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Involvement of cysteinyl leukotrienes in biphasic increase of nasal airway resistance of antigen-induced rhinitis in guinea pigs

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Abstract

We examined the effect of a specific cysteinyl leukotriene (LT) receptor antagonist, 4-oxo-8-[4-(4-phenylbutoxy)benzoylamino]-2-(tetrazol-5-yl)-4*H*-1-benzopyran hemihydrate (pranlukast), on a novel model of allergic rhinitis induced by repeated intranasal ovalbumin challenge in actively sensitized guinea pigs. Repeated intranasal ovalbumin challenge caused a biphasic increase of nasal airway resistance, peaking 0.5 and 4 h after the final challenge. The early-phase response was accompanied by an increase in sneezing and nasal secretion, while that in the late phase was associated with edema and eosinophil infiltration of the nasal mucosa. Analysis of nasal lavage fluid showed that cysteinyl LTs increased in both phases. Pranlukast, when administered 1 h before every ovalbumin challenge, dose-dependently suppressed the increase of nasal airway resistance in the early- and late phase with evidence of histopathological improvements in the late phase. Pranlukast, however, failed to suppress sneezing and nasal secretion. We suggest that cysteinyl LTs play an important role in allergic rhinitis especially in the nasal obstruction due to edema of the nasal mucosa membrane.

Keywords: Rhinitis; Ovalbumin; Pranlukast; Leukotriene; Histamine

1. Introduction

Allergic rhinitis is a common immunologic disease, affecting 20% of the world population (Settipane, 1986). Nasal symptoms, appearing when allergic persons are exposed to antigens, often display a biphasic response (Pelikan, 1978). Both types of nasal response, marked by changes in the nasal mucosa, are accompanied by nasal mucosal infiltrates consisting mainly of eosinophils (Pelikan and Pelikan-Filipek, 1988, 1989). While the early-phase nasal response associated with sneezing, itching, hypersecretion and nasal obstruction lasts for minutes, the late-phase nasal response associated predominantly with hypersecretion and nasal obstruction lasts for some hours (Pelikan, 1978; Davies et al., 1992; Pelikan et al., 1997). Although the current main therapeutics, histamine H1 receptor antagonists, are effective in, and prevent, only some of the nasal symptoms such as sneezing, itching and partly hypersecretion, they are not significantly effective to influence and prevent nasal obstruction due to edema of the nasal mucosa membrane (Oosten et al., 1997; Pelikan et al., 1997). Among the possible chemical mediators which contribute to nasal symptoms, such as histamine and arachidonic acid metabolites, cysteinyl leukotrienes (LTs) may be of particular interest in edema of the nasal mucosa membrane causing nasal obstruction (Naclerio et al., 1991).

Cysteinyl LTs such as leukotriene C_4, D_4 and E_4 (LTC_4, LTD_4 and LTE_4) are released from various inflammatory cells including mast cells and eosinophils. These mediators are able to increase nasal vascular permeability (Shirasaki et al., 1992) and nasal blood flow (Bisgaard et al., 1986). A recent human study has shown that nasal provocation by LTD_4 but not histamine can induce nasal obstruction, as indicated by a prolonged increase of nasal airway resistance (Okuda et al., 1988). Furthermore, it has been demonstrated that the nasal congestion in the early-phase and the late-phase is accompanied by a significant

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increase of cysteinyl LTs in nasal lavage fluid in patients with allergic rhinitis (Naclerio et al., 1991). These findings suggest the hypothesis that cysteinyl LTs play an important role in allergic rhinitis, especially in nasal obstruction due to edema of the nasal mucosa membrane. However, results of clinical study using structurally different cysteinyl LT receptor antagonists are controversial (Flowers et al., 1990; Donnelly et al., 1995). To further define the role of cysteinyl LTs in allergic rhinitis, we have developed a novel model of allergic rhinitis in sensitized guinea pig which exhibits both the early-phase and the late-phase nasal response and examined the effects of a cysteinyl LT receptor antagonist, pranlukast (Nakagawa et al., 1992), on nasal symptoms associated with this model.

2. Materials and methods

2.1. Animals

Male Hartley guinea pigs (Nihon Rabbit, Osaka, Japan), weighing approximately 350 g, were used for active sensitization. The animals were housed in an air-conditioned room at 23°C ± 2 and 55 ± 5% humidity with alternating 12 h light/dark cycles and were given food and water ad libitum. Animal experiments were performed in accordance with the institutional animal care guideline of Ono Pharmaceutical.

2.2. Chemicals

Synthesis of the cysteinyl LT receptor antagonist, 4-oxo-8-[4-(4-phenylbutoxy) benzoylamino]-2-(tetrazol-5-yl)-4*H*-1-benzopyran hemihydrate (pranlukast), and extraction of an histamine H1 receptor antagonist, epinastine, from Allesion® tablets (Nippon Boehringer Ingelheim, Hyogo, Japan) were conducted in our laboratories. Ovalbumin was purchased from Seikagaku Kogyo (Tokyo, Japan) and a multispecific enzyme immunoassay kit for LTC_4, LTD_4 and LTE_4 was purchased from Amersham International (Buckinghamshire, UK). Ovalbumin was dissolved in saline. Pranlukast and epinastine for oral administration were suspended in 0.5% sodium carboxymethylcellulose solution.

2.3. Active sensitization and challenge

Guinea pigs were actively sensitized by an intraperitoneal injection of 0.5 mg ovalbumin in 0.5 ml saline on day 0 and 1 mg ovalbumin in 0.5 ml saline on day 2. Sensitized guinea pigs were repeatedly instilled with either 40 μl of ovalbumin solution (0.1, 0.2, 0.4, 0.5, 1 and 1%) or the same volume of saline into each nasal cavity on days 22, 24, 27, 31, 36 and 41, respectively. The ovalbumin-induced nasal symptoms were evaluated using the following methods.

2.4. Effects of repeated intranasal ovalbumin challenge in actively sensitized guinea pigs

2.4.1. Changes in nasal airway resistance

Respiratory resistance, using the modified method described by Yamauchi et al. (1984), was considered to be the nasal airway resistance (Narita and Asakura, 1993). Briefly, after the challenge, the guinea pig was placed inside a body plethysmograph with its head outside the chamber. The gap between the animal and the chamber was sealed with silicone rubber. The respiratory airflow from the face mask at the snout and the oscillating pressure in the body chamber at 2 cm H_2O with 30 Hz sine wave pressure were recorded (ANIMAL-ASTO, Model TMC-2100, Chest, Tokyo, Japan) with a differential pressure transducer at various time points. These signals were displayed simultaneously on an *X–Y* oscilloscope and recorded on a polygraph. The nasal airway resistance was calculated using the following formula:

$$\text{nasal airway resistance} = \text{pressure}/\text{flow}\left(\text{cm}\,H_2O\,\text{ml}^{-1}\,\text{s}^{-1}\right).$$

After baseline measurement of nasal airway resistance, the percentage increase in nasal airway resistance vs. time was measured 0.5, 2, 3, 4, 5 and 6 h after ovalbumin instillation. The animals were placed in the chamber several minutes before the measurement to allow them to settle down and become familiarized with the conditions of the experiment.

2.4.2. Sneezing and nasal secretion

After the final ovalbumin challenge, sneezing and nasal secretion were measured in the same animal at various time points. After sneezing was counted manually, cotton threads measuring 3 cm long by 1 mm in diameter and weighing approximately 15 mg (Kitagawa Eisei Zairyou, Osaka, Japan) were inserted in both nostrils and nasal secretion was absorbed for 1 min. The increase in thread weight was regarded as the quantity of nasal secretion.

2.4.3. Changes in cysteinyl LTs concentration and histopathological features

The animals were killed under urethane anesthesia by bleeding either 0.5 or 4 h after the final ovalbumin challenge. A cannula was inserted into the nasal side-section from the bronchi for nasal lavage. Nasal cavities were then washed five times with 5 ml of saline containing 0.1% bovine serum albumin (25 ml in total) and the nasal lavage fluid was collected. The cysteinyl LTs in nasal lavage fluid were extracted as follows. The nasal lavage fluid was centrifuged for 10 min ($100 \times g$, 4°C) and 20 ml of the supernatant was adjusted to pH 3–4 by adding 100 μl of 1 M acetic acid. The samples were then applied to a SEP-PAK® C_{18} cartridge column (Waters Assoc., Milford, MA, USA) which had been washed with methanol-distilled water (2:4, v/v). After washing the column with ethyl

acetate-distilled water (3:10, v/v), cysteinyl LTs were eluted with 2 ml of methanol and were freeze-dried. The dehydrated eluate was then dissolved in the buffer of the enzyme immunoassay kit and used in the assay of cysteinyl LTs. The concentration of cysteinyl LTs was determined with a multispecific enzyme immunoassay kit for LTC_4, LTD_4 and LTE_4. Briefly, aliquots of samples or standards were incubated with anti-LTC_4, LTD_4 and LTE_4 serum in 96-well plates precoated with anti-IgG. The peroxidase ligand that bound to the anti-IgG was immobilized for 3 h at −4°C. Wells were washed with the buffer supplied and the substrate of peroxidase, 3′,3′,5′,5′-tetraethylbenzidine hydrogen peroxide, was added to each well. After 30-min incubation, the intensity of the color developed was measured spectrophotometrically at 450 nm. The amount of cysteinyl LTs in nasal lavage fluid was expressed as picogram per 20 ml per lavage. The cross-reactivities of this anti-serum to LTC_4, LTD_4 and LTE_4 were 100%, 100% and 70%, respectively. This assay is sensitive to 0.75 pg/50 μl of cysteinyl LTs.

The procedure for the histopathological study was as follows. After the nasal lavage at 4 h post-ovalbumin challenge, the animals were killed by bleeding and their nasal cavities were flushed with 10–20 ml of 10% neutral buffered formalin from the posterior opening of the nasopharinx. The heads were immediately immersed in fresh fixative (10% neutral buffered formalin) for at least 7 days. After decalcification with 10% EDTA solution, the heads were sectioned vertically at the vestibulum nasi, the respiratory part and the olfactory part. Samples were embedded in paraffin, cross-sectioned and stained with hematoxylin and eosin. The degree of cellular infiltration, hypersecretion in respiratory epithelium, edema and retention in the nasal cavity was examined under light microscopy (200×) using 2-μm sections of nasal mucosa.

2.5. Statistical analysis

The results were expressed as the means ± S.E.M. Either Student's *t*-test or two-way analysis of variance followed by Dunnett's *t*-test was used to confirm the statistical significance of differences between any two groups. *P* values of less than 0.05 were considered to be statistically significant.

3. Results

3.1. Characterization of allergic rhinitis induced by repeated intranasal ovalbumin challenge

3.1.1. Changes of nasal airway resistance

A time course study showed that actively sensitized guinea pigs exhibited a transient increase in nasal airway resistance, peaking 0.5 h after the first two ovalbumin challenges (0.1% on day 22 and 0.1 and 0.2% on days 22 and 24 of post-sensitization, respectively) as compared with guinea pigs after saline challenge. However, as ovalbumin challenges were repeated, the animals began to exhibit a biphasic increase of the nasal airway resistance following peaks at 0.5 h post-ovalbumin challenge (Fig. 1). Since biphasic nasal airway resistance was reproducibly induced after the 6th ovalbumin challenge, we used six repeated intranasal ovalbumin challenges (0.1, 0.2, 0.4, 0.5, 1 and 1% on days 22, 24, 27, 31, 36 and 41 of post-sensitization, respectively) in subsequent experiments.

3.1.2. Changes in sneezing and nasal secretion

In a parallel experiment, actively sensitized guinea pigs also displayed transient sneezing and nasal secretion, peaking 10 to 30 min after the final ovalbumin challenge,

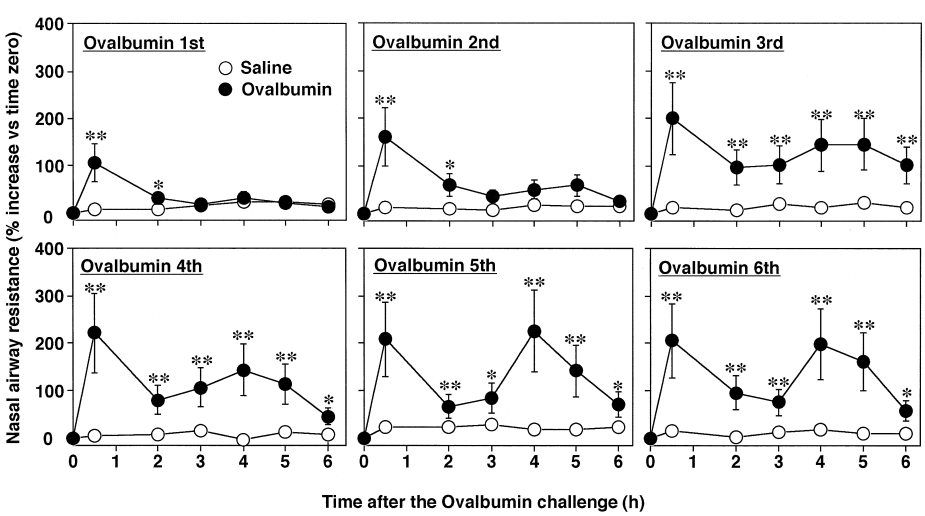

Fig. 1. Change of nasal airway resistance induced by repeated ovalbumin challenge in actively sensitized guinea pigs. Either saline (○) or 0.1 to 1% ovalbumin (●) was instilled repeatedly into both nostrils on days 22, 24, 27, 31, 36 and 41 post-sensitization, respectively. Changes in nasal airway resistance were measured after each challenge by the method described in the text. Data are expressed as percent increases of nasal airway resistance from 'pre (time 0)-value'. Each point represents the mean ± S.E.M. for seven animals. $^{*}P < 0.05$ and $^{**}P < 0.01$ vs. saline (Student's *t*-test).

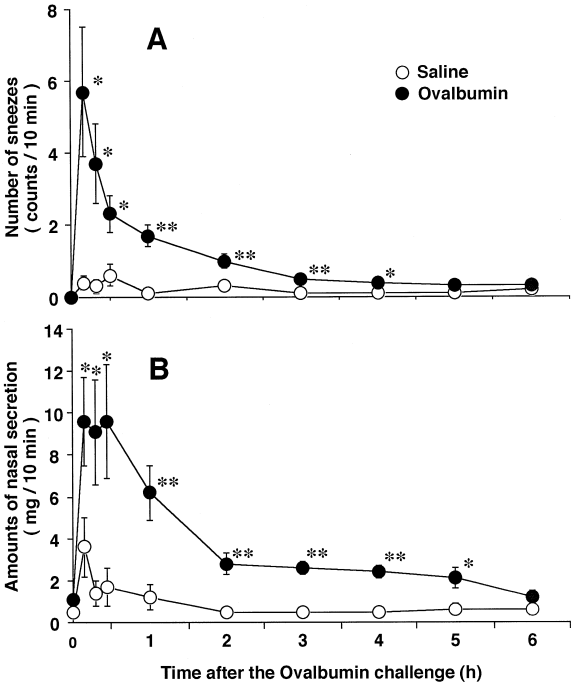

Fig. 2. Time course of sneezing (A) and nasal secretion (B) induced by the final (6th) ovalbumin challenge in actively sensitized guinea pigs. Either saline (○) or 1% ovalbumin (●) was instilled into both nostrils as described in Fig. 1. Changes in the number of sneezes and the amount of nasal secretion were measured at the time points indicated. Each point represents the mean ± S.E.M. for seven animals. $^{*}P < 0.05$ and $^{**}P < 0.01$ vs. saline (Student's *t*-test).

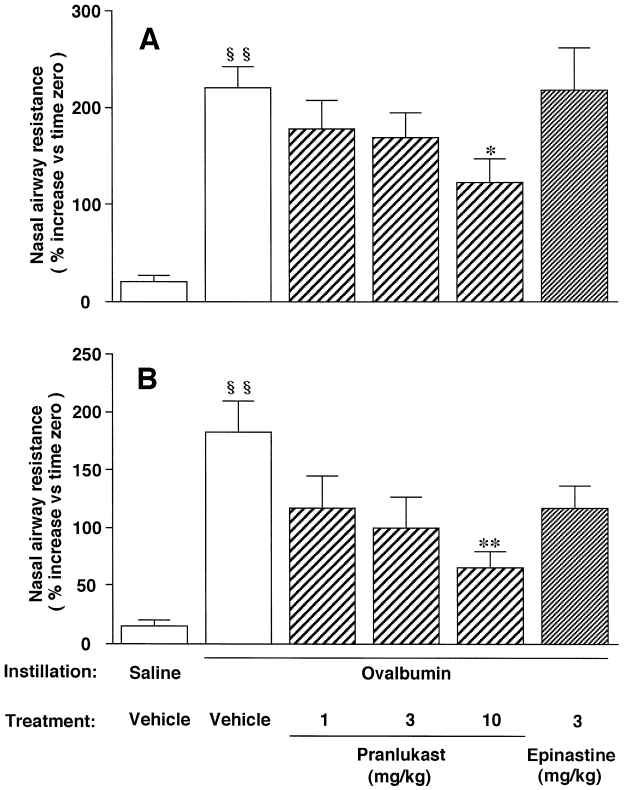

Fig. 3. Effects of pranlukast and epinastine on the increase of nasal airway resistance in the early (A) and the late phase (B) after the final (6th) ovalbumin challenge. Pre-sensitized guinea pigs were repeatedly instilled with either saline or ovalbumin into both nostrils as described in Fig. 1. After the final ovalbumin challenge, nasal airway resistance was measured at 0.5 and 4 h post-ovalbumin challenge. Pranlukast and epinastine were administered orally 1 h before each ovalbumin challenge. Each point represents the mean ± S.E.M. for 14 animals. $^{\S\S}P < 0.01$ vs. saline + vehicle (Student's *t*-test) and $^{*}P < 0.05$, $^{**}P < 0.01$ vs. ovalbumin + vehicle (Dunnett's *t*-test).

respectively. Although these nasal symptoms rapidly subsided within 2 h after the ovalbumin challenge, the nasal secretion remained slightly higher than that in the saline-challenged animals until 5 h post-challenge (Fig. 2).

3.1.3. Changes of cysteinyl LTs concentration in nasal lavage fluid and histopathological features

Analysis of the nasal lavage fluid using a different set of animals indicated that cysteinyl LTs concentrations were significantly increased, 10-fold and 5-fold, respectively, at 0.5 and 4 h after the final ovalbumin challenge as compared with those in animals challenged with saline (Table 1). Microscopic examination (200 ×) of the nasal tissue slides showed that the animals at 4 h post-ovalbumin challenge had lesions characterized by edema and hypersecretion of respiratory epithelium accompanied by eosinophil infiltration. Such changes were minor or negligible in saline-challenged animals (data not shown).

3.2. Effects of pranlukast and epinastine

3.2.1. Changes of nasal airway resistance

Baseline values of nasal airway resistance (mean ± S.D., $cmH_2O\ ml^{-1}\ s^{-1}$) were 0.9 ± 0.1, 1.0 ± 0.1, 1.0 ± 0.1,

Table 1
Cysteinyl LTs concentration in nasal lavage fluid after the final ovalbumin challenge in actively sensitized guinea pigs

Challenge	*n*	Time (h) after the final ovalbumin challenge	
		Early phase (0.5 h) (picogram per 20 ml lavage)	Late phase (4 h) (picogram per 20 ml lavage)
Saline	10	37.6 ± 14.3	14.4 ± 6.3
Ovalbumin	9	374.5 ± 88.2[a]	76.5 ± 6.5[a]

Guinea pigs were killed at 0.5 and 4 h post-ovalbumin challenge and nasal lavage was done as described in the text. Values are means ± S.E.M. for 9 to 10 animals.
[a] $P < 0.01$ vs. saline (Student's *t*-test).

1.1 ± 0.2, 1.1 ± 0.2 and 1.0 ± 0.2, respectively, for saline-instilled, vehicle-treated, pranlukast-treated (1, 3 and 10 mg kg^{-1}) and epinastine-treated animals. Vehicle-treated animals exhibited a significant and biphasic increase of nasal airway resistance both at 0.5 and 4 h post-ovalbumin challenge that was similar to the increase in the characterization study. When administered orally 1 h before every ovalbumin challenge, pranlukast (1, 3 and 10 mg kg^{-1}) suppressed the early and the late increase of nasal airway resistance by 22.5, 22.7 and 50.0%, and 36.7, 47.1 and 68.5%, respectively, in a dose-dependent fashion. The effects of pranlukast were significant at 10 mg kg^{-1} as compared with the vehicle-treated animals. An antiallergic agent, epinastine (3 mg kg^{-1}), administered orally in the same manner, reduced neither the earlier nor the later increase of nasal airway resistance (Fig. 3).

3.2.2. Changes in sneezing and nasal secretion

Since pranlukast had significant efficacy on the increase of nasal airway resistance at 10 mg kg^{-1}, this dose was chosen for the following experiments. Oral administration of pranlukast (10 mg kg^{-1}) suppressed neither sneezing nor nasal secretion which appeared in the early phase. However, epinastine (3 mg kg^{-1}) significantly suppressed sneezing by 59.8%, although its effect on nasal secretion was not clear (Fig. 4).

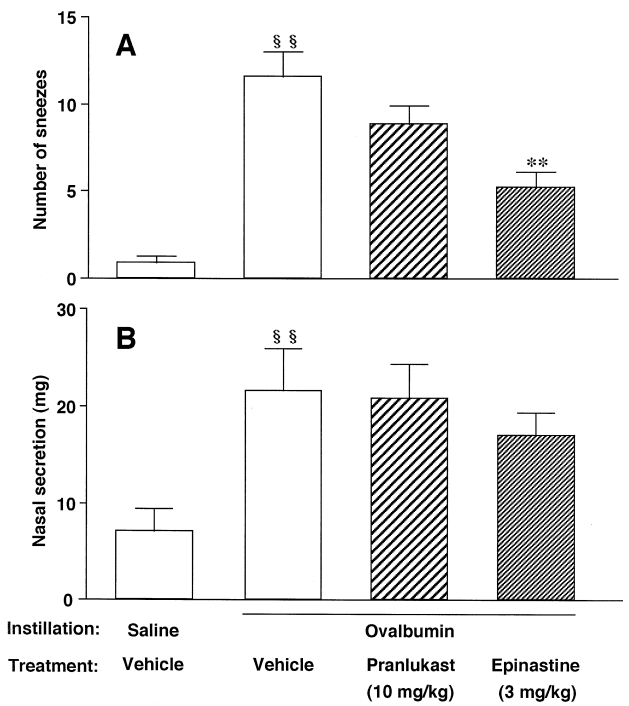

Fig. 4. Effect of pranlukast and epinastine on sneezing (A) and nasal secretion (B) in the early phase after the final (6th) ovalbumin challenge. Pre-sensitized guinea pigs were repeatedly instilled with either saline or ovalbumin into both nostrils as described in Fig. 1. Data in panel A are expressed as the total number of sneezes during the first 60 min of the final ovalbumin challenge. Data in panel B are expressed as the total amount of nasal secretion that was measured 10, 20, 30 and 60 min after the final ovalbumin challenge. Each point represents the mean ± S.E.M. for 13–14 animals. $^{§§}P < 0.01$ vs. saline + vehicle (Student's *t*-test) and $^{**}P < 0.01$ vs. ovalbumin + vehicle (Student's *t*-test).

3.2.3. Histopathological changes

The effects of pranlukast (10 mg kg^{-1}, p.o.) and epinastine (3 mg kg^{-1}, p.o.) on the histopathological changes in the late phase were examined in a separate experiment. Typical microscopic findings for the nasal mucosa are shown in Fig. 5. These micrographs indicated that the vehicle-treated animals showed mild to moderate eosinophil infiltration and hypersecretion of respiratory epithelium and mild edema. In animals pretreated with pranlukast, these changes were milder than those in the vehicle-treated animals. However, in epinastine-pretreated animals, eosinophil infiltration and edema were similar to those in the vehicle-treated animals, although improvement of hypersecretion in the respiratory epithelium and retention in the nasal cavity were more evident than those in the pranlukast-pretreated animals.

4. Discussion

The current study has provided (a) a novel model of allergic rhinitis in guinea pigs that yields a biphasic nasal response similar to the early-phase and the late-phase nasal response observed in patients with allergic rhinitis, and (b) evidence that the biphasic increase of nasal airway resistance in this model can be prevented by pretreatment with the cysteinyl LT receptor antagonist, pranlukast.

There are only few animal models which allow the study of nasal obstruction in the late-phase nasal response. Using repeated nasal ovalbumin challenge, we were able to induce biphasic nasal obstruction evidenced by an increase in nasal airway resistance in sensitized guinea pig. This increase of nasal airway resistance was accompanied by a transient expression of sneezing and nasal secretion in the early phase, and edema and eosinophil infiltration in nasal mucosa in the late phase. These symptoms are similar to some of the clinical symptoms observed in the early-phase and the late-phase nasal response in patients with allergic rhinitis (Pelikan, 1982; Pelikan and Pelikan-Filipek, 1982, 1989). Since dye solution, which was instilled nasally in the same manner as ovalbumin solution, localized only within the nasal cavity, it is likely that nasal ovalbumin challenge affects only the upper airway and not the lower airway. Therefore, it may be reasonable to assume that changes in respiratory resistance reflect those in nasal airway resistance at least in our experiments. This model, thus, may be useful for the study of nasal symptoms, particularly the nasal obstruction associated with the late-phase nasal response in allergic rhinitis.

Our study of the nasal airway resistance showed that the biphasic increase was prevented by oral administration of pranlukast. The prevention in the late phase was also associated with a histopathological improvement of edema

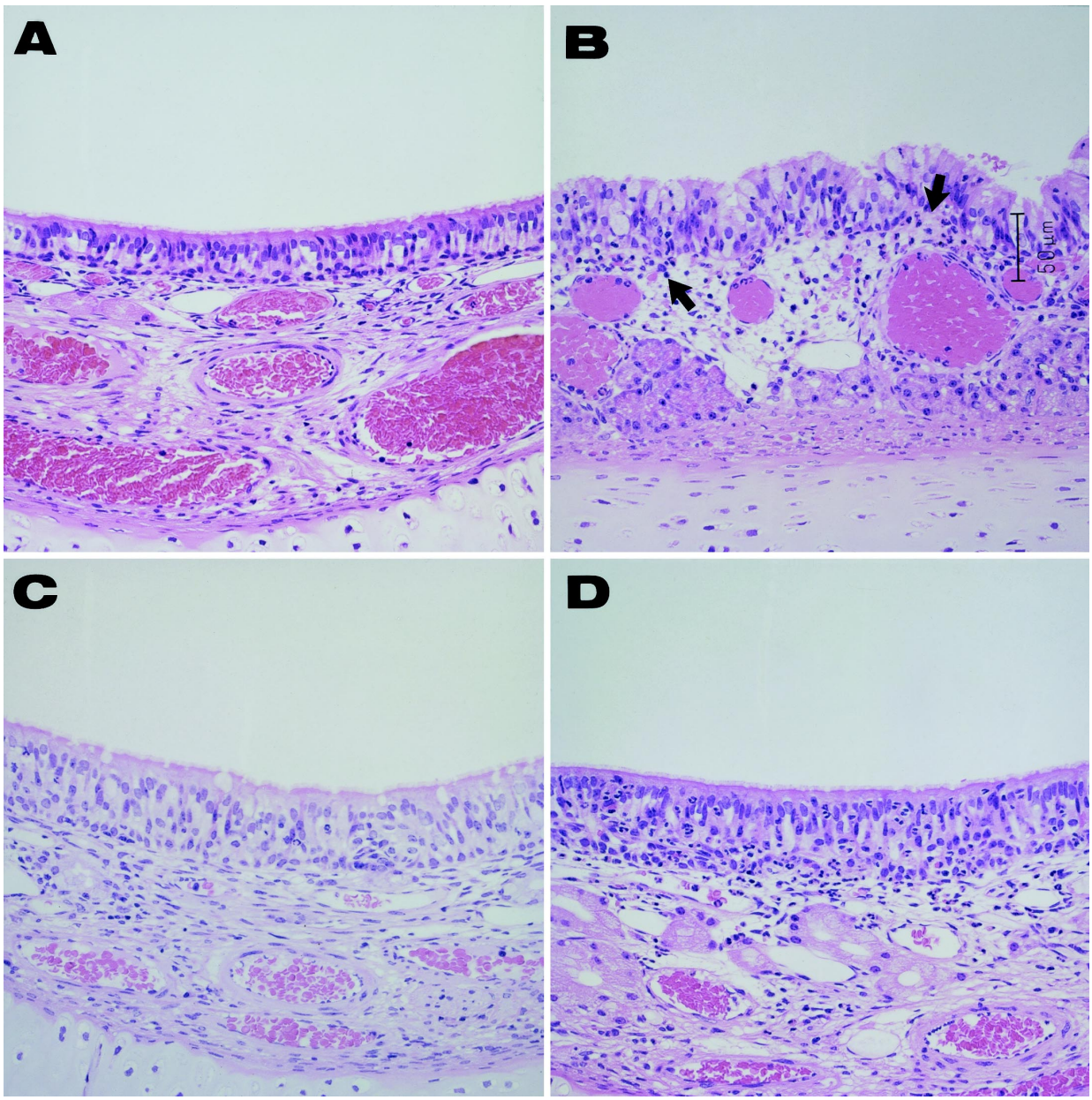

Fig. 5. Effects of pranlukast and epinastine on histopathological changes of nasal mucosa in the late phase. Pre-sensitized guinea pigs were repeatedly instilled with either saline or ovalbumin into both nostrils as described in Fig. 1. After the final ovalbumin instillation, the animals were killed at 4 h post-ovalbumin challenge and sections of nasal mucosa were examined microscopically (200 ×) as described in the text. Each picture shows representative histopathological changes of nasal mucosa taken from saline + vehicle (A), ovalbumin + vehicle (B), ovalbumin + pranlukast (C) and ovalbumin + epinastine (D)-treated animals.

and eosinophil infiltration in the nasal mucosa. However, in agreement with recent clinical findings (Oosten et al., 1997; Pelikan et al., 1997); the biphasic increase was not prevented by the antiallergic agent, epinastine, whose main action is histamine H1 receptor blockade (Kamei et al., 1992). Since pranlukast at the doses used in this study has been shown to specifically antagonize endogenous cysteinyl LTs in guinea pigs (Fujita et al., 1997a), the prevention may be attributed to the antagonism of cysteinyl LTs activity. The increase in cysteinyl LTs concentration accompanied by an increase of nasal airway resistance also supports the possibility of a contribution of cysteinyl LTs in this response. These results are in agreement with those of recent clinical studies (Donnelly et al., 1995), and support the current hypothesis that cysteinyl LTs play an important role in nasal obstruction.

In contrast to the prevention of nasal airway resistance increase, sneezing in the early phase was not reduced by pranlukast. This result is consistent with the recent findings that LTD_4 does not induce sneezing in human (Okuda et al., 1988) and guinea pigs (Fujita et al., 1997b). Although these studies also indicate that LTD_4 induces nasal

secretion, in the present study, nasal secretion was not reduced by pranlukast. Thus, it appears that cysteinyl LTs play a minor, direct, role in sneezing and hypersecretion. Nevertheless, the possibility that cysteinyl LTs participate indirectly in sneezing and hypersecretion via an increase in hypersensitivity to specific and/or non-specific stimulus may not be totally excluded. Recently, it has been suggested that LTD_4 enhances the responsiveness of capsaicin-sensitive afferent fibers in the guinea pig airway (Undem, 1993).

One of the discrepancies between our findings and those of another study is the inhibitors' response to the early phase increase of nasal airway resistance. Whereas in our model, the increase of nasal airway resistance in the early phase was prevented by pranlukast, that in the other study was not prevented by a cysteinyl LT receptor antagonist, FPL-55712 (Narita and Asakura, 1993). Several reasons, such as the difference in models and pharmacokinetics of the two agents, can be offered to explain this discrepancy. However, although our results suggest that cysteinyl LTs contribute to the increase in nasal airway resistance, not only in the late-phase nasal response but also in the early-phase nasal response, the relative contribution of cysteinyl LTs in these two responses may not be the same. This notion is supported by the different protective effects of disodium cromoglycate and of the glucocorticoids on the early-phase and the late-phase nasal response against allergen challenge (Pelikan, 1982; Pelikan and Pelikan-Filipek, 1982).

Interestingly, the degree of eosinophil infiltration in the nasal mucosa associated with the late phase was improved by pranlukast. This result is consistent with our previous findings that LTD_4, but not histamine, causes a significant eosinophil infiltration in the nasal mucosa which persists for up to 24 h after the topical challenge in guinea pigs (Fujita et al., 1997b). A recent report that cysteinyl LTs induce eosinophil infiltration in lower airway in human (Laitinen et al., 1993) and guinea pigs (Underwood et al., 1996) also supports the suggestion that cysteinyl LTs contribute to eosinophil infiltration. Thus, it appears that cysteinyl LTs are involved in eosinophil infiltration in allergic rhinitis. However, in spite of its potent chemotactic activity (as low as 10^{-10} M) in human eosinophils (Spada et al., 1994), LTD_4 up to 10 μM had no apparent chemotactic activity in guinea pig eosinophils in vitro (unpublished data). Therefore, its action is not attributable to direct chemotactic activity in guinea pig eosinophils. It is speculated that cells other than eosinophils and/or tissues produce eosinophil chemoatractant(s) in response to LTD_4. Thromboxane A_2 may be one of the candidate mediators, as it can be released from tissues by LTD_4 stimulation (Cheng et al., 1990) and its involvement has been suggested in nasal eosinophil migration in guinea pigs (Narita et al., 1996).

Cysteinyl LTs and histamine are mediators, both capable of inducing nasal obstruction (reviewed by White and Kaliner, 1992). As shown in an earlier study, LTD_4 is approximately 5000 times more potent than histamine to induce an increase of nasal airway resistance in humans. It is noteworthy that the increase is more prolonged than that with histamine and is similar to that induced by antigens (Okuda et al., 1988). These findings were also confirmed in our previous study in that nasal LTD_4 challenge increases nasal airway resistance in normal guinea pigs by a cysteinyl LTs receptor-mediated mechanism (Fujita et al., 1997b). Although the discrepancy between the level of cysteinyl LTs in nasal lavage fluid and nasal airway resistance in this study remains unexplained, these observations, together with our present results, strongly suggest that cysteinyl LTs are potential mediators of allergic rhinitis, especially in nasal obstruction.

Besides the biphasic nasal response, isolated types of the early and the late-phase nasal response (i.e., delayed nasal response), have also been demonstrated in patients with allergic rhinitis after allergen provocation (Pelikan, 1978; Pelikan and Pelikan-Filipek, 1995). These responses defined by different types of hypersensitivity contribute, in a complex manner, to the physiology of allergic rhinitis. Among these responses, the late-phase nasal response has been recognized as an important clinical phenomenon in patients with chronic allergic rhinitis associated with lasting edema of nasal membrane mucosa, and nasal mucosal infiltrates consisting mainly of eosinophils (Pelikan and Pelikan-Filipek, 1989). Glucocorticoids, which are powerful anti-allergic drugs, can reduce the changes in nasal mucosa membrane in the late phase, but not those in the early phase (Pelikan, 1982; Pelikan and Pelikan-Filipek, 1982; Davies et al., 1992), emphasizing the importance of the late-phase nasal response in allergic rhinitis. Although the involvement of cysteinyl LTs in isolated types of nasal response remains unclear, cysteinyl LTs, via induction of edema of the nasal membrane mucosa and/or eosinophil infiltration, may have an important role in the pathogenesis of allergic rhinitis.

In summary, we have developed a novel model of allergic rhinitis which allows the study of the early-phase and the late-phase nasal response. The results of the present study with this model support the involvement of cysteinyl LTs in allergic rhinitis, especially in edema of nasal membrane mucosa causing nasal obstruction. Cysteinyl LT receptor antagonists, such as pranlukast, may have therapeutic potential in the treatment of allergic rhinitis.

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