Effects of a Specific Cysteinyl Leukotriene Antagonist, Pranlukast, on Antigen-Induced Cysteinyl Leukotriene-Mediated Rhinitis in Guinea Pigs

Manabu Fujita, Yasuo Yonetomi, Hiroshi Takeda, Naoki Nakagawa, Kazuhiro Kawabata* and Hiroyuki Ohno
Minase Research Institute, Ono Pharmaceutical Co., Ltd., 3-1-1 Sakurai, Shimamoto-cho, Mishima-gun, Osaka 618, Japan

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ABSTRACT—To examine the effects of a specific cysteinyl leukotriene (cysLT) antagonist, pranlukast, on allergic rhinitis, antigen-induced rhinitis in guinea pigs was modified by pretreatment with an cyclooxygenase inhibitor (indomethacin) followed by an H1-blocker (pyrilamine). Intranasal ovalbumin (OVA) administration in actively sensitized guinea pigs resulted in concentration-dependent increases in nasal permeability and nasal airway resistance (NAR). Although pyrilamine (1 mg/kg, i.v.) abolished these antigen-induced changes, pretreatment with indomethacin (5 mg/kg, i.v.) followed by pyrilamine enhanced these responses to a degree similar to that observed with OVA challenge alone. Analyses of nasal perfusate in indomethacin/pyrilamine-pretreated animals showed that cysLTs increased by 270.8%, whereas thromboxane B2 decreased by 88.3% as compared with those on challenged with OVA alone. Oral administration of pranlukast (1–10 mg/kg) dose-dependently prevented increases in nasal permeability and NAR of indomethacin/pyrilamine-pretreated animals. However, an anti-allergic agent, azelastine, did not affect these responses. These results indicate that pranlukast suppresses antigen-induced cysLT-mediated responses of allergic rhinitis in actively sensitized guinea pigs. A cysLT antagonist, pranlukast, may thus prevent cysLT-mediated symptoms of allergic rhinitis.

Keywords: Pranlukast, Rhinitis, Leukotriene, Allergic, Ovalbumin

Nasal symptoms of allergic rhinitis often display a biphasic pattern after antigen provocation. While the early-phase symptoms (up to 1 hr after antigen provocation) such as sneezing and rhinorrhea are transient, the late-phase symptom (4–8 hr after antigen provocation) characterized by nasal congestion is persistent (1). Mediators that have been suggested to participate in these symptoms include histamine, cyclooxygenase metabolites of arachidonic acid and cysteinyl leukotrienes (cysLTs) such as leukotriene C4, D4 and E4 (LTC4, LTD4 and LTE4), main components of the slow-reacting substance of anaphylaxis (SRS-A) (2). Among these mediators, cysLTs are of particular interest as they have been suggested to play a role in nasal congestion (3, 4), a symptom which is resistant to many therapeutic agents including current anti-histaminergics (5). Thus, agents that may antagonize or inhibit the action of cysLTs (anti-cysLTs) may be useful in the treatment of allergic rhinitis.

Although interest has focused on the therapeutic effects of anti-cysLTs against allergic rhinitis, current findings remain inconsistent. Clinical studies to evaluate the effects of anti-cysLTs on allergic rhinitis have produced controversial results (6, 7), and there is lack of an appropriate animal model for allergic rhinitis to evaluate relevant effects of anti-cysLTs. This study was designed to examine the effects of a specific cysLT antagonist, pranlukast, which has previously been demonstrated to be a useful agent for treatment of bronchial asthma (8) with a novel model of antigen-induced rhinitis in guinea pigs. We thus employed pretreatment with indomethacin followed by pyrilamine to pharmacologically modify and develop an antigen-induced cysLT-mediated response in guinea pigs (9).

MATERIALS AND METHODS

Animals
Male Hartley guinea pigs (Nihon Rabbit, Osaka),
weighing 300 to 600 g, were used throughout the experiments. Animals were housed in an air-conditioned room at 23 ± 2°C and 55 ± 5% humidity with a 12-hr light/dark cycle. Animals were given food and water ad libitum.

Chemicals

The synthesis of pranlukast (4-oxo-8-[4-(4-phenylbutoxy)benzoylaminol]-2-(tetrazol-5-yl)-4H-1-benzopyran hemihydrate) and extraction of azelastine hydrochloride from Azeptine® (Eizai, Tokyo) were conducted in our laboratories. Other chemicals used in this study were: indomethacin, pyrilamine and ovalbumin (OVA) (Sigma, St. Louis, MO, USA); a specific radioimmunoassay kit for LTC₄, LTD₄ and LTE₄ (Amersham International plc., Buckinghamshire, UK); Evans blue (Tokyo Kasei, Tokyo) and killed Bordetella pertussis (Kaketu Lab., Kumamoto). OVA, pyrilamine and Evans blue were dissolved in saline. Indomethacin was dissolved in 7% sodium bicarbonate solution. Pranlukast and azelastine were suspended in 0.5% sodium carboxymethylcellulose solution for oral administration.

Active sensitization

Guinea pigs were actively sensitized by i.p. injection of 1 mg of OVA containing 5 × 10⁹ killed Bordetella pertussis in 0.5 ml saline on day zero. The sensitized animals on day 14–21 post-OVA injection were used for experiments.

Antigen-induced rhinitis

Changes in nasal permeability: Sensitized guinea pigs, anesthetized with Nembutal (75 mg/kg, i.p.), were placed on a warm bed and cannulation of lungs and nasal cavities and other surgical procedures were repeated as described above. The lung cannula was connected to a constant volume respirator (Model SN-480-7; Shinano Apparatus, Tokyo), and the animals were artificially ventilated with a constant volume of 5 ml at 70 strokes/min. The intranasal flow of nasal cavity cannula was aerated with an air pump at a rate of 500 ml/min. Changes in nasal airway resistance (NAR) under conditions of constant airflow were measured with a pressure transducer (Valdyne: Model DP 45-24-2114; Gould, Northridge, CA, USA) connected to the side-arm of the nasal cavity cannula. After stabilization of the air flow (5 min), the basal nasal airway pressure was measured. The air pump was then paused and OVA (0.03–1%, 1 ml) was instilled into the nasal cavity for 5 min. After completely flushing out OVA with air, NAR was measured for 30 min. Indomethacin (5 mg/kg, i.v.) and pyrilamine (1 mg/kg, i.v.) were pretreated as described above.

Measurement of cysLTs and TXB₂: Contents of cysLTs were determined with a LTC₄/D₄/E₄ multispecific RIA kit (Amersham International plc). The procedure for extraction was as follows: Briefly, 0.5 ml of nasal perfusate was mixed with 2 ml of ethanol, and then the mixture was allowed to stand for 30 min at 4°C followed by mixing. After centrifugation at 1700 × g for 5 min at 4°C, the supernatant was evaporated to dryness. The dehydrated supernatant was then dissolved in the assay buffer (0.05 M phosphate buffer, pH 7.4, containing 0.14 M NaCl and 0.01% gelatin) and thereafter used as samples. Radioimmunoassay was performed according to the kit manual. Briefly, aliquots of samples or standards were incubated with tracer ([³H]LTC₄) and anti-cysLTs serum at 4°C for 18–24 hr. Dextran-coated charcoal was added to remove unbound cysLTs. After centrifugation of the suspension at 1700 × g for 10 min, the supernatants were transferred to a scintillation vial with 10 ml of scintillation fluid. Radioactivity was measured with a scintillation counter for 1 min. The cross-reactivities of this anti-serum to LTC₄, LTD₄ and LTE₄ were 100%, 100% and 41%, respectively. This RIA is sensitive to 15.6 pg/50 μl of cysLTs.

Concentrations of TXB₂ in the nasal lavage fluid were measured by the enzyme immunoassay manufacturer's protocol (Cayman Chemical Co., Ann Arbor, MI, USA) according to the kit manual. Briefly, aliquots of sample or standards were incubated with tracer TXB₂ linked to acetylcholinesterase and anti-TXB₂ serum on 96-well plates precoated with mouse monoclonal antibody for OVA perfusion, eliminated possible effects attributed to endogenous prostaglandins and histamine, respectively.
18 hr at room temperature. The wells were washed with the supplied buffer and the substrate of acetylcholine, Ellman's reagent, was added to each well. After a 60- to 90-min incubation, the intensity of developed color was measured spectrophotometrically at 412 nm (range: 405–420 nm). The lower detection limit of this assay is 7.8 pg/ml.

Drug administration
Pranlukast and azelastine were orally administered 1 hr before OVA challenge in overnight-fasted animals.

Statistical analyses
Results are expressed as the mean ± S.E.M. Student's t-test or two-way analysis of variance followed by Dunnett's t-test were used to verify statistical significance of difference between pairs of groups. P values of less than 0.05 were considered to be significant.

RESULTS

Nasal permeability in OVA-induced rhinitis
Nasal perfusion with OVA concentration-dependently increased the permeability in actively sensitized animals at a concentration of less than 2%. The total amounts of dye recovered at 10-min intervals with 40-min perfusion with saline, 0.5%, 0.75%, 1% and 2% OVA were 4.3, 32.3, 56.1, 98.2 and 96.6 μg/40 min, respectively (N=3-5). The significant increase in nasal permeability with 1% OVA perfusion was comparable to that seen in a preliminary study on the concentration-response with OVA. The increase in permeability peaked 10 to 20 min after OVA perfusion and decreased thereafter. Pretreatment of sensitized animals with the H₁-blocker, pyrilamine (1 mg/kg, i.v.), markedly reduced the increase in permeability due to OVA perfusion. However, in contrast, pretreatment with a cyclooxygenase inhibitor, indomethacin (5 mg/kg, i.v.) followed by pyrilamine (1 mg/kg, i.v.), enhanced the permeability to a degree similar to that with OVA perfusion alone (Fig. 1).

NAR in OVA-induced rhinitis
We used 0.1%-OVA for instillation in this experiment as this concentration maximized the increase in NAR: For the area under the curve (AUC) analysis with saline, the NAR values induced by saline, 0.03%, 0.1%, 0.3% and 1%-OVA were 139.3, 242.4, 313.7, 332.7 and 332.3 cmH₂O·min, respectively (N=5). As shown in Fig. 2, OVA (0.1%) administration into the nasal cavity increased NAR in actively sensitized guinea pigs with the effect peaking at 10 min post-antigen administration and persisting at high levels thereafter. Similar to nasal permeability, pretreatment of sensitized animals with pyrilamine (1 mg/kg, i.v.) markedly reduced the NAR response. However, in contrast, pretreatment with indomethacin (5 mg/kg, i.v.) followed by pyrilamine (1 mg/kg, i.v.) enhanced the NAR to a value similar to that induced by OVA alone.

Contents of cysLTs and TXB₂ in the nasal perfusate of OVA-induced rhinitis
Nasal OVA (1%) perfusion increased the concentrations of TXB₂ (Fig. 3), a stable metabolite of thromboxane A₂ (TXA₂), and cysLTs (Fig. 4) in the perfusate of sensitized guinea pigs. Both mediators peaked 10 to 20 min after OVA perfusion. Indomethacin (5 mg/kg)/pyrilamine (1 mg/kg)-pretreatment abolished the TXA₂ increase (Fig. 3) but enhanced the cysLTs contents (Fig. 4).
Fig. 2. Changes in nasal airway resistance (NAR) in actively sensitized guinea pigs after nasal OVA challenge. Control (●), pyrilamine-pretreated (■) and indomethacin/pyrilamine-pretreated (▲) animals were nasally instilled with 0.1% OVA solution. Nasal airway resistance was measured at 5-min intervals for the indicated periods. Saline-challenged animals (○) were instilled with saline instead of OVA solution. Animals were pretreated with pyrilamine and indomethacin as described in Fig. 1. Results are shown as the means±S.E.M. of five animals. Statistical comparisons based on AUC between challenged and either saline or control animals where P<0.01 with Student's t-test are marked by ** and †, respectively.

Fig. 3. Changes in thromboxane B₂ concentrations in nasal perfusate of actively sensitized guinea pigs after nasal OVA challenge. Control (●) and indomethacin/pyrilamine-pretreated (■) animals were nasally perfused with 1% OVA solution for 10 min. Saline-challenged animals (○) were nasally perfused with saline instead of OVA solution. Animals were pretreated with pyrilamine and indomethacin as described in Fig. 1. Results are shown as the means±S.E.M. of ten animals. Statistical comparisons based on AUC between challenged and either saline or control animals where P<0.01 with Student's t-test are marked by ** and †, respectively.

Fig. 4. Changes in cysteinyl leukotrienes (cysLTs) in nasal perfusate of actively sensitized guinea pigs after nasal OVA challenge. Control (●) and indomethacin/pyrilamine-pretreated (■) animals were nasally perfused with 1% OVA solution for 10 min. Saline-challenged animals (○) were nasally perfused with saline instead of OVA solution. Animals were pretreated with pyrilamine and indomethacin as described in Fig. 1. Results are shown as the means±S.E.M. of ten animals. Statistical comparisons based on AUC between challenged and either saline or control animals where P<0.01 (**) and where P<0.05 (*), respectively, were verified with Student's t-test.
by 270.8% when the values of AUC from 0 to 60 min after OVA challenge were compared with those of OVA alone.

**Effects of pranlukast and azelastine**

Ovalbumin challenge resulted in increases of nasal permeability and NAR in sensitized and indomethacin (5 mg/kg)/pyrilamine (1 mg/kg)-pretreated animals. Oral

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**Fig. 5.** Effects of pranlukast and azelastine on increases in nasal permeability of actively sensitized guinea pigs after OVA (1%) perfusion. Guinea pigs were pretreated with pyrilamine and indomethacin as described in Fig. 1. In the saline-challenged group, sensitized animals were challenged with saline. Either pranlukast or azelastine was administered 1 hr before OVA challenge. Results are shown as the means ± S.E.M. of total dye content 0–60 min after OVA challenge in three to five animals. Statistical comparisons of the challenged group with either saline-treated animals where P < 0.05 (†) or control animals where P < 0.01 (**) were verified with Student's t-test or Dunnett's t-test, respectively.

**Fig. 6.** Effects of pranlukast and azelastine on increases in nasal airway resistance (NAR) of actively sensitized guinea pigs after OVA (0.1%) challenge. Guinea pigs were pretreated with pyrilamine plus indomethacin as described in Fig. 1. In the saline-challenged group, sensitized animals were challenged with saline. Pranlukast or azelastine was administered 1 hr before OVA challenge. Results are shown as the means ± S.E.M. of AUC of NAR 0–30 min after OVA challenge in six animals. Statistical comparisons of the challenged group with either saline-treated animals where P < 0.01 (#) or control animals where P < 0.01 (**) were verified with Student's t-test or Dunnett's t-test, respectively.
administration of a specific cysLT antagonist, pranlukast, suppressed the increase in nasal permeability (Fig. 5) and NAR (Fig. 6) in a dose-dependent manner. Suppression of permeability and NAR with 1, 3 and 10 mg/kg of pranlukast were 0.1%, 71.3% and 88.5% and 37.5%, 75.6% and 95.6%, respectively. Based on the results of AUC analysis, suppression of nasal permeability and NAR were significant at doses higher than 3 mg/kg. The ED50 values of pranlukast were 4.4 and 1.4 mg/kg (p.o.) for inhibition of the increases in nasal permeability and NAR, respectively. However, an anti-allergic agent, azelastine, failed to reduce either nasal permeability or NAR even at a p.o. dose of 10 mg/kg.

**DISCUSSION**

This study shows that indomethacin/pyrilamine-pretreatment followed by nasal OVA challenge facilitated nasal permeability and NAR in actively sensitized guinea pigs. These increases, comparable to those induced by OVA alone, were associated with significantly enhanced cysLTs and decreased TxB2 in the nasal perfusate. The specific cysLT antagonist, pranlukast (10) dose-dependently attenuated the nasal symptoms induced in indomethacin/pyrilamine-pretreated animals, whereas the anti-allergic agent azelastine (11) did not affect these symptoms. This is the first report on a cysLT-dependent model of allergic rhinitis and its prevention by a cysLT antagonist.

Evaluation of agents that prevent the action of cysLTs (anti-cysLTs) in antigen-induced allergy models has been unreliable, because single antigen challenge generally induces only the histamine-dominated early-phase responses but not the late-phase response, where the involvement of cysLTs has been considered to be more prominent. In fact, the histamine-dependency of the early-phase response is supported by our results: pyrilamine pretreatment potently blocked the OVA-induced increases in nasal permeability and NAR in sensitized guinea pigs (Figs. 1 and 2), whereas pranlukast did not prevent these responses at least at 10 mg/kg (p.o.) (unpublished data). In contrast, the cysLT-dependency of late phase response is best illustrated by the observation that cysLT antagonists more efficiently block the late-phase bronchoconstriction than that in the early-phase in experimental models of bronchial asthma (12).

Our findings, however, suggest that a single OVA challenge in sensitized guinea pigs can induce significant nasal symptoms even after eliminating the contributions of histamine and cyclooxygenase products of prostaglandins by pyrilamine and indomethacin, respectively. These symptoms are cysLTs-dependent, as indicated by enhanced cysLTs in nasal perfusate and marked suppression of these symptoms by pranlukast. The cysLT-dependency is similar to that reported by Anderson et al. (13) who showed that pharmacological modification causes antigen-induced cysLT-mediated bronchoconstriction in sensitized guinea pigs. It has been considered that pretreatment with indomethacin diverts metabolites of arachidonic acid to cysLTs by an alternate pathway, possibly by the lipoxygenase pathway (14). Thus, our models may be useful for the study of agents with in vivo antagonistic or inhibitory activities against cysLTs in antigen-induced rhinitis. However, one must be careful to note that agents demonstrating efficacy in this model are not necessary considered to be effective on antigen-induced rhinitis.

It has been reported that antigen-induced cysLT-mediated increases in bronchial airway resistance display slower onset (1 to 2 min after antigen challenge) and development when compared with those induced by antigens alone in guinea pigs (9), depicting the characteristic responses of cysLTs as SRS-A (9, 13). However, we did not observe such delays in pyrilamine/indomethacin-pretreated animals, at least within the observation period of this study. This discrepancy may be due the different mechanisms of cysLTs action in increasing airway resistance at different sites. This suggests that LTD4 increases bronchial airway resistance via bronchoconstriction (15) and increases NAR via edema probably due to increases in vascular permeability (16) and volume of nasal mucosa (17). Follow-up studies within different time intervals are warranted to further characterize the cysLT-mediated response of antigen-induced rhinitis in guinea pigs.

An earlier study has shown that, on a weight basis, LTD4 is approximately 5000 times more potent than histamine in inducing nasal secretion and NAR increases in humans. Although the maximum response induced by LTD4 is less potent than that of histamine, the increase in NAR (an index of nasal congestion) is notably more persistent and similar to that induced by antigens (3). Cysteinyl leukotrienes have been found during the late-phase in human nasal lavage fluid (18). Although histamine is widely recognized as the major mediator of early-phase responses such as sneezing and rhinorrhea and anti-histaminergic are effective against early-phase responses, these agents have negligible effects on nasal congestion (19, 20). These results together with our observations that pranlukast potently reduced the increases in NAR and permeability support a potential role of cysLTs in allergic rhinitis, especially in relation to nasal congestion.

As shown in this study, pranlukast suppressed OVA-induced increases in nasal permeability and NAR with ED50 values of 4.4 and 1.4 mg/kg in pyrilamine/indomethacin-pretreated animals, respectively. These values
of pranlukast are similar to those reported previously in preventing antigen-induced cysLT-mediated increases in bronchial airway resistance (8). These findings suggest that the antagonistic activities of this compound coincide with those effective against antigen-induced cysLT-mediated bronchoconstriction and rhinitis symptoms. Thus, pranlukast may potently antagonize endogenous cysLTs in both the lower and upper airway.

Previous clinical studies with anti-cysLTs have been inconclusive. Although A-64077, a selective 5-lipoxygenase inhibitor, attenuates nasal congestion (21), this compound also inhibits the synthesis of LTB4 (22). The effects of ICI 204,219 and L-649923 (potent cysLT antagonists) are controversial (6, 7). Furthermore, since the antagonistic activities of these compounds on antigen-induced rhinitis are unclear, the contributions of other pharmacological effects cannot be excluded in their dosings. Therefore, cautious interpretation of results is warranted, especially in understanding the effects of cysLTs on allergic rhinitis. On the other hand, azelastine has been reported to have both anti-cysLT and anti-histaminergic activity (23), however this agent did not prevent cysLT-mediated symptoms of allergic rhinitis at least in our study. In contrast to these agents, pranlukast may specifically antagonize the action of cysLTs in allergic rhinitis at a dose that has previously been used in clinical trials on bronchial asthma. Thus, it may be useful in understanding the effects of specific cysLT antagonism in allergic rhinitis. Results of a clinical trials with pranlukast against allergic rhinitis, which are currently under way, may provide answers to these questions.

REFERENCES