Effect of Oxatomide on Experimental Allergic Rhinitis in Guinea Pigs

Toshihiko Kaisé, Yukino Akamatsu and Kenji Ohmori

*Drug Development Research Laboratories, Pharmaceutical Research Institute, Kyowa Hakko Kogyo Co., Ltd.,
1188 Shimotogari, Nagaizumi-cho, Sunto-gun, Shizuoka 411, Japan*

Received September 22, 1997 Accepted December 5, 1997

ABSTRACT—We have investigated the effect of oxatomide, an antiallergic agent, on experimental allergic rhinitis in sensitized guinea pigs. Oxatomide (1 and 10 mg/kg, p.o.) significantly inhibited the sneeze response and nasal rubbing after antigen challenge. Oxatomide (10 and 30 mg/kg) reduced the increase in nasal vascular permeability induced by the antigen-antibody reaction. The decreases in nasal cavity volume caused by nasal mucosal swelling 10 min, 30 min and 6 hr after antigen challenge were significantly inhibited by oxatomide (30 mg/kg). These results indicate that oxatomide inhibits the experimental allergic rhinitis in guinea pigs.

Keywords: Oxatomide, Experimental allergic rhinitis

Oxatomide, 1-{3-[4-(diphenylmethyl)-1-piperazinyl]-propyl}-1,3-dihydro-2*H*-benzimidazol-2-one, is an anti-allergic agent that inhibits both the release and the action of chemical mediators such as histamine and peptide leukotrienes (LTs) (1–5). Oxatomide has been clinically demonstrated to have therapeutic efficacies in the treatment of allergic rhinitis (6). In order to support the clinical efficacy of oxatomide, we investigated the effect of the drug on experimental allergic rhinitis in sensitized guinea pigs.

Oxatomide (Janssen, Beerse, Belgium) was suspended in a 0.3% carboxymethylcellulose solution (0.3% CMC) for oral administration. Reagents used were ovalbumin (OVA; Sigma Chemical, St. Louis, MO, USA), *Ascaris suum* allergenic extract (Funakoshi, Tokyo), 2,4-dinitrobenzenesulfonic acid sodium salt, carbamic acid ethylester (urethane) and pontamine sky blue (Tokyo Kasei, Tokyo).

The sneeze response, nasal rubbing and nasal vascular permeability were assessed as previously described (7). Male Hartley guinea pigs (5-week-old; Japan SLC, Shizuoka) were passively sensitized by intravenous injection of anti-OVA guinea pig serum whose 8-day homologous PCA titer was 1 : 128. Eight days after the sensitization, the sneeze response and nasal rubbing were induced by instilling 50 μ l of the 1% OVA solution into a unilateral nostril of the conscious guinea pigs. Oxatomide was administered orally 2 hr before antigen challenge. Numbers of the sneeze response and nasal rubbing after antigen challenge were counted for 30 min. In the measurement of nasal vascular permeability, the nasal cavity

was perfused with saline under anesthesia with urethane. After the dye (2% pontamine sky blue) was injected intravenously and saline was nasally perfused for 10 min (period 1), the perfusion was stopped and 20 μ l of the 1% OVA solution was instilled into the bilateral nostrils. After 10 min, the nasal cavity was perfused again with saline for 10 min (period 2). The dye concentration in each intranasal perfusate (periods 1 and 2) was quantified by spectrometry with the absorbance at 620 nm. The difference in dye concentration between periods 1 and 2 was defined as dye leakage.

Nasal mucosal swelling was evaluated as previously reported (8). *Ascaris suum* allergenic extract was coupled with dinitrophenyl (DNP-*Ascaris*) by the method of Eisen et al. (9). Guinea pigs (5-week-old) were actively sensitized by intraperitoneal injection of DNP-*Ascaris* (3.12 μ g protein) with alumina gel adjuvant 4 times at 2-week intervals. Animals were boosted by inhalation of DNP-*Ascaris* (15.6 μ g protein/ml, for 3 min) beginning 2 weeks after the fourth intraperitoneal injection, and this was repeated every day for 5 days. Animals were used at least 10 days after the final inhalation. The mean antibody titer was 1 : 200 as estimated by 8-day homologous PCA. The antisera were inactivated by heating at 56°C for 2 hr, indicating that these sera mainly contained IgE antibody. The nasal volume of the guinea pig was measured by acoustic rhinometry (GJ Elektronik, Skanderborg, Denmark) under anesthesia with urethane. The details of the acoustic reflection technique have been reported else-

Fig. 1. Effects of oxatamide on sneeze response (A) and nasal rubbing (B) after antigen challenge in passively sensitized guinea pigs. Oxatamide or 0.3% CMC was administered orally 2 hr before the antigen challenge. Sham challenge was made by intranasal instillation of saline instead of the antigen solution. Results are the mean $\pm$ S.E. of 13 animals. +++ P < 0.001, compared with the control group by Wilcoxon's test, and # P < 0.05, ## P < 0.01, compared with the control group by the least-significant difference test.

where (10). Nasal mucosal swelling was evaluated by the volume between the nostril and 2 cm into the nasal cavity. Changes in the sum of the volume of left and right nasal cavities after antigen challenge are expressed as the percentage changes from the pre-challenge volume. Antigen challenge was performed by instilling 20 μ l of the DNP-*Ascaris* solution (1.8 mg protein/ml) into the bilateral nostrils. Nasal volume was measured 10 min, 30 min, 3 hr and 6 hr after antigen challenge.

Data are shown as the mean $\pm$ S.E. Differences were regarded as significant at P < 0.05.

Figure 1 shows the effect of oxatamide on the sneeze response and nasal rubbing developed a few min after antigen challenge. In the control group, 16.8 ± 3.7 sneeze responses (Fig. 1A) and 55.9 ± 13.7 times nasal rubbings

Fig. 2. Effect of oxatamide on the dye leakage after antigen challenge in passively sensitized guinea pigs. Oxatamide or 0.3% CMC was administered orally 2 hr before the antigen challenge. Sham challenge was made by intranasal instillation of saline instead of the antigen solution. Results are the mean $\pm$ S.E. of 8–19 animals. +++ P < 0.001, compared with the control group by Wilcoxon's test, and # P < 0.05, ## P < 0.01, compared with the control group by the least-significant difference test.

(Fig. 1B) were observed 30 min after antigen challenge. Both the sneeze response and nasal rubbing almost completely disappeared by 30 min after antigen challenge.

Fig. 3. Effect of oxatamide on the decrease in the nasal cavity volume after antigen challenge in actively sensitized guinea pigs. Oxatamide or 0.3% CMC was administered 2 hr before the antigen challenge. Sham challenge was made by intranasal instillation of saline instead of the antigen solution. Results are the mean $\pm$ S.E. of 8–9 animals. * P < 0.05, ** P < 0.01, compared with the control group at each time by Wilcoxon's test, and # P < 0.05, compared with the control group at each time by Steel's test. Sham (□), control (■), oxatamide: 0.3 mg/kg (◇), 3 mg/kg (△), 30 mg/kg (○).

Oxatomide significantly inhibited the sneeze response and nasal rubbing at doses of 1 and 10 mg/kg.

Figure 2 shows that dye leakage in the control group ($1.33 \pm 0.32 \mu\text{g/ml}$) was significantly higher than that in the sham group ($0.11 \pm 0.03 \mu\text{g/ml}$). Oxatomide significantly reduced the increase in the dye leakage at doses of 10 and 30 mg/kg.

The basal volume of the nasal cavity in the control group ($126.9 \pm 2.7 \mu\text{l}$) and the sham group ($122.3 \pm 3.3 \mu\text{l}$) did not differ significantly. The percentage changes in nasal cavity volume 10 min, 30 min, 3 hr and 6 hr after antigen challenge were significantly greater in the control group than in the sham group (Fig. 3). Oxatomide significantly inhibited the decreases in nasal cavity volume 10 min, 30 min and 6 hr after antigen challenge at a dose of 30 mg/kg. At a dose of 3 mg/kg, oxatomide suppressed the decrease in volume 10 min and tended to inhibit the decreases at 30 min and 6 hr after antigen challenge.

The inhibitory effect of oxatomide on the sneeze response and nasal rubbing after antigen challenge in guinea pigs suggests that oxatomide may inhibit the sneezing and the nasal pruritus in humans. The mechanisms of the sneeze response and nasal rubbing in animals are considered to be as follows (11): Histamine released from mast cells in nasal mucosa after antigen challenge stimulates trigeminal nerve endings. This stimulation causes nasal pruritus, leading to the behavior of nasal rubbing, and induces the sneeze response via a nerve reflex. The inhibitory effect of oxatomide on the sneeze response and nasal rubbing, therefore, may chiefly result from the inhibition of the release and action of histamine (1).

The inhibitory effect of oxatomide on the dye leakage in guinea pigs after antigen challenge suggest that oxatomide reduces the increase in the nasal vascular permeability. It has been reported that LTs and bradykinin as well as histamine cause an increase in the nasal vascular permeability (12). Oxatomide has been demonstrated to inhibit the release and action of LTs (2–4) and the action of bradykinin (5); therefore, these effects as well as inhibition of the release and action of histamine (1) may be involved in the suppression of the increase in nasal vascular permeability.

Nasal obstruction was assessed by the decrease in nasal cavity volume caused by nasal mucosal swelling. In the present study, the nasal mucosal swelling occurred 6 hr as well as 10 min and 30 min after antigen challenge. It is reported that the late-phase response in the nose associated with nasal swelling occurs in humans several hours after antigen challenge (13). The nasal mucosal swelling observed 6 hr after antigen challenge in guinea pigs may correspond to the late-phase response in the human nose.

The inhibitory effects of oxatomide on the decreases in the nasal cavity volume 10 min, 30 min and 6 hr after antigen challenge suggest that oxatomide inhibits the nasal mucosal swelling in both the early- and late-phase responses. The nasal mucosal swelling is induced by both mucosal edema as a result of increased vascular permeability and dilation of capacitance vessels; therefore, the inhibitory effect of oxatomide on the increase in nasal vascular permeability is likely to contribute to the suppression of the nasal mucosal swelling.

Studies on the pathogenesis of nasal mucosal swelling such as evaluation of swelling after intranasal instillation of chemical mediators and measurement of concentration of each chemical mediator in nasal lavage fluid after antigen challenge in patients with allergic rhinitis have shown that histamine, LTs, bradykinin and prostaglandin D_2 (PGD_2) play important roles in the development of nasal mucosal swelling in both early- and late-phase responses (12, 14, 15). Oxatomide has been shown to inhibit the release and action of histamine (1), the release and action of LTs (2–4), the action of bradykinin (5) and the release of PGD_2 (3). These effects of oxatomide may contribute to the inhibition of nasal mucosal swelling. Our previous study using the same model demonstrated that ketotifen, an anti-allergic agent, inhibited the nasal mucosal swelling in the early-phase response but did not suppress that in the late-phase response (8). In the nasal mucosal swelling in the late-phase response, LTs are deeply involved in the pathogenesis. The source of the LTs' release in the late-phase response is suspected to be eosinophils (13). Oxatomide has been demonstrated to inhibit the release of LTs from eosinophils (4) as well as that from mast cells (3). The time course of infiltration of eosinophils into the nasal mucosa after antigen challenge and the effect of oxatomide on the infiltration of eosinophils in this guinea pig model remain to be elucidated.

In conclusion, oxatomide inhibits the sneeze response, nasal rubbing, increase in nasal vascular permeability and nasal mucosal swelling after antigen challenge in sensitized guinea pigs. These results suggest that oxatomide may inhibit sneezing, pruritus rhinorrhoea and nasal obstruction in humans and support the clinical efficacy of oxatomide in the treatment of allergic rhinitis.

Acknowledgments

We would like to thank Dr. Y. Sakakura (Mie University School of Medicine) for his help and advice on these experiments and manuscript.

REFERENCES

- 1 Awouters F, Niemegeers CJE, Van Den Berk J, Van Neuten JM, Lenaerts FM, Borgers M, Schellekens KHL, Broeckaert A, De Cree J and Janssen PAJ: Oxatomide, a new orally active

- drug which inhibits both the release and the effects of allergic mediators. *Experientia* **33**, 1657–1659 (1977)
- 2 Ohmori K, Ishii H, Kubota T, Shuto K and Nakamizo N: Inhibitory effects of oxatomide on several activities of SRS-A and synthetic leukotrienes in guinea-pigs and rats. *Arch Int Pharmacodyn Ther* **275**, 139–150 (1985)
 - 3 Kosaka Y, Kawabe H and Ishii A: Oxatomide inhibits the release of bronchoconstrictor arachidonic acid metabolites ($iLTC_4$ and PGD_2) from rat mast cells and guinea-pig lung. *Agents Actions* **21**, 32–37 (1987)
 - 4 Manabe H, Ohmori K, Tomioka H and Yoshida S: Oxatomide inhibits the release of chemical mediators from human lung tissues and from granulocytes. *Int Arch Allergy Immunol* **87**, 91–97 (1988)
 - 5 Ohmori K, Ishii H, Nito M, Shuto K and Nakamizo N: Pharmacological studies on oxatomide (KW-4354) (7). Antagonistic effects on chemical mediators. *Folia Pharmacol Jpn* **81**, 399–409 (1983) (Abstr in English)
 - 6 Juniper EF, Cartier A, Trebilcock AL, Frith PA, Dolovich J and Hargreave FE: Effects of oxatomide compared with chlorpheniramine in allergic rhinoconjunctivitis. *Clin Allergy* **11**, 61–66 (1981)
 - 7 Kaise T, Manabe H and Ohmori K: The effect of KW-4679, an anti-allergic drug, on experimental allergic rhinitis. *Jpn J Allergol* **44**, 1229–1233 (1995) (Abstr in English)
 - 8 Kaise T, Ohmori K, Sakakura Y and Ukai K: The effect of KW-4679, an antiallergic drug, on experimental allergic rhinitis in guinea pigs: Effects on nasal blockage. *Jpn J Pharmacol* **69**, 435–438 (1995)
 - 9 Eisen HN, Belman S and Carsten ME: The reaction of 2,4-dinitro-benzenesulfonic acid with free amino groups of proteins. *J Am Chem Soc* **75**, 4583–4585 (1953)
 - 10 Pedersen OF, Yamagiwa M, Miyahara Y and Sakakura Y: Nasal cavity dimensions in guinea pigs measured by acoustic reflections. *Am J Rhinol* **8**, 299–304 (1994)
 - 11 Saito H, Asakura K and Kataura A: The in vivo effects of anti-ICAM antibody on OA sensitized rats. *Jpn J Allergol* **43**, 579–584 (1994) (Abstr in English)
 - 12 White MV and Kaliner MA: Mediators of allergic rhinitis. *J Allergy Clin Immunol* **90**, 699–704 (1992)
 - 13 Terada N, Konno A and Togawa K: Biochemical properties of eosinophils and their preferential accumulation mechanism in nasal allergy. *J Allergy Clin Immunol* **94**, 629–642 (1994)
 - 14 Howarth P, Walsh S and Robinson C: The comparative nasal effects of prostaglandin D_2 in normal and rhinitic subjects. *Adv Prostaglandin Thromboxane Leukot Res* **21A**, 449–452 (1991)
 - 15 Naclerio RM, Proud D, Togias AG, Adkinson NF Jr, Meyers DA, Kagey-Sobotka A, Plaut M, Norman PS and Lichtenstein LM: Inflammatory mediators in late antigen-induced rhinitis. *N Engl J Med* **313**, 65–70 (1985)