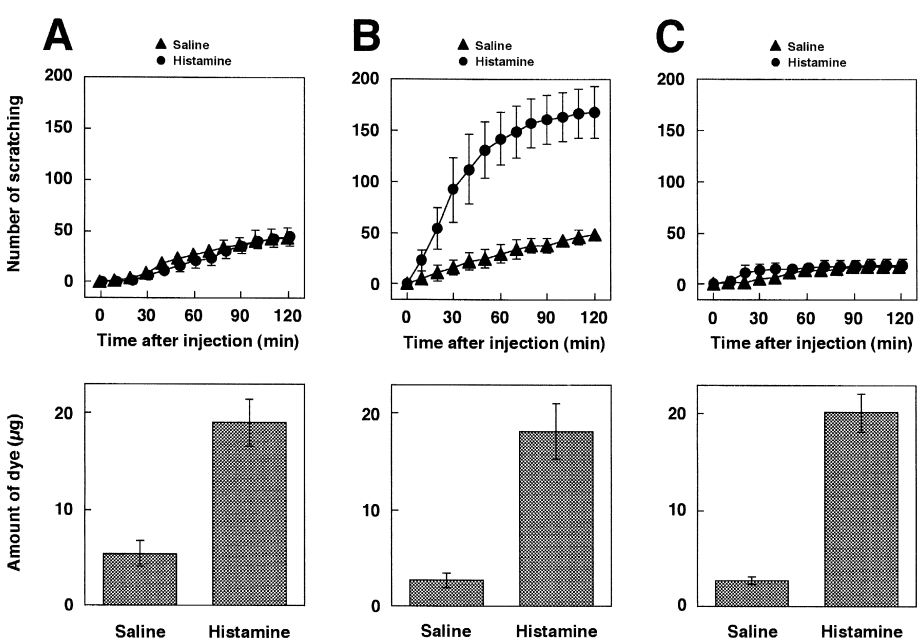

Fig. 5. Scratching behavior and vascular permeability increase induced by histamine injection in BALB/c, ICR and ddY mice. Mice received an intradermal injection of histamine. Scratching was counted for 120 min, and vascular permeability increase was measured at 30 min after histamine injection. Each value represents the mean ± S.E.M. for six mice. Number of scratching is indicated cumulatively. (A) BALB/c, (B) ICR, (C) ddY.

scratching behavior was only induced in ICR mice (Fig. 5B).

4. Discussion

In the present study, we induced scratching behavior and cutaneous reactions in three strains of mice, BALB/c, ICR and ddY, as reported previously (Kuraishi et al., 1995; Musoh et al., 1997; Inagaki et al. 1999), and effects of oxatomide and epinastine on the responses were examined.

Dinitrofluorobenzene application in BALB/c mice, passive cutaneous anaphylaxis in ICR mice, and substance P injection in ddY mice caused frequent scratching behavior and substantial cutaneous reactions. Histamine, however, induced scratching behavior only in ICR mice, although it caused a potent vascular permeability increase in all three strains of mice. It is considered, therefore, that histamine does not participate in the scratching behavior observed in BALB/c mice after dinitrofluorobenzene application and in ddY mice after substance P injection.

Dinitrofluorobenzene application to sensitized BALB/c mice causes ear edema, which is inhibited by anti-histamines and anti-allergic drugs (Nagai et al., 1995; Musoh et al., 1997; Inagaki et al., 1998), indicating that immunoglobulin E-dependent mast cell activation and mediator release are evoked at the lesion. However, vehicle application caused a relatively high incidence of scratching behavior even in intact mice, and histamine was not an effective stimulus for scratching behavior in BALB/c mice. Therefore, the contribution of an immunoglobulin E-dependent allergic mechanism to the induction of scratching behavior in BALB/c mice is partial, although it is actually responsible for the edema formation. Because the scratching behavior is inhibited by cyproheptadine (Musoh et al., 1997), serotonin may play some role. In the present study, oxatomide and epinastine never affected the scratching behavior in BALB/c mice, although they significantly inhibited the ear edema, as reported previously for cetirizine and terfenadine (Musoh et al., 1997). These drugs, therefore, inhibited edema formation mainly through an antagonistic action against histamine H_1 receptors.

Passive cutaneous anaphylaxis is an immediate type allergic cutaneous reaction as described by Ovary (1958a,b), and involves mast cell activation and vascular permeability increase caused by mast cell mediators. As reported previously, as ICR is a relatively high responder strain for passive cutaneous anaphylaxis, we selected this strain of mice for examining the scratching behavior associated with passive cutaneous anaphylaxis (Inagaki et al., 1986b, 1999). Furthermore, we confirmed that only histamine-sensitive ICR mice exhibit scratching behavior upon induction of passive cutaneous anaphylaxis (unpublished observation). In ICR mice, histamine from mast cells is a dominant mediator for causing not only a vascular permeability increase but also scratching behavior (Inagaki et al., 1999). Furthermore, we demonstrated that histamine induces scratching behavior in ICR mice not only through histamine H_1 receptors but also through histamine H_2 receptors. In the present experiments, oxatomide and epinastine significantly inhibited both the vascular permeability increase and the scratching behavior. In the case of epinastine, although the vascular permeability increase was completely inhibited at the highest dose (30 mg/kg), the inhibition of scratching behavior was only 80%. Similar results have been observed with cetirizine and terfenadine (Inagaki et al., 1999). These results indicate that the vascular permeability increase associated with passive cutaneous anaphylaxis is caused by histamine through histamine H_1 receptors, and that histamine H_1 receptors also play a major role in the induction of scratching behavior, but other mediators or histamine H_2 receptors are also partially involved.

Although histamine did not cause scratching behavior in the present experiments with ddY mice, substance P injection was an effective stimulus for inducing scratching behavior as reported by Kuraishi et al. (1995). It is well known that substance P stimulates skin mast cells to release mediators (Johnson and Erdös, 1973; Fewtrell et al., 1982). However, cetirizine and terfenadine failed to affect, not only scratching behavior, but also the vascular permeability increase caused by substance P in ddY mice. These results suggest that substance P injection does not cause mast cell mediator release in ddY mice under the present experimental condition, which is consistent with a previous report that substance P could cause scratching behavior in mice independently of mast cell activation (Andoh et al., 1998). Therefore, not only does histamine not participate in scratching behavior but it is also not involved in the vascular permeability increase upon substance P injection in ddY mice. Oxatomide and epinastine, however, apparently inhibited both the scratching behavior and vascular permeability increase. In contrast to the results for passive cutaneous anaphylaxis, these two drugs inhibited the scratching behavior more potently than the dye leakage. These drugs, therefore, inhibit the responses by a mechanism independent of histamine H_1 receptor antagonism. Oxatomide is slightly more potent than epinastine. Neither of these drugs affects the substance P-induced bronchoconstriction in guinea pigs (Misawa and Kanai, 1991), suggesting that these drugs might not have a direct antagonistic action against substance P.

In the present study, we examined the anti-scratch properties of oxatomide and epinastine in mice, and found that both these drugs have an additional anti-scratch character, which is independent of the histamine H_1 receptor antagonistic property. Cetirizine and terfenadine seemed not to share this property. Therefore, we suggest the possibility that oxatomide and epinastine could be applied to the treatment of itch in humans more widely than other anti-histamines, although the mechanism is still to be elucidated.

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