

Short Communication

Prostaglandin D₂ Inhibits IgE-Mediated Scratching by Suppressing Histamine Release From Mast Cells

Yuki Hashimoto¹, Iwao Arai^{1,*}, Makoto Tanaka¹, and Shiro Nakaike¹

¹Department of Pharmacology Laboratory, Medicinal Research Laboratory, Taisho Pharmaceutical Co., Ltd., 1-403, Yoshino-cho, Kita-ku, Saitama City, Saitama 331-9530, Japan

Received December 27, 2004; Accepted March 3, 2005

Abstract. Effects of prostaglandin (PG) D₂, PGE₂, and PGI₂ on itch-associated scratching responses of mice and histamine release from the rat basophilic leukemia cell line RBL-2H3 were examined. PGD₂ and ketotifen but not PGE₂ and PGI₂ suppressed the scratching caused by ovalbumin injected into ovalbumin-sensitized mice. Ketotifen also suppressed compound 48/80-induced scratching but not PGD₂, PGE₂, and PGI₂. In vitro, PGD₂ suppressed the antigen-induced histamine release from RBL-2H3 cells, but PGE₂ and PGI₂ did not. These findings suggest that PGD₂ specifically suppressed IgE-mediated scratching by inhibiting IgE-mediated histamine release from mast cells.

Keywords: prostaglandin D₂, scratching behavior, mast cell

Itching is a characteristic symptom in various forms of dermatitis, especially atopic dermatitis; consequently, it is a major diagnostic criteria (1). To date, itch-associated scratching responses have been induced in mice via intradermal injections of histamine, compound 48/80, substance P, leukotriene B₄, and other irritants (2–4). Histamine is also a potent inducer of itching in humans (5) and is released from mast cells when they are stimulated by an antigen. Histamine is usually determined as a degranulation marker in experiments on immediate allergic reactions. In our previous study, prostaglandin (PG) D₂, PGE₂, and PGI₂ suppressed scratching of NC/Nga mice with atopic dermatitis, but not the histamine-induced scratching in ICR mice (6). It was reported that PGD₂ inhibited histamine release from anti-IgE-activated rat peritoneal mast cells (7). We investigated effects of PGD₂, PGE₂, and PGI₂ on itch-associated responses of mice and histamine release from the rat basophilic leukemia cell line RBL-2H3, a cultured analogue of mucosal mast cells (8).

We used male BALB/c mice (Japan SLC, Inc., Shizuoka), which were 7 weeks of age at the start of the experiments. All were housed 8 per cage in our laboratory [lights on (7:00–19:00)–dark (19:00–7:00) cycle at a controlled temperature (23 ± 3°C) and

humidity 55 ± 15%]. Food and tap water were provided ad libitum. All experiments complied with the Guidelines for Care and Use of Laboratory Animals by Taisho Pharmaceutical Co., Ltd.

Measurement of scratching behavior was done, as described (9). In brief, the number of scratchings was automatically recorded and objectively evaluated using Micro Act (Neuroscience, Tokyo). A small magnet was implanted subcutaneously into both hind paws of the mice under diethyl ether anesthesia at least 24 h before the measurement of scratching behavior. Magnets were implanted for every individual measurement of scratching behavior. The mouse with magnets was placed in the observation area with food and tap water, surrounded by a round coil. This food and tap water were provided ad libitum. Results of scratching behavior are given as the total-scratching counts during 30 min after scratching behavior was elicited. Analysis parameters for detecting waves were threshold: 0.1 V, event gap: 0.2 s, minimum duration: 0.3 s, maximum frequency: 20 Hz, minimum frequency: 2 Hz. Each PG was dissolved in ethanol (Kokusan Kagaku, Tokyo) at 0.1 w/v% and applied 0.1 ml/mouse to the skin of the back, and ketotifen fumarate (Sigma-Aldrich, St. Louis, MO, USA) was suspended with 5% gum arabic in distilled water and given orally at 30 mg/kg at 30 min before the scratching was elicited.

*Corresponding author. FAX: +81-48-654-6650
E-mail: i.arai@po.rd.taisho.co.jp

Ovalbumin (OVA)-induced scratching behavior was elicited as follows: mice were sensitized with OVA (Seikagaku Kogyo, Tokyo). OVA (50 μ g) dissolved in phosphate-buffered saline, with 2 mg of aluminum hydroxide gel, was given intraperitoneally, twice at 2-week intervals. Two weeks after the second sensitization, 50 μ g OVA (dissolved in saline) given as an intradermal injection to the back of the neck significantly increased the scratching counts compared with non-sensitized mice (91.1 ± 8.3 and 22.8 ± 7.1 counts/30 min). PGD₂ and ketotifen statistically decreased OVA-induced scratching (19.1 ± 5.1 and 4.9 ± 1.0 counts/30 min, respectively), but PGE₂ and PGI₂ did not (52.6 ± 12.0 and 87.3 ± 18.5 counts/30 min, respectively) (Fig. 1).

Compound 48/80 (Sigma-Aldrich)-induced scratching behavior was elicited as follows: compound 48/80 dissolved in physiological saline was injected (15 mg/ml, 50 μ g/mouse) intradermally. Compound 48/80 injection significantly increased the scratching counts compared with the findings for the saline injections (167.8 ± 13.9 and 15.0 ± 2.8 counts/30 min). Ketotifen also statistically decreased the compound 48/80-induced scratching (115.0 ± 11.4 counts/30 min), but PGD₂, PGE₂, and PGI₂ did not (130.4 ± 14.6 , 146.4 ± 15.2 , and 184.1 ± 9.5 counts/30 min, respectively) (Fig. 2).

RBL-2H3 cells (Health Science Research Resources Bank, Osaka) were placed in RPMI 1640 supplemented

with FBS (GIBCO, Grand Island, NY, USA), L-glutamine (2 mmol/l), penicillin (100 U/ml), and streptomycin (100 μ g/ml) in a 37°C humidified incubator with an atmosphere of 95% air and 5% CO₂. A 24-well plate of cultured cells (5×10^5 cells/well) was used for the deregulation assay. After an overnight sensitization of RBL-2H3 cells with 1 μ g/ml monoclonal anti-dinitrophenyl (DNP)-IgE (Seikagaku Co.), the antibody was washed out, and then the cells were incubated with 10^{-5} mol/l of ibuprofen (Sigma-Aldrich) for 10 min, which was then washed out. Cells were then stimulated with 1 μ g/ml of antigen (DNP-BSA; LSL Co., Ltd., Tokyo) and PGD₂, PGE₂, or PGI₂ (dissolved in 0.1% ethanol) for 30 min. Histamine concentrations in the supernatants and cells were measured with an histamine EIA kit (Oxford Biomedical Research, Oxford, MI, USA), according to the manufacturer's instructions. Antigen stimulation significantly ($P < 0.001$) increased the histamine contents compared with the vehicle control (76.9 ± 3.7 and 36.2 ± 2.8 ng/ml). PGD₂ statistically decreased antigen-induced histamine release from RBL-2H3 cells, but PGE₂ and PGI₂ did not (Fig. 3).

In response to a specific antigen, chemical mediators were released by IgE-mediated activation of mast cells in immunized animals. In this study, PGD₂ decreased the number of OVA-induced scratching behaviors but not so compound 48/80-induced scratching behavior, while ketotifen (30 mg/kg, p.o. did not show sedative properties) statistically decreased both. Furthermore, PGD₂ inhibited antigen-induced histamine release from

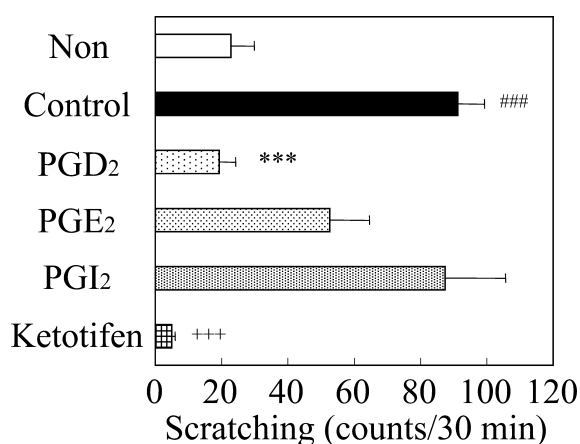


Fig. 1. Effects of PGD₂, PGE₂, PGI₂, and ketotifen on ovalbumin-sensitized/ovalbumin-challenged scratching by BALB/c mice. Values are the mean and S.E.M. for 8 mice. Non: non-sensitized, Control: vehicle, PGD₂: 0.1% prostaglandin D₂, PGE₂: 0.1% prostaglandin E₂, PGI₂: 0.1% prostaglandin I₂, Ketotifen: ketotifen fumatate at 30 mg/kg, p.o. ### $P < 0.001$, when compared with the non-sensitized group (Student's *t*-test), *** $P < 0.001$, when compared with the vehicle and ovalbumin-sensitized (Control) group (Dunnnett's test), +++ $P < 0.001$, when compared with the Control group (Student's *t*-test).

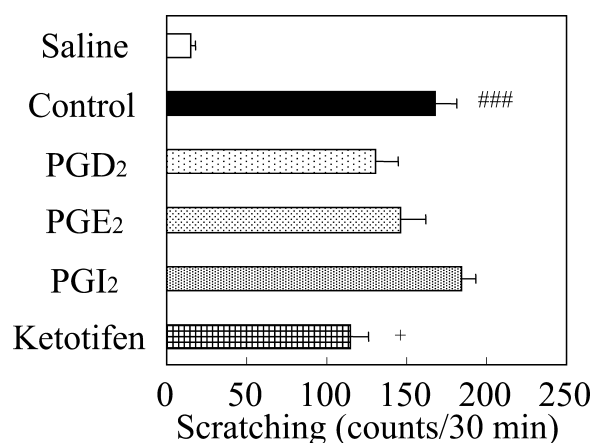


Fig. 2. Effects of PGD₂, PGE₂, PGI₂, and ketotifen on compound 48/80-induced scratching by BALB/c mice. Values are the mean and S.E.M. for 8 mice. Non: saline injection, Control: vehicle, PGD₂: 0.1% prostaglandin D₂, PGE₂: 0.1% prostaglandin E₂, PGI₂: 0.1% prostaglandin I₂, Ketotifen: ketotifen fumatate at 30 mg/kg, p.o. ### $P < 0.001$, when compared with the saline-injected group (Student's *t*-test). + $P < 0.05$, when compared with the Control group (Student's *t*-test).

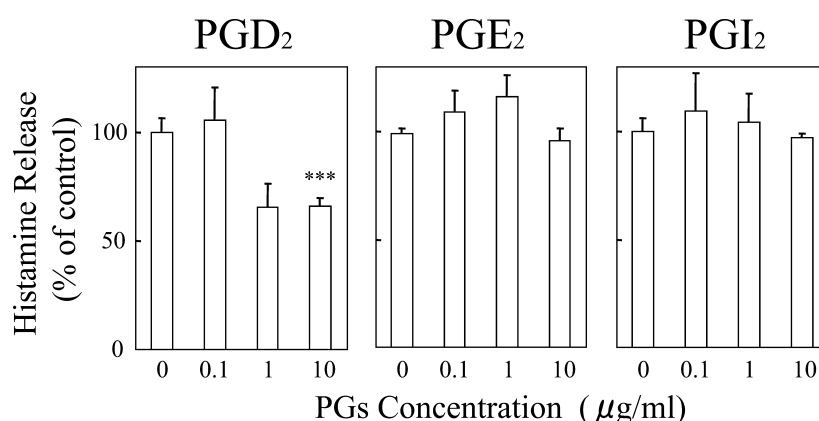


Fig. 3. Effects of PGD₂, PGE₂, and PGI₂ on histamine release from rat basophilic leukemia cell line RBL-2H3. Values are the mean and S.E.M. for 6 experiments. PGD₂: prostaglandin D₂, PGE₂: prostaglandin E₂, and PGI₂: prostaglandin I₂. *** $P < 0.001$, when compared with the vehicle group (Dunnett's test).

RBL-2H3 cells. There was a report that mast cell-deficient WBB6F1-W/W^v mice did not exhibit the IgE-mediated allergic skin reaction (10). In our preliminary experiment, mast cell-deficient WBB6F1-W/W^v mice did not exhibit scratching behavior after injection of OVA. Moreover, anti-histaminergics, ketotifen, and chlorpheniramine completely inhibited OVA-induced scratching behavior in OVA-sensitized mice (9). These results clearly indicated that OVA-induced scratching behavior is mast cell-dependent. It was already reported that PGD₂ inhibited the histamine release from anti-IgE-activated rat peritoneal mast cells (7). Our findings supported previous findings that PGD₂ suppressed a specific receptor that is the predominant prostanoid receptor in rat peritoneal mast cells. These findings suggest that PGD₂ decreased the number of IgE-mediated scratching behaviors by inhibiting IgE-mediated histamine release from mast cells, *in vivo*. As we previously reported, PGD₂ inhibits the scratching behaviors not via histamine H₁ (6). Hence there was a possibility that PGD₂ inhibited the release of other mediators from mast cells in OVA-induced scratching behaviors, but even if this is possible, its potency would be weak. Compound 48/80 is a potent activator of connective tissue-type mast cells and skin mast cells (11), and the cutaneous reaction caused by compound 48/80 has been discussed in relation to mast cell mediators such as histamine (12). Moreover, RBL-2H3 cells have been used in experiments on histamine release (13, 14). To determine the difference between IgE-mediated and non-mediated scratching behaviors, we used 2 types of secretagogues. However, RBL-2H3 cells do not normally respond to basic secretagogues such as compound 48/80 (14). As reported, an histamine H₁ receptor antagonist could not completely inhibit compound 48/80-induced scratching

behaviors (4); hence, scratching behavior occurred mainly via histamine H₁ but other mediators were also involved. In this study, ketotifen weakly inhibited compound 48/80-induced scratching behaviors. Our results support the data (4).

These findings suggest that, PGD₂ suppresses IgE-mediated scratching behavior by inhibiting IgE-mediated histamine release from mast cells.

References

- Williams HC, Burney PGJ, Perbroke AC, Hay RJ. The UK. Working party's diagnostic criteria for atopic dermatitis, III. Independent hospital validation. *Br J Dermatol.* 1994;131:406–416.
- Kuraishi Y, Nagasawa T, Hayashi K, Satoh M. Scratching behavior induced by pruritogenic but not algisogenic agents in mice. *Eur J Pharmacol.* 1995;275:229–233.
- Andoh T, Kuraishi Y. Intradermal leukotriene B₄, but not prostaglandin E₂, induces itch-associated responses in mice. *Eur J Pharmacol.* 1998;353:93–96.
- Sugimoto Y, Umakoshi K, Nojiri N, Kamei C. Effects of histamine H₁ receptor antagonists on compound 48/80-induced scratching behavior in mice. *Eur J Pharmacol.* 1998;351:1–5.
- Jensen C, Norn S, Stahl Skov P, Espersen F, Koch C, Permin H. Bacterial histamine release by immunological and non-immunological lectin-mediated reactions. *Allergy.* 1984;39:371–377.
- Arai I, Takano N, Hashimoto Y, Futaki N, Sugimoto M, Takahashi N, et al. Prostanoid DP₁ receptor agonist inhibits the pruritic activity in NC/Nga mice with atopic dermatitis. *Eur J Pharmacol.* 2004;505:229–235.
- Chan CL, Jones RL, Lau HY. Characterization of prostanoid receptors mediating inhibition of histamine release from anti-IgE-activated rat peritoneal mast cells. *Br J Pharmacol.* 2000;129:589–597.
- Seldin DC, Adelman S, Austen KF, Stevens RL, Hein A, Caulfield JP, et al. Homology of the rat basophilic leukemia cell and the rat mucosal mast cell. *Proc Natl Acad Sci USA.*

- 1985;82:3871–3875.
- 9 Takano N, Arai I, Kurachi M. Analysis of the spontaneous scratching behavior by NC/Nga mice: a possible approach to evaluate antipruritics for subjects with atopic dermatitis. *Eur J Pharmacol.* 2003;471:223–228.
 - 10 Katayama I, Tanei R, Yokozeki H, Nishioka K, Dohi Y. Induction of eczematous skin reaction in experimentally induced hyperplastic skin of Balb/C mice by monoclonal anti-DNP IgE antibody: possible implications for skin lesion formation in atopic dermatitis. *Int Arch Allergy Appl Immunol.* 1990;93: 148–154.
 - 11 Benyon RC, Robinson C, Church MK. Differential release of histamine and eicosanoids from human skin mast cells activated by IgE-dependent and non-immunological stimuli. *Br J Pharmacol.* 1989;97:898–904.
 - 12 Metys J, Frycova H, Soucek R. Inhibition of passive cutaneous anaphylaxis by several histamine (H1) and serotonin antagonists in the rat. *Agents Actions.* 1988;23:331–333.
 - 13 Saito SY, Maruyama Y, Kamiyama S, Nakahata N, Ohizumi Y. Ephedrae herba in Mao-Bushi-Saishin-To inhibits IgE-mediated histamine release and increases cAMP content in RBL-2H3 cells. *J Pharmacol Sci.* 2004;95:41–46.
 - 14 Morimoto Y, Yasuhara T, Sugimoto A, Inoue A, Hide I, Akiyama M, et al. Anti-allergic substances contained in the pollen of *Cryptomeria japonica* possess diverse effects on the degranulation of RBL-2H3 cells. *J Pharmacol Sci.* 2003;92: 291–295.