

Inhibition of hematopoietic prostaglandin D synthase improves allergic nasal blockage in guinea pigs

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ARTICLE INFO

Article history:

Received 3 December 2010

Received in revised form 6 April 2011

Accepted 3 May 2011

Available online 10 May 2011

Keywords:

Allergic rhinitis

Nasal blockage

Prostaglandin D₂

H-PGDS

Guinea pig

ABSTRACT

Although it has been suggested that prostaglandin (PG) D₂ is involved in the pathogenesis of allergic rhinitis, whether the inhibition of hematopoietic PGD₂ synthase (H-PGDS) shows beneficial effects on allergic rhinitis has been unclear. We evaluated the effects of a selective H-PGDS inhibitor, TFC-007, on nasal symptoms on Japanese cedar pollen-induced allergic rhinitis of guinea pigs. Sensitized animals were challenged with the pollen once a week. TFC-007 (30 mg/kg, p.o.) given once before a challenge almost completely suppressed PGD₂ production in the nasal tissue early and late after the challenge. Although pre-treatment did not affect the incidences of sneezing and early phase nasal blockage, late phase nasal blockage was partially but significantly attenuated; however, nasal eosinophilia was not suppressed. In contrast, when TFC-007 was given once 1.5 h after the challenge, the late phase response was not affected. Collectively, PGD₂ produced by H-PGDS early after an antigen challenge can participate in the induction of late phase nasal blockage, although the mechanism may be independent of eosinophil infiltration. The strategy for H-PGDS inhibition may be beneficial for allergic rhinitis therapy.

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1. Introduction

Prostaglandin (PG) D₂ is a major cyclooxygenase (COX) metabolite produced mainly by mast cells after crosslinking of an allergen with the specific IgE antibody on the cell membrane [1]. PGD₂ is formed from its precursor, PGH₂, by PGD₂ synthase (PGDS) [2], which has been classified into two distinct types: lipocalin-type PGDS and hemopoietic-type PGDS (H-PGDS) [3]. The former is known to be expressed mainly in the brain, and H-PGDS has been reported to be expressed on mast cells, Th2 cells, basophils [4], which are known to be major players in allergic responses. Biological functions of PGD₂ are exerted by activation of two distinct G-protein-coupled receptors, DP₁ and CRTH2 (also known as DP₂) receptors [5–7]. Both receptors have been demonstrated to be expressed in the nasal mucosa of patients with allergic rhinitis [8]. PGD₂ has been suggested to be involved in the pathogenesis of allergic rhinitis because intranasal treatment with PGD₂ induced decrease in the nasal patency, nasal congestion and nasal secretion in both atopic and non-atopic subjects although sneezing was not evoked by the treatment with PGD₂ [9]. Furthermore, it has been

demonstrated that nasal antigen challenge produced PGD₂ in the local tissues of patients suffering from allergic rhinitis [10,11].

PGD₂ receptor antagonists have been developed to relieve allergic diseases such as allergic rhinitis. S-5751, a DP₁ receptor antagonist, has also been reported to prevent ovalbumin-induced allergic rhinitis symptoms in sensitized guinea pigs [12,13]. Another DP₁ receptor antagonist, laropiprant (MK-0524) has also been developed. Laropiprant effectively suppressed nasal congestion induced by intranasal instillation of PGD₂ in healthy subjects [14]. However, unexpectedly, the DP₁ receptor antagonist did not demonstrate efficacy in patients with allergic rhinitis [15]. On the other hand, as an antagonist against another PGD₂ receptor, CRTH2, ramatroban has been shown to suppress allergic rhinitis symptoms in guinea pigs [16], mice [17] and humans [18]. Ramatroban is being marketed in Japan as a therapeutic drug for allergic rhinitis. However, ramatroban showed antagonistic activity against not only CRTH2 but also TP receptors [19]. Regarding selective CRTH2 receptor antagonist, TM30089 was developed, and tested whether asthma pathology is affected by the compound. Consequently, the CRTH2 antagonist effectively attenuated antigen-induced eosinophilic airway inflammation in mice [20]. Additionally, it was reported that another novel selective CRTH2 antagonist, AM156 inhibited nasal symptoms in allergic rhinitis in mice [21]. Taken together, because PGD₂ can play significant roles in allergic rhinitis through CRTH2 receptors, attenuation of PGD₂ production by inhibition of PGDS could alleviate symptoms of the

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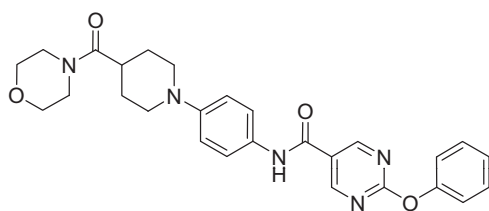


Fig. 1. Chemical structure of TFC-007.

disease. It was demonstrated that an H-PGDS inhibitor, HQL-79 inhibited antigen-induced nasal vascular permeability in guinea pigs [22], whereas the compound had potent anti-histamine and anti-5-hydroxytryptamine activities [23]. Thus, whether inhibition of H-PGDS improves allergic nasal symptoms has never been clear.

On the other hand, we have established a guinea pig model of allergic rhinitis, in which sensitized animals were repetitively exposed to Japanese cedar pollen once a week for more than 6 months [24–26]. This model allows us to repetitively conduct pharmacological evaluations of compounds that may possess anti-allergic activities because almost reproducible nasal allergic symptoms, immediate sneezing, and immediate and late phase nasal blockage, are evoked after challenges with the pollen. We have reported that the induction of immediate sneezing was potently suppressed by anti-histaminic drugs [27,28], and that the late phase nasal blockage was mediated by arachidonic acid metabolites, cysteinyl leukotrienes (CysLTs) and thromboxane (TX) A_2 , because the nasal blockage was partially suppressed by either a CysLT₁ receptor antagonist, pranlukast [25,27,29], or a TP receptor antagonist, seratrodist [25,27].

In this study, in order to identify the role of PGD₂ produced by H-PGDS in the induction of allergic rhinitis symptoms, we assessed pre-treatment effects of a selective H-PGDS inhibitor, TFC-007, on the induction of nasal signs in Japanese cedar pollen-induced experimental allergic rhinitis. The post-treatment effect of TFC-007 on late phase nasal blockage was also assessed.

2. Materials and methods

2.1. H-PGDS inhibitor

An H-PGDS inhibitor, TFC-007 [N-(4-(4-(morpholine-4-carbonyl)piperidine-1-yl)phenyl)-2-phenoxy-pyrimidine-5-carboxamide], was synthesized at Taiho Pharmaceutical Co. Ltd. (Tokyo, Japan). Chemical structure of TFC-007 is represented in Fig. 1.

2.2. Inhibitory effect of TFC-007 on activities of H-PGDS and L-PGDS

Effects of TFC-007 on the activities of H-PGDS and L-PGDS were evaluated according to Urade et al. [30]. Briefly, TFC-007 (final concentration of 0.1–100 μ M) or the vehicle (1% dimethylsulfoxide) was pre-incubated with human recombinant H-PGDS or L-PGDS at 25 °C for 5 min in γ -globulin (0.1 mg/ml)-containing Tris–HCl buffer (pH 8.0), which included 1 mM reduced form glutathione (for H-PGDS) or 1 mM dithiothreitol (for L-PGDS). For H-PGDS, the assay was conducted in the presence of 2 mM MgCl₂ because H-PGDS is activated by Mg²⁺ [31]. As a substrate, [¹⁴C] PGH₂ (final concentration of 10 μ M) was added followed by incubation for 1 min. The enzyme reaction was stopped by addition of a mixture of diethyl ether, methanol and 1 M citric acid (diethyl ether:methanol:1 M citric acid = 30:4:1). The organic solvent layer was applied to thin layer chromatography (TLC), followed by developing in a solvent containing diethyl ether, methanol and acetic

acid (diethyl ether:methanol:acetic acid = 90:2:1) at –20 °C for 45 min. The TLC plate was exposed to an imaging plate, and the radioactivity was analyzed by an imaging analyzer (BAS-2500; Fuji-film, Tokyo, Japan).

2.3. Effect of TFC-007 on activities of other arachidonic acid-related enzymes

Effect of TFC-007 on COX-1 activity was examined as reported by Chan et al. [32]. Briefly, TFC-007 (10 μ M) or the vehicle (1% dimethylsulfoxide) was pre-incubated with COX-1 purified from human platelets at 37 °C for 15 min in Hanks' balanced salt solution (HBSS) buffered with 15 mM HEPES (pH 7.4). Then, 100 μ M arachidonic acid was added as a substrate, followed by further incubation at 37 °C for 15 min. Amount of produced PGE₂ was measured by enzyme immunoassay. IC₅₀ value of indomethacin, a positive control drug, in this assay was 0.0261 μ M.

For assay of effect on COX-2 activity [33], TFC-007 (10 μ M) or the vehicle was pre-incubated with human recombinant COX-2 at 37 °C for 15 min in 100 mM Tris–HCl (pH 7.7) supplemented with 1 mM glutathione, 1 μ M hematin and 500 μ M phenol. Then, 0.3 μ M arachidonic acid was added, followed by incubation at 37 °C for 5 min. PGE₂ produced was assayed by enzyme immunoassay. IC₅₀ value of a positive control drug, rofecoxib in this assay was 0.125 μ M.

Effect of TFC-007 on 5-lipoxygenase (LOX) activity was evaluated by a method reported by Carter et al. [34], which was modified by us. Briefly, 5-LOX derived from human peripheral blood mononuclear leukocytes was treated with TFC-007 (10 μ M) or the vehicle in HBSS at 37 °C for 15 min, followed by addition of a substrate, arachidonic acid. Then, the mixture was incubated at 37 °C for 15 min. Amount of produced LTB₄ was measured by enzyme immunoassay. IC₅₀ value of a positive control compound, nordihydroguaiaretic acid in this assay was 0.122 μ M.

Effect on LTC₄ synthase activity was assayed as follows: TFC-007 (10 μ M) or the vehicle was pre-incubated with LTC₄ synthase derived from guinea pig lung at 37 °C for 15 min in 15 mM potassium phosphate buffer (pH 7.8) supplemented with 4.5 mM L-serine, 1.5 mM glutathione and 0.06% bovine serum albumin. Then, 2.5 μ g/ml LTA₄ methyl ester was added, followed by incubation at 37 °C for 30 min. LTC₄ produced was assayed by enzyme immunoassay. IC₅₀ value of a positive control compound, sulfasalazine in this assay was 117 μ M.

Effect of TFC-007 on TX synthase activity was evaluated by a method reported elsewhere [35]. Briefly, TX synthase derived from human platelets was incubated with TFC-007 or the vehicle in 10 mM Tris–HCl (pH 7.4) at 25 °C for 15 min. A substrate, PGH₂ was added at 10 μ M, followed by incubation at 25 °C for 3 min. Amount of formed TXB₂ was quantified by enzyme immunoassay. IC₅₀ value of a positive control compound, 1-(7-carboxyheptyl)-imidazole in this assay was 0.0115 μ M.

Effect on microsomal PGE synthase (mPGES) was also assessed according to a method [36], which was modified by us. mPGES was prepared from interleukin-1 β -stimulated 1321N1 astrocytoma cells. TFC-007 (100 μ M) or the vehicle was pre-incubated with the mPGES at 25 °C for 5 min in 100 mM potassium phosphate buffer (pH 7.0) supplemented with 2.5 mM glutathione. Then, a substrate, [¹⁴C] PGH₂ (10 μ M) was added. One min after the substrate addition, the reaction was stopped by addition of a mixture of diethyl ether, methanol and 1 M citric acid (diethyl ether:methanol:1 M citric acid = 30:4:1). The organic solvent layer was developed by TLC, followed by measurement of radioactivity at a band corresponding to PGE₂. IC₅₀ value of MK-886 used as a positive control as reported by Mancini et al. [36] in this assay was 54.5 μ M.

2.4. Effect of TFC-007 on the binding of each ligand to H_1 , CysLT₁ and TP receptors

For the study of H_1 receptors, based on a literature reported by De Backer [37], a membrane fraction prepared from human recombinant CHO cells, [³H] pyrilamine (1.2 nM), and TFC-007 (10 μ M) or the vehicle (1% dimethylsulfoxide) were incubated at 25 °C for 3 h in 50 mM Tris–HCl (pH 7.4) supplemented with 2 mM MgCl₂, 100 mM NaCl and 250 mM sucrose, and then filtrated over glass microfiber filters. Subsequently, radioactivity retained on the filters was counted. For assessment of the non-specific binding, 1 μ M of unlabelled pyrilamine was used. K_D and B_{max} in this binding assay were 1.1 nM and 6.7 pmol/mg protein, respectively. IC₅₀ value of pyrilamine in this assay was 1.92 nM.

Binding assay of CysLT₁ receptors was conducted as reported [38]. A membrane fraction prepared from human recombinant CHO-K1 cells, [³H] LTD₄ (0.3 nM), and TFC-007 (10 μ M) or the vehicle were incubated at 25 °C for 30 min in 50 mM Tris–HCl (pH 7.4) supplemented with 5 mM CaCl₂, 5 mM MgCl₂, 100 μ g/ml bacitracin, 1 mM benzamidine and 0.1 mM phenylmethanesulfonyl fluoride, and then filtrated over glass microfiber filters. Subsequently, radioactivity retained on the filters was counted. For assessment of the non-specific binding, 0.3 μ M of unlabelled LTD₄ was used. K_D and B_{max} in this binding assay were 0.21 nM and 3 pmol/mg protein, respectively. IC₅₀ value of LTD₄ in this assay was 1.48 nM.

Binding study for TP receptors using a TP receptor ligand, SQ-29,548 was conducted as reported [39]. A membrane fraction prepared from human recombinant HEK-293 cells, [³H] SQ-29,548 (5 nM), and TFC-007 (10 μ M) or the vehicle were incubated in 50 mM Tris–HCl (pH 7.4) supplemented with 154 mM NaCl at 25 °C for 30 min, and then filtrated over glass microfiber filters. Subsequently, radioactivity retained on the filters was counted. For assessment of the non-specific binding, 1 μ M of unlabelled SQ-29,548 was used. K_D and B_{max} in this binding assay were 9.4 nM and 5.1 pmol/mg protein, respectively. IC₅₀ value of a TP receptor antagonist, ICI-192,605 in this assay was 2.3 nM.

2.5. Sensitisation and challenge with cedar pollen

Japanese cedar pollen (*Cryptomeria japonica*) was harvested in Gifu and Shiga Prefectures in Japan in 1998. The cedar pollen extract and Al(OH)₃ gel were prepared as previously described [40,41]. Twice each day for 7 days, male, 4-week-old, Hartley guinea pigs (Japan SLC, Hamamatsu, Japan) were sensitised by intranasal instillation of the cedar pollen extracts adsorbed on Al(OH)₃ gel (100 μ g protein/100 mg Al(OH)₃ per mL) into the bilateral cavities at a volume of 3 μ L/nostril [24]. Sensitized animals were intranasally challenged once every week by inhalation of 1.8 mg cedar pollen per nostril (3.6 mg/animal) under spontaneous breathing without any anaesthesia using a hand-made inhalation apparatus [40].

All animal procedures and experimental protocols complied with the NIH Guidelines and those of the Experimental Animal Research Committee at Kyoto Pharmaceutical University.

2.6. Study design

In the first series of experiments, effects of a selective PGDS inhibitor, TFC-007, on activities of H-PGDS and L-PGDS were evaluated in vitro.

We next examined whether inhibition of H-PGDS improves allergic rhinitis symptoms using the guinea pig model in vivo. We have previously reported that biphasic nasal blockage was almost reproducibly induced during the 4th–30th challenges [24]. In this study, subsequent experiments were conducted during those challenges. In order to evaluate the single pre-treatment effect of

Table 1

Effects of TFC-007 on activities of H-PGDS and L-PGDS.

	H-PGDS (IC ₅₀ value)	L-PGDS (inhibitory rate at 100 μ M)
TFC-007	0.083 μ M	2.2%

TFC-007 (p.o.) on antigen-induced sneezing and biphasic nasal blockage, the compound was administered before the 7th challenge. Effects of pre-treatment with TFC-007 (p.o.) on increases in leukocyte numbers, and amounts of PGD₂, CysLTs and TXB₂ in nasal cavity lavage fluid (NCLF) were assessed at the 10th, 13th, 15th or 20th challenge. Post-treatment effects of TFC-007 (p.o.) on antigen-induced nasal blockage and the increase in PGD₂ amount in NCLF were also evaluated at the 7th and 8th challenges, respectively.

2.7. Treatment with TFC-007

Pre-treatment: TFC-007 (30 mg/kg) was orally administered 1 h before the 7th, 10th, 13th, 15th or 20th challenge.

Post-treatment: TFC-007 (30 mg/kg) was orally administered 1.5 h after the 7th or 8th challenge.

Pharmacokinetic data after an oral administration of 30 mg/kg of TFC-007 in guinea pigs were as follows: C_{max} , 6.8 μ M; T_{max} , 2 h; $T_{1/2}$, 6.6 h; AUC_{0–24h}, 68.6 μ Mh. Even at 10 h after the oral administration, the plasma concentration of TFC-007 was 2.8 μ M. As demonstrated in this study, the IC₅₀ value of TFC-007 for H-PGDS was 0.083 μ M (Table 1). In addition, the plasma protein bound rate of TFC-007 was 80%. Taken together, it is clear that the free form of TFC-007 exists in the circulation at sufficient concentrations for blocking H-PGDS within 10 h after the oral administration.

2.8. Measurement of specific airway resistance (sRaw)

As an indicator of respiratory function (nasal blockage), the sRaw in conscious guinea pigs was measured using a two-chambered, double-flow plethysmograph system (Pulmos-I, -II, -III; M.I.P.S., Osaka, Japan), as has been previously described [42].

Elevation of sRaw after pollen inhalation in the sensitised guinea pig appeared to reflect total (upper and lower) airway resistance, as the guinea pig functionally respire through the nose and not through the mouth. We have previously demonstrated that our inhalation procedure deposits 99.999% of the pollen in the upper airway [40]. Furthermore, after the pollen inhalation challenge, accumulation of eosinophils in the lung, which is a characteristic feature of allergic bronchial asthma, was not observed in this model [43], even though nasal eosinophilia was markedly induced [43]. Thus, the changes in sRaw induced by antigen challenge in the present model exclusively reflect the nasal response and not the lower airway response.

2.9. Counting of sneezing frequency

Sneezing frequency was determined 0–10 min and 10–60 min after a pollen inhalation challenge.

2.10. Nasal cavity lavage (NCL)

The nasal cavity of the sensitized animal was lavaged with physiologic saline before, 20 min after or 5 h after a pollen challenge. NCL was performed once for each guinea pig, as previously described [40]. In brief, warm saline (1 mL at 37 °C) was aspirated from the left nostril under reduced light pressure produced by an air pump. NCLF was collected from the right nostril into a tube, followed by centrifugation. The NCLF recovered from the left nostril was centrifuged at 120 $\times$ g for 5 min at 4 °C. The resultant pellet was used for counting the number of leukocytes. The supernatant was stored

at -80°C until assayed for the concentration of PGD_2 , CysLTs and TXB_2 .

2.11. Counting of cells in NCLF

The total leukocyte number in the cell pellet was determined by staining with Turk's solution (Nacalai Tesque, Kyoto, Japan). For the numbers of differentiated leukocytes, the cell suspension was centrifuged in a Cell Settling Chamber (Neuro Probe, Cabin John, MD) at $50 \times g$ for 30 s at 4°C , and then the settled leukocytes were stained with Diff-Quik® solution (Sysmex International Reagent, Kobe, Japan), followed by microscopic counting of at least 500 cells.

2.12. Measurement of concentrations of PGD_2 , CysLTs and TXA_2 in the NCLF

The amount of PGD_2 in the supernatant was measured using the PGD_2 -MOX Express kit (Cayman Chem.) after deproteinization with 50% acetone according to the manufacturer's instructions. CysLTs and TXB_2 were measured using the Cysteinyl Leukotriene express EIA kit and TXB_2 EIA kit (both from Cayman Chem.) after deproteinization with 80% ethanol, as previously reported by us [27]. Recoveries during the deproteinization procedures were more than 80% as estimated by radiolabelled materials.

2.13. Statistical analyses

Data were statistically evaluated by one-way analysis of variance. If a difference appeared to be significant, the individual group difference was determined using Bonferroni's correction for multiple tests. *P* values less than 0.05 were considered significant.

3. Results

3.1. Effects of TFC-007 on activities of H-PGDS and L-PGDS, and other arachidonic acid-related enzymes

Table 1 shows the effects of TFC-007 on the activities of H-PGDS and L-PGDS in vitro. TFC-007 concentration-dependently inhibited H-PGDS activity, and the IC_{50} value of TFC-007 was $0.083 \mu\text{M}$. On the other hand, L-PGDS activity was hardly affected by the compound, even at $100 \mu\text{M}$.

Table 2
Effects of TFC-007 on activities of various arachidonic acid-related enzymes.

	% of inhibition at 10 or 100 μM					
	COX-1	COX-2	5-LOX	LTC ₄ synthase	TX synthase	mPGES
TFC-007	-5	5	-3	15	12	-1

The final concentration of TFC-007 for assays of cyclooxygenase (COX)-1, COX-2, 5-lipoxygenase (LOX), leukotriene (LT) C₄ synthase or thromboxane (TX) synthase was $10 \mu\text{M}$. The assay for microsomal prostaglandin E₂ synthase (mPGES) was performed using $100 \mu\text{M}$ TFC-007.

Table 3
Effects of TFC-007 on binding of each ligand to H₁, CysLT₁ and TP receptors.

	% of inhibition at $10 \mu\text{M}$		
	H ₁	CysLT ₁	TP
TFC-007	17	-1	-2

In order to further evaluate the specificity of TFC-007 on H-PGDS, effects of the compound on activities of various other arachidonic acid-related enzymes were assessed. As shown in Table 2, activities of COX-1, COX-2, 5-LOX, LTC₄ synthase and TX synthase were not affected by TFC-007 at $10 \mu\text{M}$. TFC-007 at $100 \mu\text{M}$ did not affect the activity of mPGES (Table 2).

Effects of TFC-007 on binding at several receptors that are related to allergic inflammation were examined. Although TFC-007 tended to affect the binding to H₁ receptors, the magnitude (17% inhibition) was small even at $10 \mu\text{M}$ (Table 3) in comparison with the IC_{50} value (1.92 nM) of a classical H₁ receptor antagonist, pyrilamine. The binding of each ligand to CysLT₁ and TP receptors was hardly affected by $10 \mu\text{M}$ TFC-007 (Table 3).

3.2. Effects of pre-treatment with TFC-007 on experimental allergic rhinitis

When TFC-007 was administered 1 h before the pollen challenge, sneezing, which was induced within 1 h after the challenge, was not affected by the treatment (Fig. 2(A)). Although we have reported that the time-course change in sRaw after a pollen inhalation challenge was clearly biphasic [24], that of the control group shown in Fig. 2(A) did not seem to be biphasic. However, the pre-treatment with TFC-007 significantly inhibited late phase nasal blockage but not early phase nasal blockage (Fig. 2(B) and (C)),

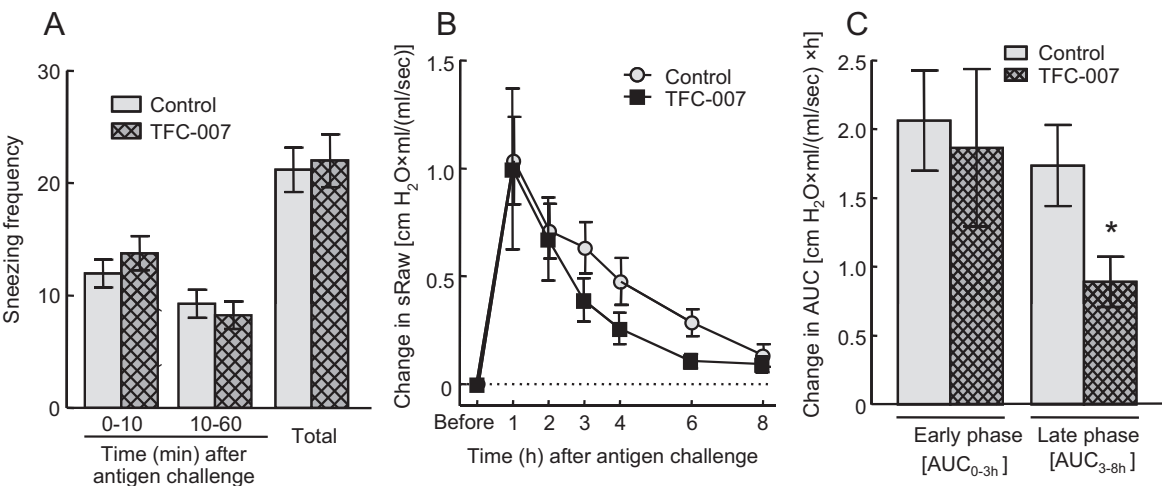


Fig. 2. Effect of pre-treatment with TFC-007 on sneezing (A) and the biphasic increase in specific airway resistance (sRaw) (B and C) induced by an antigen challenge in sensitized guinea pigs. TFC-007 (30 mg/kg, p.o.) was administered once 1 h before the 7th antigen challenge. Panel C shows the effect on nasal blockage as the area under the response curve (AUC) for sRaw during the early (0–3 h) and late (3–8 h) phases. Each column and point represents the mean $\pm$ S.E. of 20 animals. Significantly different from the control (**P* < 0.05).

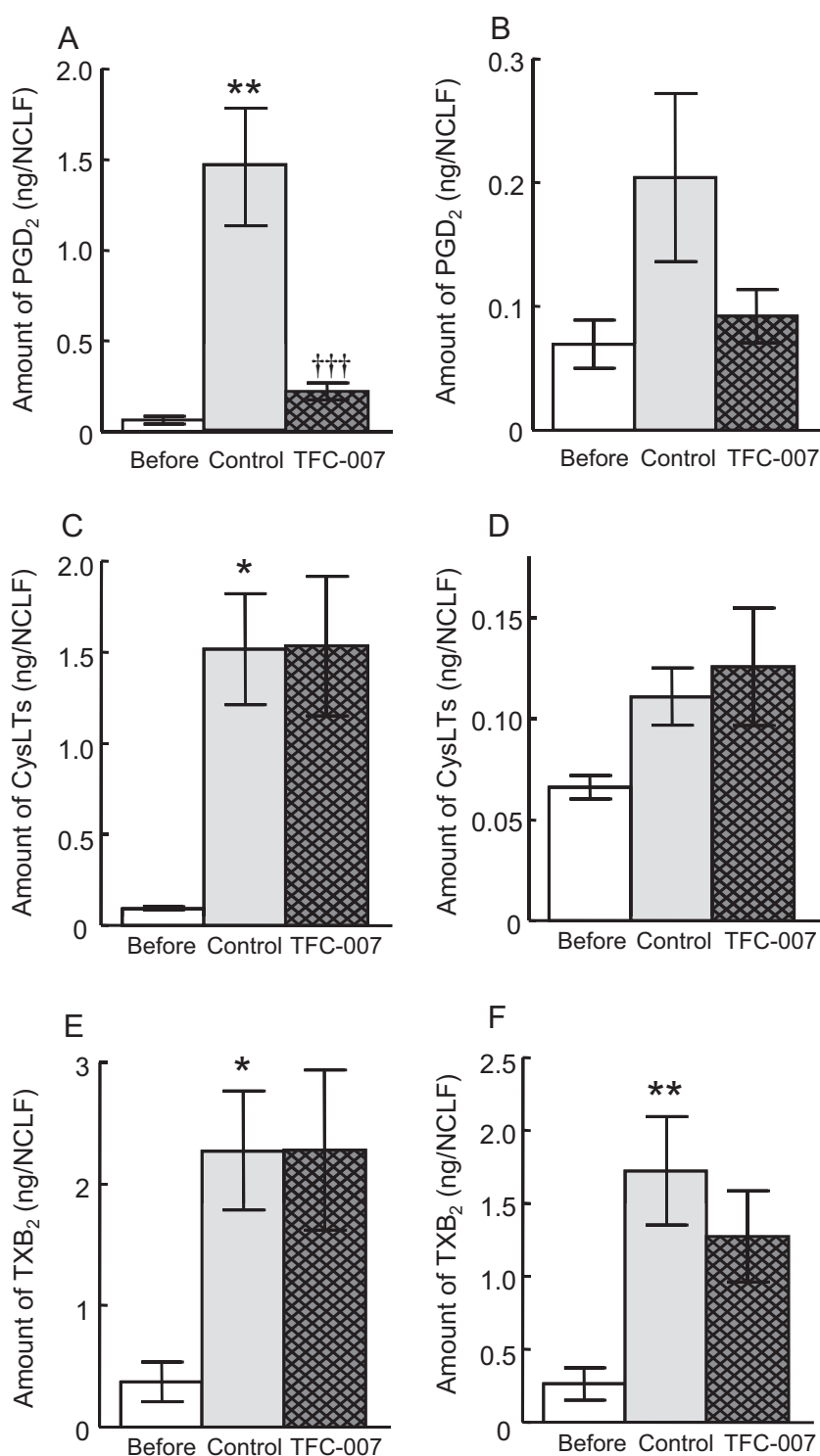


Fig. 3. Effect of pre-treatment with TFC-007 on increases in prostaglandin (PG) D₂ (A and B), cysteinyl leukotrienes (CysLTs, C and D) and thromboxane (TX) B₂ (E and F) in the nasal cavity lavage fluid (NCLF) collected 20 min (A, C and E) and 5 h (B, D and F) after an antigen challenge in sensitized guinea pigs. TFC-007 (30 mg/kg, p.o.) was administered once 1 h before the 10th–20th antigen challenge. Each column represents the mean $\pm$ S.E. of 8–28 animals. Significantly different from the value before the challenge (* P < 0.05 and ** P < 0.01) and control (††† P < 0.001).

suggesting that the nasal blockage during the period consists of 2 phases as has been reported in our previous literatures [27,29].

The significant increase in PGD₂ in NCLF collected 20 min after the challenge was almost completely inhibited by pre-treatment with TFC-007 (Fig. 3(A)). In addition, a relatively small increase in PGD₂ was observed 5 h after the challenge. The increase in PGD₂ observed in the late phase was also reduced by pre-treatment with

the H-PGDS inhibitor (Fig. 3(B)). In contrast, neither the CysLTs nor TXB₂ increase in the NCLF collected 20 min and 5 h after the challenge was inhibited by pre-treatment (Fig. 3(C)–(F)).

As we have previously reported [43], eosinophils were markedly increased in the NCLF 5 h after the challenge (Fig. 4). The increased number of eosinophils in NCLF was highly variable. The pre-treatment with TFC-007 did not affect the eosinophilia (Fig. 4).

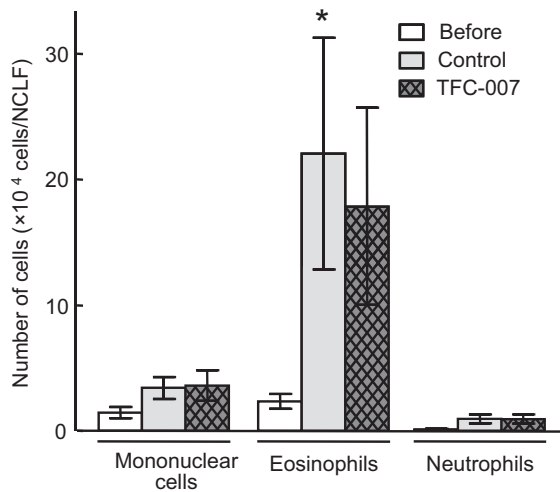


Fig. 4. Effect of pre-treatment with TFC-007 on increases in numbers of mononuclear cells, eosinophils and neutrophils in nasal cavity lavage fluid (NCLF) collected 5 h after an antigen challenge in sensitized guinea pigs. TFC-007 (30 mg/kg, p.o.) was once administered 1 h before the 10th–20th antigen challenge. Each column represents the mean $\pm$ S.E. of 16–32 animals. Significantly different from before the challenge (* $P < 0.05$).

Mononuclear cells and neutrophils also tended to increase (Fig. 4); however, pre-treatment with TFC-007 did not affect increases in the leukocytes as well (Fig. 4).

3.3. Effects of post-treatment with TFC-007 in experimental allergic rhinitis

The post-treatment effect of the H-PGDS inhibitor was assessed by oral administration of the compound at 1.5 h after the antigen challenge. The treatment failed to affect the nasal blockage at 2–8 h after the challenge (Fig. 5(A)), whereas the increase in PGD₂ in the NCLF was strongly suppressed, even by post-treatment (Fig. 5(B)).

4. Discussion

A single pre-treatment with a specific H-PGDS inhibitor almost completely prevented the increase in PGD₂ both at 20 min and

5 h after a pollen challenge. On the other hand, we have reported that both a CysLT₁ receptor antagonist and a TP receptor antagonist partially but significantly attenuated late phase nasal blockage [25,27,29], and that both CysLTs and TXB₂ were increased in NCLF at 20 min and 5 h after a pollen challenge [27]. However, increases in the amounts of CysLTs and TXB₂ were not affected by H-PGDS inhibition, confirming that TFC-007 is a specific inhibitor of PGD₂ production. In addition, although it has been reported that CysLTs can stimulate macrophages to secondarily generate TXA₂ in guinea pig lung tissue [44], the present finding indicates that PGD₂ cannot generate CysLTs and TXA₂ in the nasal tissue of guinea pigs.

Under the condition in which allergen-induced PGD₂ production was markedly suppressed, the nasal blockage during 3–6 h after a pollen challenge was effectively attenuated, whereas neither sneezing induced immediately nor early phase nasal blockage during 0–2 h after the challenge was affected by the compound. Pollen challenge-induced eosinophilia in the nasal tissue was also not prevented by the single pre-treatment. On the other hand, when the H-PGDS inhibitor was administered 1.5 h after the challenge, the late phase nasal blockage was not suppressed even though PGD₂ increase during the late phase was inhibited. Taken together, the late phase nasal blockage could be induced by PGD₂ produced early after the pollen challenge within 1.5 h. It is possible that PGD₂ indirectly induced the nasal blockage, probably through the recruitment of certain effector cells in the nasal mucosa. However, pre-treatment with the H-PGDS inhibitor did not affect the increase in eosinophils that have been reported to express CRTH2 [7], indicating that eosinophils are not involved in PGD₂-mediated late phase nasal blockage. Consistent with this, we have previously reported that even when eosinophilia was almost completely suppressed by an anti-IL-5 mAb in the model of allergic rhinitis, neither early nor late phase nasal blockage was inhibited [45]. Thus, eosinophil infiltration is not basically associated with the induction of late phase nasal blockage. Besides eosinophils, Th2 lymphocytes and basophils also express CRTH2 [7], implying that these cells may be responsible for PGD₂-induced nasal blockage.

In agreement with the speculation that CRTH2 could be involved in the induction of nasal blockage, it has been recently reported that selective CRTH2 antagonists, TM30089 [20] and AM-156 [21] attenuated antigen-induced pulmonary inflammation in mice. Furthermore, interestingly, Gyles et al. [46] reported that PGD₂ anaphylactically produced from mast cells play a central role

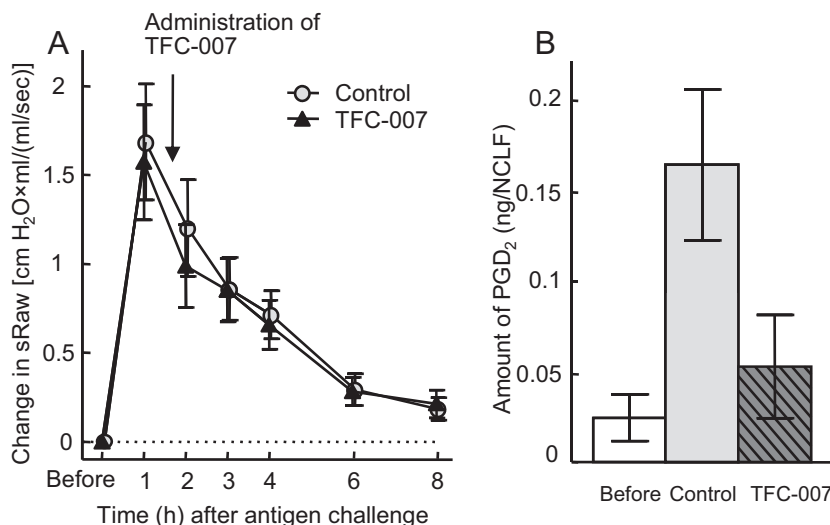


Fig. 5. Effect of post-treatment with TFC-007 on biphasic increase in specific airway resistance (sRaw) induced by an antigen challenge (A), and increase in prostaglandin (PG) D₂ level in the nasal cavity lavage fluid (NCLF) collected 5 h after an antigen challenge (B) in sensitized guinea pigs. Effects on the nasal blockage and PGD₂ production were assessed at 7th or 8th, and 18th challenges, respectively. TFC-007 (30 mg/kg, p.o.) was administered once 1.5 h after an antigen challenge. Each column and point represents the mean $\pm$ S.E. of 37 (panel A) and 8–16 (panel B) animals.

in the activation of CRTH2⁺ CD4⁺ Th2 lymphocytes through a CRTH2-dependent mechanism. This finding further strengthens the speculation in this study that PGD₂ produced within 1.5 h after the challenge may induce the late phase nasal blockage through recruitment of other leukocytes than eosinophils, such as Th2 lymphocytes, to the nasal tissue.

Although a relatively large amount of PGD₂ was produced during early phase nasal blockage in comparison with the late phase, early phase nasal blockage was not inhibited by the inhibition of PGD₂ generation. It is well known that PGD₂ activates Gs protein-coupled DP₁ receptors on vascular smooth muscle cells of the nasal mucosa leading to vasodilatation associated with nasal blockage mainly through an increase in intracellular cyclic AMP [47,48]. Our present finding suggests that PGD₂-dependent nasal blockage may not be a major mechanism of early phase nasal blockage. This ineffectiveness on early phase nasal blockage may imply that antagonism against DP₁ receptors produces no beneficial effect on nasal blockage, as demonstrated by the recent clinical study that a DP₁ antagonist, laropiprant did not improve symptoms of allergic rhinitis [15]. On the other hand, we have reported that a non-selective nitric oxide (NO) synthase inhibitor, N^w-nitro-L-arginine methyl ester, completely inhibited early phase nasal blockage [49]. Since NO directly activates guanylyl cyclase, which increases intracellular cyclic GMP, cyclic GMP-dependent vasodilatation might be an important event in late phase nasal blockage.

Among H-PGDS inhibitors developed to date, HQL-79 has been demonstrated to exhibit anti-allergic effects, such as inhibition on passive cutaneous anaphylaxis, antigen-induced bronchoconstriction and nasal vascular permeability, and antigen-induced late asthmatic response in experimental animals [22]. However, because HQL-79 has anti-histamine and anti-5-hydroxytryptamine effects that were comparable to activities of other anti-histamines, epinastine and ketotifen [23], we have not used HQL-79 as a positive control H-PGDS inhibitor. In contrast, our radiobinding assay revealed that TFC-007 exerted no binding to H₁ receptors. We have reported that sneezing induced immediately after a pollen inhalation challenge in this experimental allergic rhinitis was mainly mediated by histamine [27,28], whereas TFC-007 did not inhibit the sneezing, suggesting that TFC-007 has no anti-histamine effect in vivo experiment as well. This finding further confirms a clinical finding that intranasal instillation of PGD₂ did not evoke sneezing in both non-atopic and atopic subjects [9].

In contrast to the effectiveness of the PGDS inhibitor, Arimura et al. [12] reported that a DP₁ receptor antagonist, S-5751, suppressed immediate nasal blockage and sneezing, plasma extravasation into the nasal mucosa during the late phase, and antigen-induced increase in eosinophils in the nasal cavity lavage fluid in OVA-induced models of guinea pigs, by either pre- or post-treatment. The reasons for the differences between our results and the literature [12] are not clear; however, we speculate that they might be due to the antigen used and sensitization/challenge protocol. A biphasic allergic rhinitis model was developed by Arimura et al. [12] by four-times intranasal dripping of 1% OVA solution at 2- or 4-day intervals, followed by a final OVA challenge 5–7 days after the end of the fourth OVA instillation, in guinea pigs that had been sensitized by i.p. administrations of OVA + Al(OH)₃. In our model, in order to mimic the pathogenesis in the clinical setting as much as possible, guinea pigs were intranasally sensitized and then challenged by inhalation of the pollen once a week. Biphasic nasal blockage could be observed in the 4th–6th challenges, followed by the induction of almost reproducible nasal blockage in subsequent challenges [24]. Thus, susceptibility to pharmacologically active compounds in allergic models may be dependent on the antigen used and/or duration and times of antigen challenge. In other words, our allergic model was more resistant to therapeutic drugs than others, such as in the model reported by Arimura et al. [12]; however, we believe

that the drug resistance in our model can reflect a chronic state of allergic rhinitis. Indeed, we have experienced that the effectiveness of an anti-histaminic drug was markedly decreased by multiple local challenges with pollen in a guinea pig model of allergic conjunctivitis [50]. It will be necessary to directly compare the effects of the H-PGDS inhibitor with those of a DP₁ or CRTH2 receptor antagonist.

In conclusion, PGD₂ produced early after an antigen challenge can participate in the induction of late phase nasal blockage, but the mechanism may not be dependent on either the production of CysLTs/TXA₂ or eosinophil infiltration. The strategy for H-PGDS inhibition may be beneficial for allergic rhinitis therapy.

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