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# 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 $D_2$ 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.



## 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 $D_2$ ($PGD_2$) 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 $PGD_2$ in lavage fluids from both upper and lower airways (Miadonna et al., 1990; Naclerio, 1991) and tears (Proud et al., 1990). Moreover, provocation of $PGD_2$ 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).

$PGD_2$ 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 mucocele, 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 $PGD_2$. 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 μM. 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

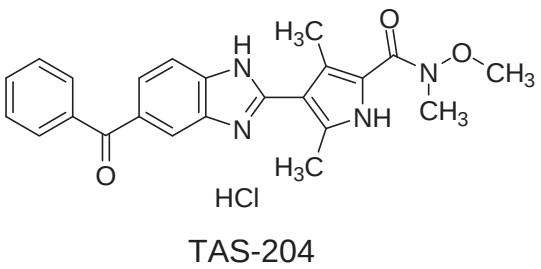
TAS-204 (purity 99.5%) was synthesized in our laboratories. CysLTs antagonist pranlukast was extracted from Onon® capsules (Ono Pharmaceutical, Osaka, Japan). Prednisolone was obtained from Sigma-Aldrich (St. Louis, MO, USA) and Nacalai Tesque (Kyoto, Japan). The chemicals were purchased commercially: 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 μg 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.




TAS-204

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

### 2.5. Measurement of $PGD_2$, $PGE_2$, 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 μM indomethacin to collect nasal lavage fluid. After centrifugation at 1200 × *g* for 10 min, the nasal lavage fluid supernatant was frozen at −80 °C. $PGD_2$, $PGE_2$, cysLT and histamine levels were measured by the following enzyme immunoassay kits according to the manufacturer's instructions; $PGD_2$, $PGE_2$ 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 × *g* for 10 min and counted in a hemocytometer. Some cells were dispersed by cytospin (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 μl 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 ± 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