



Immunopharmacology and Inflammation

Role of hematopoietic prostaglandin D synthase in biphasic nasal obstruction in guinea pig model of experimental allergic rhinitis

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ABSTRACT

We investigated the role of hematopoietic prostaglandin D synthase (H-PGDS) in biphasic nasal obstruction in allergic rhinitis using a new specific inhibitor, (N-methoxy-N-methyl)-4-(5-benzoylbenzimidazole-2-yl)-3,5-dimethylpyrrole-2-carboxamide hydrochloride (TAS-204). First, we developed a novel guinea pig model of allergic rhinitis. Guinea pigs sensitized to ovalbumin without adjuvant were challenged with intranasal exposure to ovalbumin once a week. After the 3rd antigen challenge, they exhibited biphasic nasal obstruction. Additionally, analysis of nasal lavage fluid revealed an increase in the level of prostaglandin D₂ in both early and late phases. Treatment with oral TAS-204 for 15 days during the period of antigen challenges suppressed increases in nasal airway resistance in both phases. It is noteworthy that the late phase nasal obstruction was almost completely abrogated by inhibiting H-PGDS alone. Eosinophil infiltration in nasal lavage fluid and nasal hyperresponsiveness to histamine was also reduced by TAS-204 administration. These findings suggest that H-PGDS plays a critical role in the development of allergic rhinitis, especially in the induction of late phase nasal obstruction.

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1. Introduction

Allergic rhinitis is a common inflammatory disease of nasal mucosa and its prevalence has increased over the last three decades. After nasal provocation with allergen, patients with allergic rhinitis show an immediate response. Following the immediate reaction, some patients develop late phase nasal response (Iliopoulos et al., 1990; Naclerio et al., 1985). The overall reaction is termed “biphasic nasal response”. It was reported that high-affinity immunoglobulin E (IgE) receptor deficient mice exhibit no early phase nasal reaction and the reduced levels of late phase nasal obstruction (Miyahara et al., 2005). Therefore, IgE-triggered release of mediators from mast cells and basophils is thought to have an important role in the development of late phase, as well as early phase, response. Currently, histamine and cysteinyl leukotrienes (cysLTs) are considered to be important proinflammatory mediators in allergic rhinitis, because histamine H1 receptor antagonists and cysLT antagonists provide improvement on sneezing, rhinorrhoea and itch (Philip et al., 2002; Scadding et al., 2008). However, neither of these agents is sufficiently efficacious for

nasal obstruction when given as monotherapy. Therefore, there is a need for new therapies that are effective in relieving nasal obstruction in allergic rhinitis.

Prostaglandin D₂ (PGD₂) is thought to be involved in allergic diseases such as asthma, allergic rhinitis and allergic conjunctivitis. Local allergen challenge in the patients has been shown to cause an increase in the level of PGD₂ in lavage fluids from both upper and lower airways (Miadonna et al., 1990; Naclerio, 1991) and tears (Proud et al., 1990). Moreover, provocation of PGD₂ has been reported to enhance Th2 type airway inflammation in mice (Honda et al., 2003) and to induce nasal obstruction in the patients (Doyle et al., 1990).

PGD₂ is formed from arachidonic acid by successive enzyme reactions mediated by cyclooxygenase (COX) and two types of PGD synthase (PGDS) isoforms. One is called lipocalin-type PGDS (L-PGDS) which is localized in the central nervous system, testis and heart (Gerena et al., 2000; Urade et al., 1985). The other one, hematopoietic PGDS (H-PGDS), is present in mast cells, Th2 cells, microglia, necrotic muscle fibers and apoptotic smooth muscle cells (Kanaoka and Urade, 2003). Higher expression of H-PGDS was observed in nasal mucosa of the patients with allergic rhinitis compared with that of patients with mucocoele, whereas the expression of L-PGDS was scarcely detected in these tissues (Okano et al., 2006). These findings suggest that H-PGDS, but not L-PGDS, may be responsible for nasal symptoms caused by PGD₂. However, verification of significance of each isoform has been difficult due to the lack of an isoform-selective inhibitor.

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We have successfully produced a novel inhibitor selective for H-PGDS, TAS-204 (Fig. 1), that inhibits recombinant human H-PGDS with a 50% inhibitory concentration of 23.0 nM. It showed no inhibitory activities against COX-1, COX-2, microsomal PGE synthase or L-PGDS up to 10 μ M. In the present study, we developed a novel allergic rhinitis model in guinea pig sensitized without adjuvant and assessed the effects of TAS-204 on the pathogenesis of this model.

2. Materials and methods

2.1. Animals

Male Hartley guinea pigs (3 weeks old) were purchased from Japan SLC (Shizuoka, Japan). The animals were kept in environmentally controlled rooms (temperature 20–26 °C, humidity 45–55%) with a 12-h light–dark cycle for 2 weeks before use. Food and water were available ad libitum. All animals were used in accordance with the guideline established by Taiho Review Committee of Animal Experiments that adopted the Declaration of Helsinki.

2.2. Drugs and materials

TAS-204 (purity 99.5%) was synthesized in our laboratories. CysLTs antagonist pranlukast was extracted from Onon® capsules (Ono Pharmaceutical, Osaka, Japan). Prednisolone was obtained from Sigma-Aldrich (St. Louis, MO, USA) and Nacalai Tesque (Kyoto, Japan). The chemicals were purchased commercially: ovalbumin (grade V), indomethacin and histamine (Sigma-Aldrich), and Diff-Quik solution (International Reagents, Kobe, Japan). TAS-204 and other drugs tested were suspended in 0.5% hydroxypropyl methylcellulose solution, and were administered orally once a day for 15 days from the 1st challenge to the 3rd challenge.

2.3. Sensitization and challenge

Animals were sensitized with a subcutaneous injection of ovalbumin (1 mg/ml saline per animal) without adjuvants. One week after the sensitization, the animals were challenged with 20 μ g of ovalbumin in saline into each nasal cavity. The antigen challenge was repeated once a week for 2 another weeks.

2.4. Measurement of specific airway resistance

After the ovalbumin challenge, specific airway resistance was measured using a two-chambered, double-flow plethysmograph system according to the method of Pennock et al. (1979). Briefly, the animals were placed with its neck extending through the partition of a two-chambered box. The gap between the animal and the chamber was sealed with silicone rubber. Specific airway resistance was measured with the data analyzer Pulmos-1 (MIPS, Osaka, Japan) after the detection of airflow by sensors installed in both front and rear chambers. The analysis was performed before and at the

indicated time (10 min, 0.5, 2, 3, 4, 5, 6 and 7 h) after the challenge. The change in specific airway resistance was expressed as the % increase from the pre-challenge baseline value.

2.5. Measurement of PGD₂, PGE₂, histamine and cysLTs concentration in nasal lavage fluid

The guinea pigs were sacrificed with an overdose of pentobarbital sodium (50 mg/ml, 1 ml intraperitoneally) before and 15, 30, 60 min, and 6 h after the 3rd ovalbumin challenge. Independent animals were prepared for each time point. A cannula was inserted into the nasal side-section from the bronchi for nasal lavage. Nasal cavities were then washed with 1 ml of phosphate buffered saline containing 3 mM ethylenediaminetetraacetic acid and 10 μ M indomethacin to collect nasal lavage fluid. After centrifugation at 1200 $\times$ g for 10 min, the nasal lavage fluid supernatant was frozen at –80 °C. PGD₂, PGE₂, cysLT and histamine levels were measured by the following enzyme immunoassay kits according to the manufacturer's instructions; PGD₂, PGE₂ and cysLT enzyme immunoassay kit were from Cayman Chemical (Ann Arbor, MI, USA) and histamine enzyme-linked immunosorbent assay kit was from Immunotech International (Westbrook, Me, USA).

2.6. Counting of leukocytes in nasal lavage fluid

The animals were sacrificed with an overdose of pentobarbital sodium (50 mg/ml, 1 ml intraperitoneally) 6 h after the 3rd ovalbumin challenge. A cannula was inserted into the nasal side-section from the bronchi for nasal lavage. Nasal cavities were then washed with 3 ml of phosphate buffered saline containing 0.1% bovine serum albumin and 0.5 U/ml heparin to collect nasal lavage fluid. The total leukocytes were pelleted by centrifugation at 300 $\times$ g for 10 min and counted in a hemocytometer. Some cells were dispersed by cyto spin (Cytospin 3; Shandon, Pittsburgh, PA, USA) and stained with Diff-Quik solution. At least 300 leukocytes were counted under the microscope to identify the number of eosinophils.

2.7. Nasal responsiveness to histamine

Nasal responsiveness was assessed 1 day after the 3rd ovalbumin challenge by counting the number of sneezing for 20 min following the provocation with histamine. Individual animal was applied to increasing dose of histamine (20 μ l of 0, 1 and 10 mg/ml saline solution) into each nasal cavity at an interval of 2 h.

2.8. Statistical analysis

Data were expressed as the mean $\pm$ S.E.M. Statistical analysis was performed by Welch's test for comparison between two groups and Dunnett's test for multiple comparison using SAS system version 8.2 (SAS Institute, Tokyo, Japan). Leukocyte and eosinophil counts in nasal lavage fluid and nasal responsiveness to histamine were assessed statistically by Wilcoxon's test for comparison between two groups and Steel's test for multiple comparisons. P values less than 0.05 were considered statistically significant.

3. Results

3.1. Time course study of specific airway resistance

We first attempted to establish an adjuvant-free guinea pig model mimicking human allergic rhinitis associated with biphasic nasal obstruction. Sensitized animals were challenged with ovalbumin into each nasal cavity once a week. A time course study revealed that sensitized guinea pigs started to exhibit a transient increase in specific airway resistance after the 2nd antigen challenge (Fig. 2A). Thus, detailed analysis of the nasal obstruction of both control and

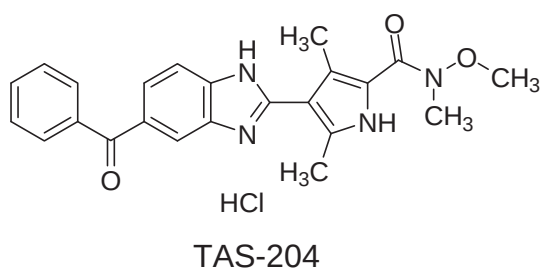


Fig. 1. Chemical structure of (N-methoxy-N-methyl)-4-(5-benzoylbenzimidazole-2-yl)-3,5-dimethylpyrrole-2-carboxamide hydrochloride (TAS-204).

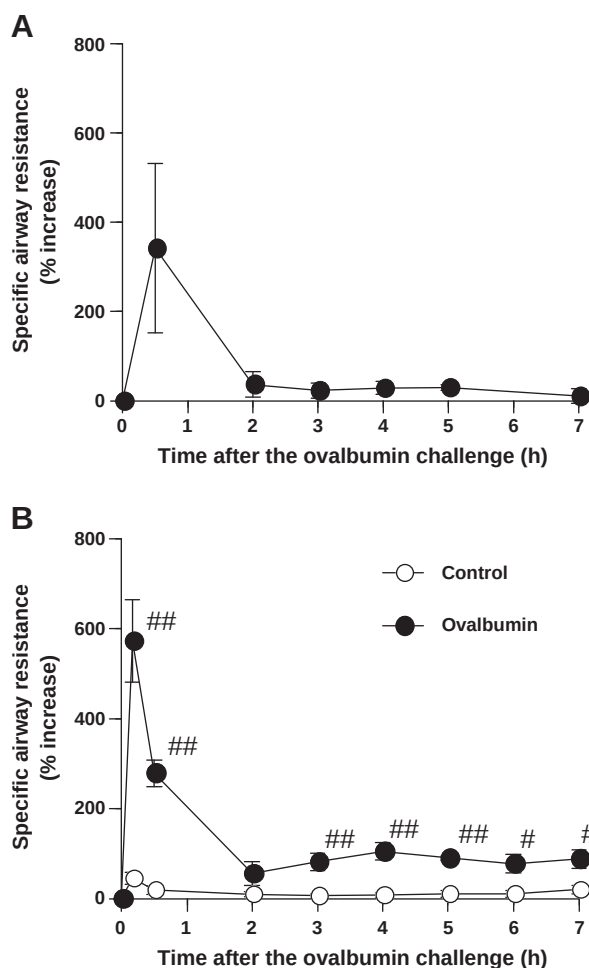


Fig. 2. Time-course changes in specific airway resistance induced by the 2nd (A) and 3rd (B) ovalbumin challenge in sensitized guinea pigs. Specific airway resistance was measured before and 10 min, 0.5, 2, 3, 4, 5, 6, and 7 h after antigen challenge. These experiments were performed with 8 animals per group. Data are expressed as the mean $\pm$ S.E.M. * $P < 0.05$, ** $P < 0.01$ vs. Control (Welch's test).

sensitized animals was performed after the 3rd ovalbumin challenge. The 3rd challenge caused a biphasic increase in specific airway resistance in sensitized animals, whereas control animals showed no alterations in specific airway resistance during the entire time course. The first peak of specific airway resistance was observed at 10 min (early phase) after the challenge and the second peak occurred at 3–7 h (late phase) (Fig. 2B). Most (>90%) sensitized guinea pigs also displayed transient sneezing and nasal secretion after the 3rd ovalbumin challenge (data not shown).

3.2. Time course study of PGD_2 , PGE_2 , $cysLTs$ and histamine concentration in nasal lavage fluid in early phase

To characterize the allergic rhinitis model in guinea pig, we monitored several mediators in nasal lavage fluid during the early phase response. PGD_2 concentration dramatically increased at 15 min after the 3rd ovalbumin challenge and reached the maximum of 2908.4 ± 684.4 pg/ml at 30 min, in contrast to low level of PGD_2 detected in nasal lavage fluid from sensitized guinea pig before the challenge (90.4 ± 25.2 pg/ml) (Fig. 3A). On the other hand, baseline PGE_2 concentration in nasal lavage fluid was high, and only a slight increase in PGE_2 level was observed after the antigen challenge (Fig. 3B). Kinetics of $cysLT$ levels followed a similar pattern to that of PGD_2 (Fig. 3C). Histamine concentration increased at 15 min after

the challenge and rapidly subsided within 30 min post-challenge (Fig. 3D).

3.3. PGD_2 , PGE_2 , $cysLTs$ and histamine concentration in nasal lavage fluid in late phase

We next examined the production of these mediators in nasal cavity during late phase response. PGD_2 concentration in nasal lavage fluid was significantly enhanced 30-fold at 6 h after the 3rd antigen challenge as compared with control animals (Fig. 4A). In contrast, only a slight, but significant, increase in the levels of PGE_2 and $cysLTs$ in nasal lavage fluid was observed (Fig. 4B and C). Histamine level was also significantly increased in late phase (Fig. 4D).

3.4. Selective inhibition of PGD_2 production by TAS-204 in vivo

To investigate the inhibitory activity of TAS-204 in vivo, we examined the effects of TAS-204 on the levels of PGD_2 and PGE_2 in nasal lavage fluid at 30 min after the 3rd ovalbumin challenge. When administered orally for 15 days from the 1st to the 3rd challenge, TAS-204 at 3.75, 7.5, 15 and 30 mg/kg dose-dependently inhibited the PGD_2 production in early phase after the 3rd challenge with the inhibitory ratio of 3.2, 32.7, 58.8 and 78.5%, respectively and statistical significance was observed with 15 and 30 mg/kg (Fig. 5A). Considering possible non-selective effect of high dose TAS-204, the inhibitory potency at 60 mg/kg was not evaluated. Single administration of TAS-204 at 30 mg/kg 1 h before the 3rd antigen challenge also inhibited the increase in PGD_2 concentration in nasal lavage fluid by 70.3% (data not shown). The data indicates that PGD_2 production in this model is mainly catalyzed by H-PGDS. On the other hand, PGE_2 concentration in nasal lavage fluid was not affected by the inhibitor (Fig. 5B). Taken together, the selective inhibitory effect of TAS-204 on H-PGDS was confirmed during an in vivo inflammatory response.

3.5. Effect of TAS-204 on biphasic nasal obstruction

Effect of TAS-204 on nasal obstruction was evaluated by the change in specific airway resistance. Based on the result of Fig. 2B, early phase response was represented by the data at 10 min after the challenge, while the area under the curve from 3 to 7 h (AUC_{3-7h}) was calculated to compare late phase response. Repeated administration of TAS-204 (3, 10 and 30 mg/kg) dose-dependently suppressed the early phase increase in specific airway resistance by 26.0, 44.0 and 60.7%, respectively (Fig. 6A). The effect was significant at 30 mg/kg as compared with ovalbumin-challenged animals. More significant inhibition was observed in the late phase nasal obstruction, which were 60.8, 76.5 and 92.8% at the dose of 3, 10 and 30 mg/kg, respectively (Fig. 6B). On the other hand, repeated administration of a $cysLTs$ antagonist, pranlukast, at 30 mg/kg also exhibited significant inhibition of both early and late phase responses by 51.8 and 81.5%, respectively. Single TAS-204 administration at 30 mg/kg 1 h before the 3rd antigen challenge was sufficient to suppress the early and late phase responses by 66.8 and 63.9%, respectively (data not shown).

3.6. Effect of TAS-204 on eosinophilic accumulation in nasal mucosa

Number of total leukocytes and eosinophils in nasal lavage fluid was significantly increased 6 h after the 3rd antigen challenge as compared with saline treated animals. These increases were significantly suppressed by repeated administration of TAS-204 (30 mg/kg). The inhibition ratios for the increases in total leukocytes and eosinophils in nasal lavage fluid were 49.4 and 43.7%, respectively (Fig. 7). Repeated administration of pranlukast (30 mg/kg) also inhibited the increase in these cell counts by 61.1 and 54.0%, respectively. In addition, repeated administration of prednisolone (20 mg/kg) strongly inhibited the increase in these cell counts.

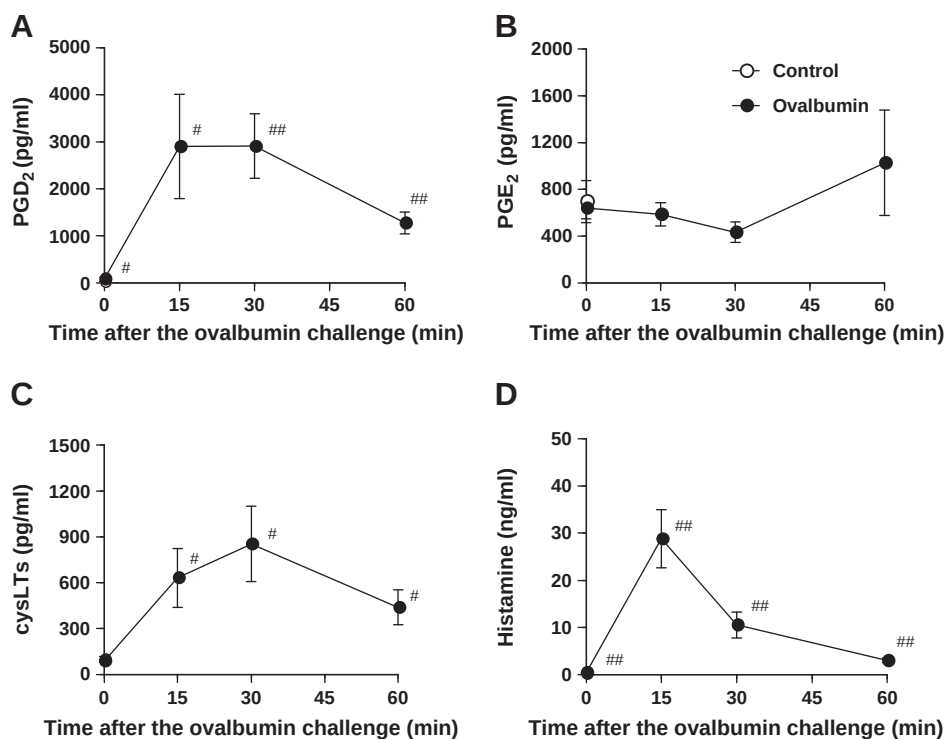


Fig. 3. Time-course changes in PGD₂ (A), PGE₂ (B), cysLTs (C) and histamine (D) concentration in nasal lavage fluid after the 3rd ovalbumin challenge in sensitized guinea pigs in early phase. Nasal lavage was performed before and 15, 30 and 60 min after the 3rd antigen challenge. Each point represents the mean ± S.E.M. for 8 to 10 animals. #P < 0.05, ##P < 0.01 vs. Control (Welch's test).

3.7. Effect of TAS-204 on nasal responsiveness to histamine

Nasal responsiveness was examined by provocation with histamine 1 day after the 3rd ovalbumin challenge. The number of sneezing after

the provocation significantly increased in sensitized guinea pigs compared with saline treated animals, indicating that repeated antigen challenges in sensitized animals caused nasal hyperresponsiveness to histamine. Repeated administration of TAS-204 (10 and 30 mg/kg)

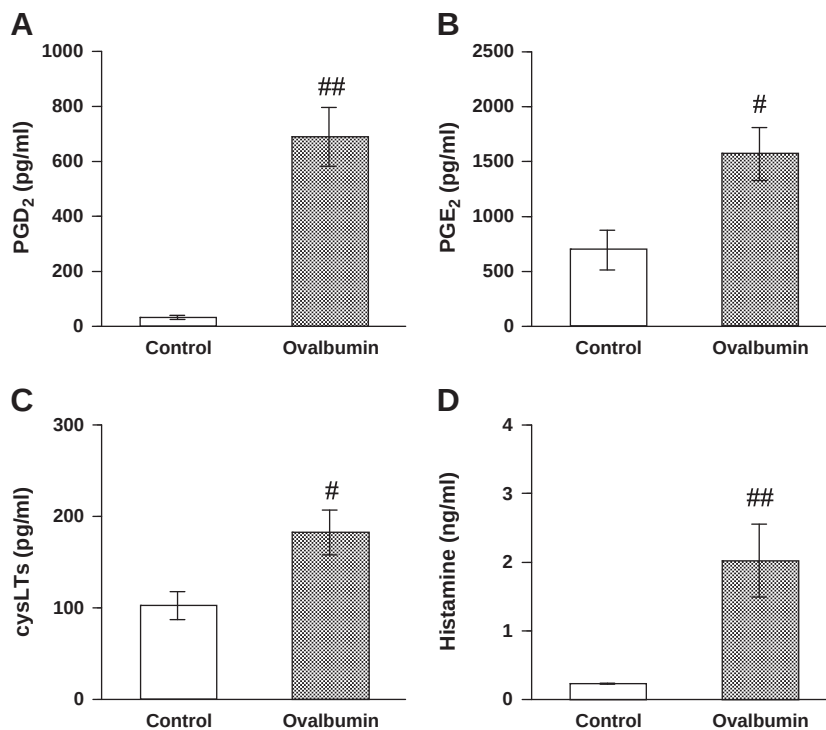


Fig. 4. PGD₂ (A), PGE₂ (B), cysLTs (C) and histamine (D) concentration in nasal lavage fluid after the 3rd ovalbumin challenge in sensitized guinea pigs in late phase. Nasal lavage was performed 6 h after the 3rd antigen challenge. Results are expressed as the mean ± S.E.M. for 8 or 9 animals. #P < 0.05, ##P < 0.01 vs. Control (Welch's test).

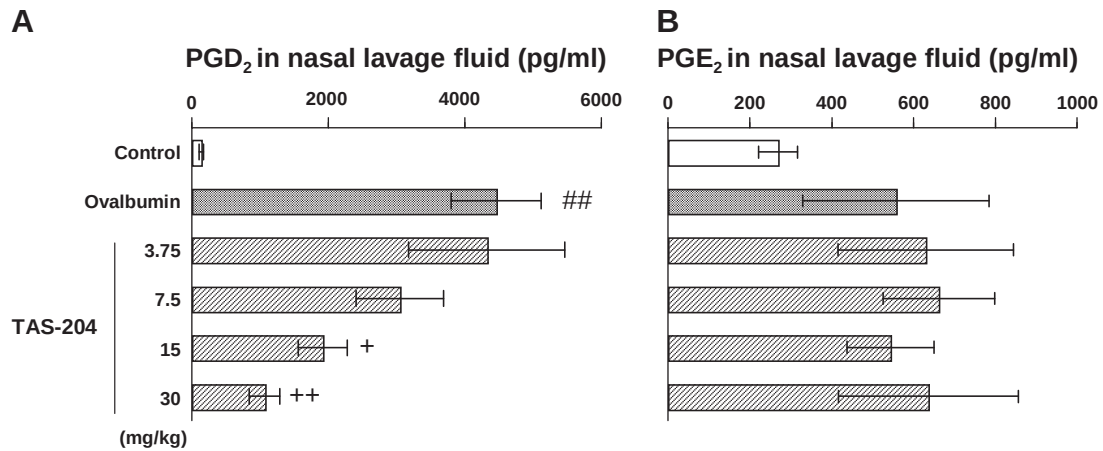


Fig. 5. A selective inhibition of PGD₂ production in vivo by oral administration of TAS-204. Nasal lavage fluid was collected 30 min after the 3rd ovalbumin challenge. TAS-204 was administered for 15 days from the 1st to the 3rd challenge. Ovalbumin-challenged animals were given vehicle alone. Results are expressed as the mean $\pm$ S.E.M. for 12 to 16 animals. ^{##} $P < 0.01$ vs. Control (Welch's test) and ⁺ $P < 0.05$, ⁺⁺ $P < 0.01$ vs. Ovalbumin (Dunnett's test).

inhibited the nasal response to 10 mg/ml histamine, and the effect at 30 mg/kg was statistically significant (Fig. 8). Repeated administration of prednisolone at 20 mg/kg exerted almost complete inhibition of the nasal response.

4. Discussion

In the present study, we for the first time demonstrated that an H-PGDS selective inhibitor TAS-204 strongly suppressed antigen-induced PGD₂ production in nasal lavage fluid and biphasic nasal obstruction in a guinea pig model of allergic rhinitis. Although the differences in the role of two-types of PGD₂ receptors, DP (prostanoid receptor for PGD₂) and CRTH2 (chemoattractant receptor-homologous molecule expressed on Th type 2 cells), have been extensively studied, there is little information regarding the contribution of two PGDS isoforms to allergic rhinitis due to the lack of an isoform-selective inhibitor. Our findings suggest that PGD₂ generated by H-PGDS acts as an important inducer of biphasic nasal obstruction.

There are a few animal models which allow the study of nasal obstruction in the late phase response. Guinea pig models of allergic rhinitis with biphasic nasal response have been generated by intranasal antigen challenge. However, these models generally

employ adjuvants or require long period as long as 2 months (Bahekar et al., 2008; Fujita et al., 1999; Nabe et al., 1997). Adjuvants such as aluminum hydroxide by themselves induce nonspecific T cell inflammatory responses (Holt et al., 1981; Victoratos et al., 1997) which may affect pathophysiology of the model. Thus, we attempted to establish an allergic rhinitis model independent of adjuvant. We reasoned that route of sensitization and the number of antigen challenges are the most influential factors and found that a subcutaneous sensitization with ovalbumin and subsequent antigen challenge for 3 times would be optimal condition. In general specific airway resistance can be taken as the total resistance of the upper and lower airways in the animal. Because we have checked that Evans blue solution, instilled nasally in the same manner as ovalbumin solution, localized only within the nasal cavity, the changes in specific airway resistance induced by ovalbumin challenge should reflect the nasal resistance but not the lower airway resistance in our model. This protocol could induce the late phase response associated with eosinophil infiltration in nasal lavage fluid and nasal hyperresponsiveness to histamine similarly to previously reported adjuvant-dependent protocol. The levels of histamine and cysLTs in nasal lavage fluid also increased after antigen challenge, consistent with previous reports in the patients with allergic rhinitis (Naclerio, 1991; Naclerio et al., 1991) and guinea pig model (Yamasaki et al., 2001). This model

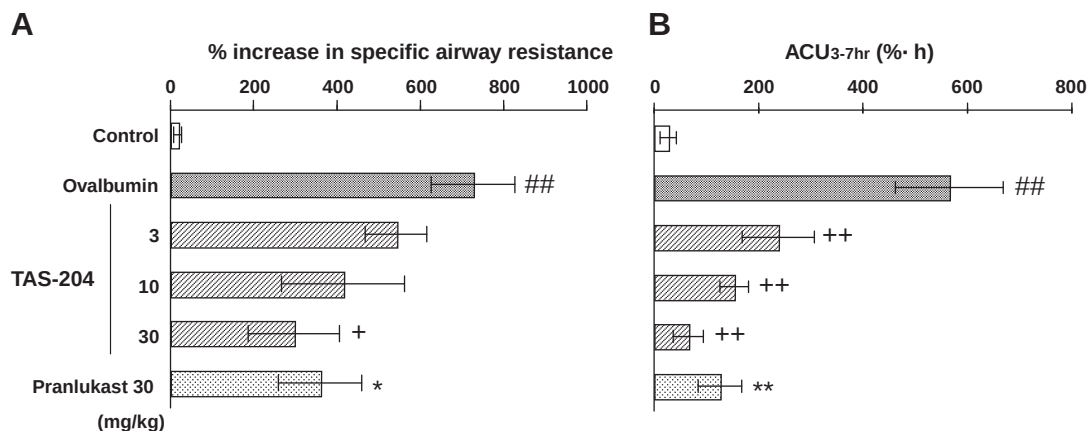


Fig. 6. Effects of TAS-204 and pranlukast on the increase in specific airway resistance in the early (A) and late phase (B) after the 3rd ovalbumin challenge. The early phase response was evaluated from the increase in specific airway resistance at 10 min. The late phase increases in specific airway resistance were determined by calculating the area under the curve (AUC) of specific airway resistance from 3 to 7 h. TAS-204 and pranlukast were administered for 15 days from the 1st to the 3rd challenge. Ovalbumin-challenged animals were given vehicle alone. Results are expressed as the mean $\pm$ S.E.M. for 8 animals. ^{##} $P < 0.01$ vs. Control (Welch's test), ^{*} $P < 0.05$, ^{**} $P < 0.01$ vs. Ovalbumin (Welch's test) and ⁺ $P < 0.05$, ⁺⁺ $P < 0.01$ vs. Ovalbumin (Dunnett's test).

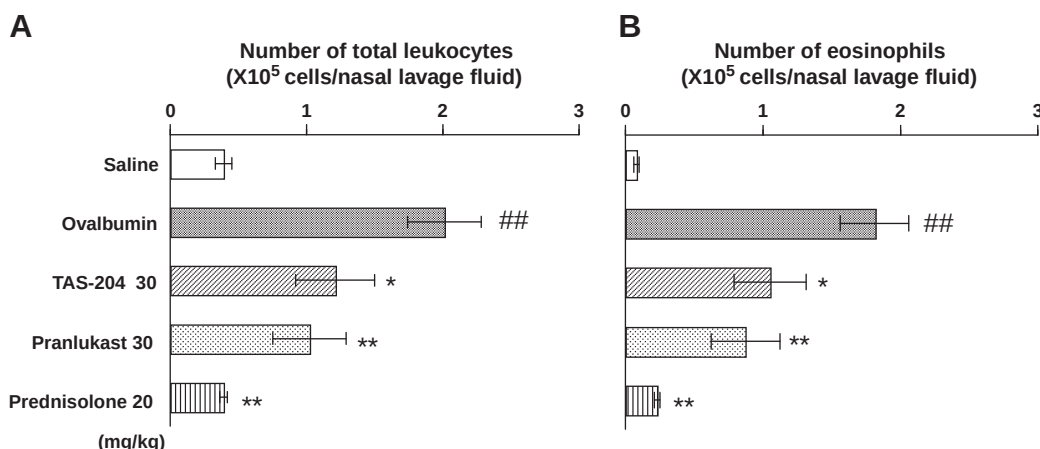


Fig. 7. Effects of TAS-204, pranlukast and prednisolone on the numbers of total leukocytes (A) and eosinophils (B) in nasal lavage fluid at 6 h after the 3rd ovalbumin challenge. TAS-204, pranlukast and prednisolone were administered for 15 days from the 1st to the 3rd challenge. Ovalbumin-challenged animals were given vehicle alone. Results are expressed as the mean $\pm$ S.E.M. for 8 to 12 animals. ## P <0.01 vs. Saline (Wilcoxon's test) and * P <0.05, ** P <0.01 vs. Ovalbumin (Wilcoxon's test).

may be useful not only for understanding the pathophysiology of allergic rhinitis but also for a development of novel therapeutics.

Several studies have been suggested the contribution of PGD₂ to early phase nasal obstruction. Nasal instillation of PGD₂ induces immediate nasal obstruction in the patients (Doyle et al., 1990). In addition, it was reported that a DP antagonist demonstrates potent inhibitory effect on early phase nasal obstruction in an adjuvant-dependent guinea pig rhinitis model (Arimura et al., 2001). However, synthase of PGD₂ responsible for nasal obstruction has not been identified. By using a specific inhibitor for H-PGDS, we have clarified this issue for the first time. TAS-204 exhibited a specific inhibition of PGD₂ production in nasal cavity and suppressed nasal obstruction not only in early phase but also in late phase after the antigen challenge. Therefore, H-PGDS, not L-PGDS, plays a critical role in both phases of nasal obstruction. Our data also demonstrated that PGD₂ participates in early and late phase responses in a different manner. Significant late phase response was observed after the 3rd, but not the 2nd, antigen challenge. This late phase response was almost completely inhibited by 15 consecutive day treatment with TAS-204, whereas the efficacy by the single treatment just before the 3rd challenge was only partial. Therefore, exposure to PGD₂ may readily induce early phase

response, whereas repetitive exposure to PGD₂ is required for the occurrence of late phase response. PGD₂ production by the first two challenges may change response/function of inflammatory cells (T cells, eosinophils and so on), thereby contributing to late phase nasal obstruction and nasal hyperresponsiveness to various chemical mediators. Predominance of nasal obstruction in patients with perennial allergic rhinitis is more remarkable than that in seasonal allergic rhinitis patients (Skoner, 2001). As perennial rhinitis patients are exposed to allergen for longer periods than seasonal rhinitis patients, more frequent PGD₂ production may be provoked in these patients. Such a repetitive exposure to PGD₂ may underlie late phase nasal obstruction. This assumption would be verified in the future when an H-PGDS inhibitor becomes clinically available.

Infiltration of eosinophils into nasal cavity is a characteristic feature of allergic rhinitis in association with the development of nasal symptoms (Linden et al., 1999). In the present study, treatment with TAS-204 significantly reduced eosinophil accumulation in nasal cavity. The effect of TAS-204 treatment was comparable with that of pranlukast, a cysLTs antagonist, already used in the clinic for allergic rhinitis. This is consistent with a previous report on augmental eosinophilic lung inflammation in an asthma model of PGDS transgenic mice (Fujitani et al., 2002). Moreover, Arimura et al. (2001) reported that a DP antagonist inhibits the number of eosinophils in nasal lavage fluid following antigen challenge in an adjuvant-dependent guinea pig rhinitis model. Equivalent effect has been observed using a CRTH2 antagonist in a murine asthma model (Uller et al., 2007). Therefore, H-PGDS inhibitor may be more potent for PGD₂-mediated eosinophilic inflammation than each of PGD₂ receptor antagonist.

Nasal hyperresponsiveness, one of major disorders in patients suffering from allergic rhinitis, is characterized by the increase of responsiveness to nonallergic stimuli, such as cold air and various irritants. This phenomenon could be ascribed, at least in part, to peripheral sensitization of nasal afferent nerves by repetitive allergen exposure. In the present study, sensitized guinea pigs displayed mild symptoms after the 1st challenge, but showed evident sneezing and nasal secretion only after the 3rd challenge. TAS-204 significantly inhibited the increase in nasal responsiveness to histamine after the repeated antigen challenge. The precise mechanism underlying the nasal hyperresponsiveness to histamine remains unclear. Kitamura et al. (2004) reported that upregulation of histamine H1 receptor in nasal mucosa is one of the mechanisms responsible for nasal hyperresponsiveness. However, this was not the case in our model because histamine H1 receptor mRNA level of ovalbumin-challenged group was similar to that of saline-treated group (data not shown). On the other hand, recent investigation has revealed that nasal

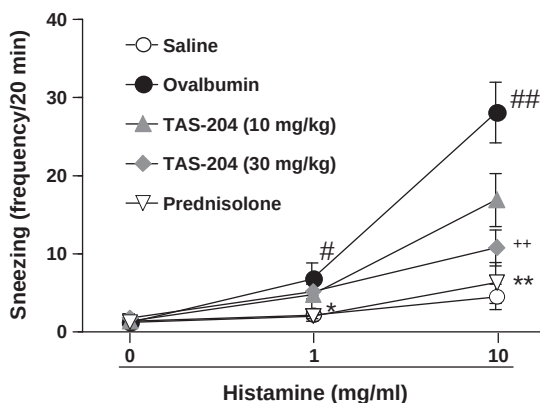


Fig. 8. Effect of TAS-204 and 20 mg/kg prednisolone on nasal hyperresponsiveness to histamine induced by the repeated antigen challenge. Nasal responsiveness to histamine was evaluated 1 day after the 3rd antigen challenge. TAS-204 and prednisolone were administered for 15 days from the 1st challenge to the 3rd challenge. Ovalbumin-challenged animals were given vehicle alone. The experiment was performed with 15 or 16 animals per group. Data are expressed as the mean $\pm$ S.E.M. # P <0.05, ## P <0.01 vs. Saline (Wilcoxon's test), * P <0.05, ** P <0.01 vs. Ovalbumin (Wilcoxon's test) and + P <0.01 vs. Ovalbumin (Steel's test).

hyperresponsiveness in guinea pigs repeatedly exposed to antigen is suppressed by DP antagonist (Yasui et al., 2008). Our present results are consistent with this report and both support the contribution of H-PGDS to nasal hyperresponsiveness in allergic rhinitis. Eosinophils produce neurotrophins, prime candidates as mediators of neural hyperresponsiveness (Sarin et al., 2006). Accumulated eosinophils in nasal cavity after the repeated antigen challenge may underlie the development of nasal hyperresponsiveness. Further studies are required to clarify the mechanism of nasal hyperresponsiveness to histamine caused by PGD₂.

In conclusion, we found that H-PGDS plays a critical role in the development of biphasic nasal obstruction, eosinophilic inflammation and nasal hyperresponsiveness to histamine in guinea pigs. From the clinical perspective, inhibition of H-PGDS may be equivalent to blocking both DP and CRTH2 receptors. H-PGDS selective inhibitor such as TAS-204 may provide a new therapeutic approach in allergic rhinitis, especially nasal obstruction.

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