Prevention of Allergic Inflammation by a Novel Prostaglandin Receptor Antagonist, S-5751

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ABSTRACT

Prostaglandin (PG) D$_2$, the major cyclooxygenase metabolite generated from immunologically stimulated mast cells, is thought to contribute to the pathogenesis of allergic diseases due to its various inflammatory effects. However, since no DP receptor antagonist has been developed as an antiallergic drug, the role of PGD$_2$ in the pathogenesis of allergic diseases remains uncertain. Here, we report the in vivo efficacy of our newly established DP receptor antagonist, S-5751 [[(Z)-7-[[1R,2R,3S,5S]-2-(5-hydroxybenzo[b]thiophen-3-ylcarbonylamino)-10-norpinan-3-yl]hept-5-enolic acid]], using various allergic inflammation guinea pig models. In allergic rhinitis models, oral administration of S-5751 dramatically inhibited not only early nasal responses, as assessed by sneezing, mucosal plasma exudation, and nasal blockage, but also late responses such as mucosal plasma exudation and eosinophil infiltration. Even when S-5751 was administered after recovery from the early responses, these late phase responses were almost completely suppressed. In addition, S-5751 alleviated allergen-induced plasma exudation in the conjunctiva in an allergic conjunctivitis model and antigen-induced eosinophil infiltration into the lung in an asthma model. These findings provide evidence for the crucial role of PGD$_2$ as a mediator of allergic inflammation in guinea pigs and suggest that DP receptor antagonists may be useful in the treatment of allergic diseases triggered by mast cell activation.

Prostaglandin (PG) D$_2$ is the major cyclooxygenase metabolite produced by mast cells responding to IgE-dependent stimuli (Lewis et al., 1982). Local allergen challenge in patients with allergic rhinitis (Naclerio et al., 1983, 1985), bronchial asthma (Murray et al., 1986), allergic conjunctivitis (Proud et al., 1990), and atopic dermatitis (Charlesworth et al., 1991) has been shown to result in rapid elevation of the PGD$_2$ level in nasal and bronchial lavage fluids, tears, and skin chamber fluids. Since PGD$_2$ has been reported to exert a variety of inflammatory effects such as an increase in vascular permeability in the conjunctiva and skin (Flower et al., 1976; Woodward et al., 1990), an increase in nasal airway resistance (Doyle et al., 1990), airway narrowing (Johnston et al., 1995), and eosinophil infiltration into the conjunctiva and trachea (Emery et al., 1989; Woodward et al., 1996), it seems to be an important mediator in various allergic diseases. However, since there have been few reports on the efficacy of DP receptor antagonists in allergic disease models, although a specific DP receptor antagonist, BW A868C (Giles et al., 1989; Hamid-Bloomfield and Whittle, 1989; Hirata et al., 1994; Boie et al., 1995; Kiriyama et al., 1997; Sharif et al., 2000), is available as a tool for biological examination of DP receptors, the contribution of PGD$_2$ to the pathogenesis of allergic diseases remains uncertain in not only human but also animal models.

Recently, we have demonstrated that a series of DP receptor antagonists, which were originally established in our laboratories by optimization of lead compounds discovered by screening of our compound library in a structure-activity relationship study, are effective for alleviating inflammatory responses in guinea pig allergic disease models (Tsuri et al., 1997). Our findings suggest that PGD$_2$ acts as an important mediator in allergic inflammation, which is also supported by recent research using DP receptor-deficient mice, established by Matsuoka et al. (2000), demonstrating that inflammatory cell infiltration into lung tissue following repeated antigen challenge was reduced in DP receptor-deficient mice compared with that in wild-type mice in the asthma model.

In the present study, to determine not only the role of PGD$_2$ in the pathogenesis of allergic diseases but also the usefulness of DP receptor antagonist as a new medication, we chose a highly potent and relatively selective DP receptor antagonist, S-5751 [[(Z)-7-[[1R,2R,3S,5S]-2-(5-hydroxybenzo[b]thiophen-3-ylcarbonylamino)-10-norpinan-3-yl]hept-5-enolic acid]] from among the compounds we had previously reported (Tsuri et al., 1997), and assessed the ability of S-5751 to alleviate allergic symptoms and inflammatory responses in guinea pig allergic animal models. Since S-5751 showed a weak antagonism for TP receptor (Tsuri et al., 1997), we also used BW A868C, and a specific TP receptor antagonist, S-1452 [a calcium salt of (+)-

ABBREVIATIONS: PG, prostaglandin; BAL, bronchoalveolar lavage.
S-145), to further clarify the role of PGD₂ in guinea pig allergic disease models.

**Materials and Methods**

**Compounds.** S-5751, BW A868C (3-benzyl-5-(6-carboxyhexyl)-1-((2-cyclohexyl-2-hydroxyethylamino)-hydantoin), (+)-S-145 ((+)-52)-7-[(1R,2S,3S)-3-(phenylsulfonylamino)bicyclo[2.2.1]hept-2-yl]-5-heptenoic acid), S-1452 [a calcium salt of (+)-S-145], and U-46619 (11α, 9α-epoxymethano-PGH₂) were synthesized in our laboratories. Terfenadine and ketotifen were purchased from Sigma (St. Louis, MO), PGD₂, PGE₂, and carbacyclin were from Funakoshi (Tokyo, Japan) and histamine was from Nacalai Tesque (Kyoto, Japan).

Guinea Pigs. Male Hartley guinea pigs, weighing 370 to 990 g, purchased from Japan Charles River (Yokohama, Japan) or Japan SLC (Hamamatsu, Japan), were used in this study.

**Preparation of Human Platelet Membranes and Ligand Binding Assay for DP and TP Receptors.** To assess the affinity of compounds for DP and TP receptors in humans, [³H]PGD₂ and [³H]-(+)-S-145 binding was performed with human platelet membranes (Cooper and Ahern, 1979; Kishino et al., 1991). Briefly, frozen-thawed human platelet membranes (80 µg), which were prepared as previously reported (Kishino et al., 1991), were incubated with 5 nM [³H]PGD₂ (115 Ci/mmol; PerkinElmer Life Science Products, Boston, MA) or [³H]-(+)-S-145 Na (26.35 Ci/mmol; synthesized in our laboratories) in the absence or presence of increasing concentrations of the compounds in the incubation buffer (50 mM Tris-HCl, pH 7.4, containing 10 mM MgCl₂) for 90 min at 4°C or for 60 min at room temperature, respectively. Nonspecific binding was determined in the presence of 10 µM PGD₂ or 10 µM (-)+S-145 Na for DP and TP receptors, respectively. The incubations were terminated by rapid vacuum filtration using glass fiber filter (GF/C; Whatman, Maidstone, UK) and washed several times with ice-cold saline, and then the radioactivities retained on the filters were measured with a liquid scintillation counter. The inhibitory activity of the compounds against the [³H]PGD₂ and [³H]-(+)-S-145 specific binding was evaluated by estimating its half-maximal inhibitory concentration (IC₅₀) from each displacement curve. The receptor-binding inhibition constants (Kᵢ values) were calculated from IC₅₀ values as previously described (Cheng and Prusoff, 1973).

**cAMP Assay in Washed Platelets for DP and IP Receptors.** To assess antagonistic activity against DP and IP receptors, human and guinea pig platelets were stimulated with PGD₂ or carbacyclin, a stable IP receptor agonist, in the presence or absence of compounds (Trist et al., 1989; Darius et al., 1994). Briefly, human and guinea pig peripheral blood samples were drawn into a syringe containing both about one-ninth volume of acid citrate-dextrose (85 mM trisodium citrate dihydrate, 70 mM citric acid, and 110 mM glucose) and 10 µg/ml PGE₂, and centrifuged at 180g for 10 min. The platelets in platelet rich plasma were washed three times with a washing buffer (100 mM NaCl, 5 mM KCl, 2 mM CaCl₂, 1 mM MgCl₂, 30 mM trisodium citrate dihydrate, 9.9% glucose, and 5% bovine serum albumin) by centrifugation at 800g for 10 min. The number of platelets was adjusted to the final concentrations of 5 x 10⁶ for humans and of 1 x 10⁹ platelets/ml for guinea pigs. The washed platelets were pretreated with 3-isobutyl-1-methylxanthine (0.5 mM) for 5 min and then treated with compounds at various concentrations. After a 10-min incubation, 0.1 µM PGD₂ or 0.1 µM carbacyclin was added to initiate the reaction. After a 2-min incubation, 1 N HCl was added to the reaction mixtures to stop the reaction, and the platelets were disrupted using an ultrasonic homogenizer. The resultant supernatants were obtained by centrifugation and stored at −20°C. The amounts of cAMP in the supernatants were determined by a radioimmunoassay method (YAMASA cAMP RIA kit, Yamasa, Japan).

**cAMP Assay in HL-60 Cells for EP2 Receptor.** To determine antagonistic action of S-5751 against human EP2 receptor, human promyelocytic leukemic HL-60 cells were used (Armstrong and Talpain, 1994). Briefly, HL-60 cells were grown in suspension cultures in RPMI 1640 medium containing l-glutamine, 10% heat-inactivated fetal calf serum, penicillin, and streptomycin and then cultured in 1.2% dimethyl sulfoxide-containing medium for 6 days to differentiate into neutrophil-like cells. These differentiated cells were resuspended in Hanks’ buffer containing 0.25 mM 3-isobutyl-1-methylxanthine (2 x 10⁶ cells/ml) and incubated with various concentrations of the compounds for 5 min at 37°C. After that, the cells were stimulated with PGE₂ (0.1 µM). The reactions were terminated by adding ethanol. The cAMP levels in the supernatant were measured as described above.

**In Vivo Responses Mediated via DP, TP, EP, and H₁ Receptor.** Since topical application of PGD₂ and U-46619 (a stable TP receptor agonist) into the eyes of guinea pigs is known to cause plasma exudation in the conjunctiva via DP and TP receptors, respectively (Woodward et al., 1990), we assessed the antagonism of compounds against conjunctival microvascular permeability induced by PGD₂ and U-46619 as well as histamine. Briefly, guinea pigs were challenged by instillation of 20 µl of 0.1% PGD₂, 0.1% U-46619, or 0.1% histamine to the eyes (10-µl volume/eye), and then Evans blue dye (20 mg/kg i.v.) was injected as a marker of plasma exudation. Thirty minutes later, the animals were exsanguinated, and the conjunctiva and eyelid were removed. The tissues obtained from both eyes were incubated in 3 ml of formamide at 60°C to extract the extravasated dye, and centrifuged. The absorption of the supernatant at 620 nm was determined, and the amount of Evans blue dye leaked into the tissues was quantified by interpolation on a standard curve for Evans blue dye. To examine the antagonism of S-5751 against EP receptor-mediated response, the mixture of histamine (0.05%) and PGE₂ (0.1%) was applied into the eyes. Histamine (0.05%) alone slightly induced plasma exudation and this response was dramatically enhanced by PGE₂ (0.1%), which alone showed no significant increase in vascular permeability (data not shown). Since S-5751 showed no antagonism of H₁ receptor, the synergistically enhanced dye leakage was determined as an EP receptor-mediated response.

In contrast to conjunctival microvascular permeability, i.v. injection of not only U-46619 but also PGD₂ is thought to cause bronchoconstriction via TP receptor (Arimura et al., 1992). Therefore, we assessed antagonism against U-46619- and histamine-induced bronchoconstriction as TP and H₁ receptor-mediated responses, respectively. Briefly, according to our previous report (Arimura et al., 1992), U-46619 (5 nmol/kg) or histamine (100 nmol/kg) was intravenously injected into the jugular vein of anesthetized guinea pigs, and maximal increase in insufflation pressure (ΔcmH₂O) was taken as an indication of bronchoconstriction.

**Allergic Rhinitis Model in Anesthetized Guinea Pigs.** The effect of S-5751 on nasal blockage immediately after exposure of the nasal cavity to the aerosol antigen was investigated according to our previously described method (Yasui et al., 1997). Briefly, guinea pigs were sensitized to ovalbumin (Sigma) twice by inhalation of an aerosol solution of 1% ovalbumin for 10 min. At 7 days after the second sensitization, the animals were anesthetized with sodium pentobarbital (30 mg/kg) and artificially ventilated through a tracheal cannula using a small animal respirator. Another glass cannula was inserted into the nasopharynx from the side of the larynx, and a fixed amount of air was continuously insufflated into the nasal cavity via the nasal cannula using another respirator. The insufflation pressure was monitored by a pressure transducer connected to the side arm of the nasal cannula as an indication of intranasal pressure. Nasal antigen challenge was performed by generating an aerosol of 3% ovalbumin between the nasal cannula and the animal respirator for 3 min using an ultrasonic nebulizer, and then the intranasal pressure was measured for 30 min. To visually assess the change in the area of the nasal airway, the nose was removed immediately after the measurement of intranasal pressure, and the nasal cavity was filled with 10% carmine dye dissolved in 2% car-
bovomethylcellulose after washing out the viscous nasal secretion with 10 ml of saline and 5 ml of 2% carboxymethylcellulose. The tissue was frozen in isopentane at −40°C. The nose was cut along the frontal plane using a cryostat at −20°C, and the frozen sections (15 μm) were thaw-mounted on poly(l-lysine)-coated glass slides and then freeze-dried at −40°C for 24 h.

**Biphasic Allergic Rhinitis Model in Conscious Guinea Pigs.**

Guinea pigs pretreated with cyclophosphamide (30 mg/kg i.p.; Sigma) 2 days earlier were sensitized by intratracheal injection of a mixture of ovalbumin and aluminum hydroxide, containing 1 mg (first injection) or 10 μg (second injection) of ovalbumin and 100 mg of aluminum hydroxide, twice at a 3-week interval. Three weeks after the second injection, topical antigen sensitization was performed by dropping 10 μl of 1% ovalbumin solution dissolved in saline into both nostrils four times at 2- or 4-day intervals. At 5 to 7 days after the end of the sensitization, nasal antigen challenge was performed by dripping 10 μl of 1% ovalbumin solution into both nostrils of conscious guinea pigs. For kinetic studies on plasma exudation in nasal mucosa, Evans blue dye (20 mg/kg i.v.) was injected immediately, 2, 4, or 6 h after antigen challenge. At 30 min, 1, 3, 5, or 7 h postchallenge, the animals were exsanguinated and then the nasoturbinates, maxilloturbinates, and septum were excised, blotted, and weighed. All tissues were incubated in 3 ml of formamide at 60°C to extract the extravasated dye, the amount of dye leaked for 30 min at 0 to 30 min postchallenge, or for 1 h at 0 to 1 h, 2 to 3 h, 4 to 5 h, and 6 to 7 h postchallenge, were estimated as nanograms of dye per milligram of wet weight tissue. For kinetic studies on inflammatory cell infiltration into the nasal cavity, the guinea pigs were exsanguinated at 30 min, 3, 5, and 7 h after antigen challenge. The nose was immediately dissected, and the nasal airway was washed by infusing 10 ml of saline into the nasal cavity from the side of the nasopharynx. The number of total cells and eosinophils present in the nasal lavage fluid was counted.

To evaluate the efficacy of compounds on early and late nasal responses, three sets of experiments were performed. Plasma exudation in the early phase was assessed by measuring dye exudation for 30 min after the challenge. In the second set, sneezing in the early phase and plasma exudation in the late phase were evaluated using the same animal. Briefly, the number of sneezes had been counted 30 min following the nasal antigen challenge, and then Evans blue dye was injected as a marker of plasma exudation. The amount of Evans blue dye extravasated in the conjunctiva and eyelid for 30 min was quantified as described above. Separately, 0.001% histamine, 0.01% PGD<sub>2</sub>, or their combination was applied to the eyes of nonsensitized guinea pigs, and the dye exudation also determined.

**Statistical Analysis.** The data are expressed as the means ± S.E.M. Statistical significance of the data was assessed by means of Dunnett’s test for multiple comparison, or Student’s or Welch’s t test for comparison between two groups (saline- versus antigen- or mediator-challenged).

**Results**

**Receptor Antagonisms in Vitro.** We examined the inhibitory activity of S-5751; a specific DP receptor antagonist, BW A868C; and a specific TP receptor antagonist, (+)-S-145 (active molecule of S-1452) against specific binding of [3H]PGD<sub>2</sub> to human platelet membrane fractions and PGD<sub>2</sub>-induced cAMP elevation in human and guinea pig platelets. As shown in Table 1, the specific binding of [3H]PGD<sub>2</sub> to human platelet membrane was suppressed by S-5751 and BW A868C with the K<sub>i</sub> values of 1.6 ± 0.9 and 640 ± 380 nM, but not by (+)-S-145. In functional second messenger assay, both S-5751 and BW A868C strongly inhibited cAMP elevation elicited by PGD<sub>2</sub> (0.1 μM) in human platelets with the IC<sub>50</sub> values of 0.9 ± 0.2 and 0.5 ± 0.1 nM despite 400-fold lower affinity of BW A868C for DP receptor than that of S-5751 in the ligand binding assay. BW A868C, but not S-5751, showed partial agonistic action in human platelets (data not shown). In guinea pig platelets, both S-5751 and BW A868C also suppressed PGD<sub>2</sub> (0.1 μM)-induced cAMP elevation. The IC<sub>50</sub> value of S-5751 was 31.3 ± 9.6 nM, but that of BW A868C could not be calculated due to partial agonistic action. However, when platelets were stimulated with PGD<sub>2</sub> at a higher concentration of 1 μM, the IC<sub>50</sub> value of BW A868C could be estimated at 118 nM. In contrast, (+)-S-145 Na showed no inhibition of PGD<sub>2</sub>-induced cAMP elevation in both human and guinea pig platelets.

S-5751 as well as (+)-S-145 suppressed the specific binding of a TP receptor specific ligand, [3H](+)-S-145, to human platelets, indicating that the TP receptor is blocked by (+)-S-145. In the membrane binding assay, (+)-S-145 Na suppressed the specific binding of [3H](+)-S-145 to human platelet membrane with IC<sub>50</sub> value of 0.90 nM despite 400-fold lower affinity of (+)-S-145 Na compared with (+)-S-145. However, (+)-S-145 Na showed no inhibition of PGD<sub>2</sub>-induced cAMP elevation in both human and guinea pig platelets.

**TABLE 1**

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Ligand</th>
<th>Species</th>
<th>Preparation</th>
<th>Assay</th>
<th>Value</th>
<th>K&lt;sub&gt;i&lt;/sub&gt; or IC&lt;sub&gt;50&lt;/sub&gt; Values</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>S-5751</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>nM</td>
</tr>
<tr>
<td>DP</td>
<td>[3H]PGD&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Human</td>
<td>Platelet membrane</td>
<td>Binding</td>
<td>K&lt;sub&gt;i&lt;/sub&gt;</td>
<td>1.6 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>PGD&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Human</td>
<td>Platelet</td>
<td>cAMP</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>0.90 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>PGD&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Guinea pig</td>
<td>Platelet</td>
<td>cAMP</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>31.3 ± 9.6</td>
</tr>
<tr>
<td>TP</td>
<td><a href="+">3H</a>-S-145</td>
<td>Human</td>
<td>Platelet membrane</td>
<td>Binding</td>
<td>K&lt;sub&gt;i&lt;/sub&gt;</td>
<td>24.2 ± 2.2</td>
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<tr>
<td></td>
<td><a href="+">3H</a>-S-145</td>
<td>Guinea pig</td>
<td>Platelet membrane</td>
<td>Binding</td>
<td>K&lt;sub&gt;i&lt;/sub&gt;</td>
<td>193 ± 20</td>
</tr>
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<td>IP</td>
<td>Carbacyclin</td>
<td>Human</td>
<td>Platelet</td>
<td>cAMP</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>&gt;10,000</td>
</tr>
<tr>
<td></td>
<td>Carbacyclin</td>
<td>Guinea pig</td>
<td>Platelet</td>
<td>cAMP</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>&gt;10,000</td>
</tr>
<tr>
<td>EP2</td>
<td>PGE&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Human</td>
<td>HL-60</td>
<td>cAMP</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>59.2 ± 7.7</td>
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N.T. not tested.

* Platelets were stimulated with 1 μM PGD<sub>2</sub> instead of 0.1 μM because the IC<sub>50</sub> value could not be determined due to partial agonistic action.
platelet membranes, with $K_i$ values of 24.2 ± 2.2 and 0.34 ± 0.04 nM, respectively, the affinity of S-5751 for TP receptor being approximately 15-fold lower than that for DP receptor in human platelet membranes. S-5751 failed to induce platelet aggregation by itself but antagonized U-46619-induced aggregation like (+)-S-145 (data not shown). BW A868C had no inhibition of the specific $^{3}$H$^{+}$]-S-145 (data not shown). BW A868C had aggregation like but antagonized U-46619-induced aggregation in human platelet membranes. S-5751 and (+)-S-145 also inhibited the specific binding of $^{3}$H$^{+}$]-S-145 to guinea pig platelet membranes with $K_i$ values of 193 ± 20 and 0.48 ± 0.08 nM, respectively.

S-5751 showed no detectable inhibition of cAMP production induced by carbachol, a specific IP receptor agonist, in human and guinea pig platelets. However, S-5751 weakly suppressed PGE$_2$-induced cAMP production of HL-60 cells, which was considered to be via EP2 receptor (Armstrong and Talpain, 1994).

Receptor Antagonisms in Vivo. Next, we investigated in vivo antagonisms of S-5751, BW A868C, S-1452, and two antihistamines against PGD$_{2r}$-U-46619-, and histamine-induced responses in guinea pigs. As shown in Table 2, orally administered S-5751 and intravenously injected BW A868C strongly suppressed PGD$_{2r}$-induced plasma exudation in the conjunctiva with ED$_{50}$ values of 0.099 and 0.12 mg/kg, respectively, but S-1452 did not affect it even at a high dose of 30 mg/kg. In contrast, S-1452 antagonized the U-46619-induced plasma exudation in the conjunctiva and bronchoconstriction with the ED$_{50}$ values of 0.017 and 0.006 mg/kg, respectively, but S-5751 and BW A868C exerted no inhibition. Histamine-induced responses were not suppressed by either S-5751, BW A868C, or S-1452, but expectedly by antihistamines, ketotifen, and/or terfenadine. Histamine-induced plasma exudation in the conjunctiva was synergistically enhanced by PGE$_2$, but the PGE$_2$-enhanced response was not affected by the pretreatment with S-5751 even at a higher dose of 30 mg/kg (data not shown).

Effect of S-5751 on Antigen-Induced Immediate Increase in Intranasal Pressure in Anesthetized Guinea Pigs. As shown in Fig. 1, A and B, both orally administered S-5751 and intravenously injected BW A868C almost completely suppressed the increase in intranasal pressure at 10 and 1 mg/kg, respectively. In contrast, terfenadine, even at a higher dose of 10 mg/kg, only showed slight inhibition (Fig. 1C). To visually confirm the inhibitory action of S-5751 against nasal blockade, we assessed the change in the area of the nasal airway. As shown in Fig. 1D, cryostat sections of the nose cut in the frontal plane clearly revealed that the nasal airway was narrowed by antigen exposure and this could be alleviated by pretreatment with S-5751.

**Effect of S-5751 on Antigen-Induced Early and Late Phase Nasal Responses in Guinea Pigs.** Next, we examined the effect of S-5751, BW A868C, and terfenadine on the early and late nasal responses elicited by intranasal antigen challenge in conscious guinea pigs that were sensitized by dripping ovalbumin repeatedly into the nostrils four times at 2- or 4-day intervals following two systemic immunizations. As shown in Fig. 2A, time course study demonstrated that the vascular permeability in nasal mucosa changed biphasically, with the first peak at either 0 to 30 min or 0 to 1 h and the second peak at 4 to 5 h after challenge. The total cell number and the eosinophil number both started increasing at 30 min after challenge and reached maximum at 5 h (Fig. 2B), when late plasma exudation was observed at a maximum magnitude. Oral administration of S-5751 at doses of 1 to 10 mg/kg 1 h before antigen challenge dose dependently inhibited the plasma exudation in nasal mucosa during 0 to 30 min after intranasal antigen challenge (Fig. 3A). Also, intravenous injection of BW A868C at 1 mg/kg markedly suppressed it (Fig. 3A). However, terfenadine also markedly suppressed this plasma exudation (Fig. 3A), and the inhibition rate at 10 mg/kg was comparable with that observed at 10 mg/kg S-5751 and 1 mg/kg BW A868C.

In another set of experiments, we assessed the effect of S-5751, BW A868C, and terfenadine on sneezing as an early response and plasma exudation in nasal mucosa during a 4- to 5-h postchallenge as a late response. As shown in Fig. 3B, S-5751 and BW A868C as well as terfenadine suppressed the antigen-provoked sneezing. After the evaluation of sneezing, we assessed the late nasal response by measuring the amount of Evans blue dye that had leaked into the nasal mucosa during 4 to 5 h after antigen challenge in the same animal. As shown in Fig. 3C, S-5751 and BW A868C dramatically inhibited the plasma exudation, whereas pretreatment with terfenadine did not even at a dose sufficient to inhibit early nasal responses. Interestingly, even when orally administered 2 h after antigen challenge, S-5751 markedly inhibited plasma exudation occurring at 4 to 5 h (Fig. 3D).

The increase in the number of total cells and eosinophils in nasal lavage fluid 5 h after challenge was significantly inhibited by pretreatment with S-5751 or BW A868C (Table 3), but was not affected by terfenadine (data not shown).

**Table 2**

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Stimuli</th>
<th>Tissue</th>
<th>Response</th>
<th>ED$_{50}$ Values</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>S-5751 p.o.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>mg/kg</td>
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<tr>
<td>DP</td>
<td>PGD$_2$</td>
<td>Conjunctiva</td>
<td>Dye exudation</td>
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<td>TP</td>
<td>U-46619</td>
<td>Conjunctiva</td>
<td>Dye exudation</td>
<td>&gt;10</td>
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<tr>
<td>H$_1$</td>
<td>Histamine</td>
<td>Conjunctiva</td>
<td>Dye exudation</td>
<td>&gt;30</td>
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<tr>
<td>H$_1$</td>
<td>Histamine</td>
<td>Lung</td>
<td>Bronchoconstriction</td>
<td>&gt;30</td>
</tr>
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</table>

N.T., not tested.
ingly, even when administered 2 h after the challenge, S-5751 suppressed inflammatory cell infiltration (Table 3).

**Effect of S-5751 on Antigen-Induced Airway Inflammatory Cell Infiltration in Guinea Pigs.** To further determine the role of PGD$_2$ in inflammatory cell infiltration, we assessed the effect of S-5751 and S-1452 on the antigen-induced increase in the number of eosinophils and macrophages in bronchoalveolar lavage fluids. As shown in Table 4, S-5751 significantly inhibited the increase in the number of not only eosinophils but also macrophages, whereas S-1452 showed no inhibition at 10 mg/kg.

**Effect of S-5751 on Allergic Conjunctivitis Model in Guinea Pigs.** We also evaluated the effect of DP, TP, and H$_1$ receptor antagonists on allergen-induced plasma exudation in the conjunctiva of sensitized guinea pigs. S-5751 and BW A868C as well as ketotifen markedly suppressed the increase in vascular permeability induced by topical allergen, but S-1452 showed no significant inhibition (Fig. 4A). The mixture of histamine and PGD$_2$ at concentrations that do not individually induce plasma exudation produced a significant increase in microvascular permeability, and this plasma exudation was completely suppressed by either DP or H$_1$ receptor antagonists (Fig. 4B).

**Discussion**

In the present study, we for the first time demonstrate the efficacy of the novel, potent, and selective DP receptor antagonist S-5751 in alleviating antigen-induced nasal blockage, plasma exudation in the conjunctiva, and inflammatory cell infiltration into upper and lower airways using guinea pigs.

In vitro study, S-5751 demonstrates a potent DP receptor antagonist in humans and guinea pigs with a weak antagonism for TP and EP2 receptors. However, oral administration of S-5751 to guinea pigs suppressed PGD$_2$-induced plasma exudation in the conjunctiva with an ED$_{50}$ value of 0.09 mg/kg, but did not affect U-46619-induced one and bronchoconstriction and PGE$_2$-enhanced histamine-mediated plasma exudation in the conjunctiva (data not shown), even at the highest dose of 10 or 30 mg/kg tested, indicating that S-5751 acts as a selective DP receptor antagonist in vivo. The specific DP receptor antagonist BW A868C strongly inhibited cAMP elevation elicited by PGD$_2$ despite the low affinity for DP receptor in the binding assay. The precise reason for this discrepancy remains uncertain, but the potent antagonistic action may be attributed to slow rate of association to and/or dissociation from DP receptors expressed on platelet mem-
branes, because platelets were stimulated with PGD2 after a 10-min preincubation with drug in the functional assay but [3H]PGD2 and compounds were simultaneously added to platelet membranes in the ligand binding assay. Since BW A868C showed no TP receptor antagonism and the TP receptor antagonist S-1452 showed no DP receptor antagonism, we also used BW A868C and S-1452 in this study to further clarify the role of PGD2 in the pathogenesis of allergic inflammation in various allergic disease models of guinea pigs.

Allergic rhinitis, the most common atopic disease, is characterized by the three major symptoms of sneezing, rhinorrhea and nasal blockage, which are presumed to be triggered by multiple mediators released from mast cells and other inflammatory cells (Naclerio et al., 1983, 1985; Howarth, 1994). Among various mediators, histamine is considered to be a main one, and antihistamines are used as the first-line therapy for nasal allergy. However, they are not efficacious against nasal blockage, which is a key symptom influencing the quality of life (Naclerio, 1991). One possible factor involved in causing nasal blockage in humans is PGD2, because it has been reported to possess 10 times more potent activity to increase nasal airway resistance than histamine and 100 times more than bradykinin (Doyle et al., 1990). In our allergen-induced nasal blockage model of anesthetized guinea pigs, orally administered S-5751 and intravenously injected BW A868C almost completely suppressed the increase in intranasal pressure at the each highest dose, whereas an antihistamine, terfenadine, orally given at a sufficient dose of 10 mg/kg to exert anti-histamine activity, only showed slight inhibition in agreement with clinical study (Wagenmann et al., 1994). The suppression by S-5751 of nasal blockage was also clearly confirmed by the cryostat sections of the nose. These findings suggest that DP receptor antagonists are effective in alleviating nasal mucosal swelling and PGD2 exclusively contributes to the onset of antigen-induced nasal blockage in guinea pigs. However, we have reported that S-1452 partially suppresses the increase in intranasal pressure in the same model (Yasui et al., 1997), and other groups have demonstrated that thromboxane A2 and leukotrienes are involved in the pathogenesis of nasal obstruction in guinea pigs and humans (Terada et al., 1998; Fujita et al.,

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**Fig. 2.** Time-dependent changes in plasma exudation in nasal mucosa (A) and the number of total cells and eosinophils in nasal lavage fluid (B) after the last nasal antigen challenge in conscious guinea pigs that were actively sensitized with ovalbumin. Plasma exudation was assessed by measuring the amount of Evans blue dye leaked into the nasal mucosa during 0 to 0.5 h, 0 to 1 h, 2 to 3 h, 4 to 5 h, and 6 to 7 h, and inflammatory cell infiltration was assessed at 0.5, 3, 5, and 7 h after antigen or saline challenge. Data represent the mean ± S.E.M. of five to eight animals. Statistical significance: *P < 0.05, **P < 0.01 versus time-matched saline-challenged control by Welch’s t test.

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**Fig. 3.** Effects of S-5751, BW A868C (BW), and terfenadine (Terf) on antigen-induced early and late nasal responses in conscious guinea pigs that were actively sensitized with ovalbumin. Plasma exudation was assessed by measuring the amount of Evans blue dye leaked into the nasal mucosa during 0 to 30 min as an early response (A). In a separate experiment, the number of sneezings was counted during 0 to 30 min postchallenge (B) as an early response, and plasma exudation in nasal mucosa was assessed during 4 to 5 h postchallenge as a late response (C). S-5751 and terfenadine were orally administered 1 and 2 h before the challenge, respectively (A–C), and BW A868C was intravenously injected 10 min before challenge. In another study, S-5751 was orally administered once 2 h after the challenge (D). Data represent the mean ± S.E.M. of 4 to 11 animals. Sa, saline-exposed control; V, vehicle control. Statistical significance: *P < 0.05, **P < 0.01 versus vehicle control by Dunnett’s test. #P < 0.05, ##P < 0.01 versus vehicle control by Student’s t test.
1999; Meltzer et al., 2000), indicating that other mediators than PGD$_2$ are involved in this model. Therefore, it appears that PGD$_2$ acts not only as a direct mediator but also as a potentiator in producing the antigen-induced increase in intranasal pressure.

In patients with allergic rhinitis, nasal provocation with antigen causes not only early nasal responses characterized by sneezing, rhinorrhea, and nasal blockage but also late phase responses characterized by nasal blockage hours later in approximately 50% of the patients, with accompanying accumulation of inflammatory cells such as eosinophils and basophils in the nasal cavity (Naclerio, 1991; Terada et al., 1994). We therefore evaluated the effect of S-5751, BW A868C, and terfenadine on the early and late nasal responses elicited by repeated intranasal antigen challenge in conscious guinea pigs. Pretreatment with either S-5751 or BW A868C dramatically inhibited the plasma exudation into nasal mucosa during 0 to 30 min after the last intranasal antigen challenge, suggesting that PGD$_2$ is a main mediator in this response. However, terfenadine also markedly suppressed this plasma exudation, and the inhibition rate at 10 mg/kg was comparable with that observed at 10 mg/kg S-5751 and 1 mg/kg BW A868C. Since PGD$_2$ is know to potentiate the increased vascular permeability in rat skin elicited by histamine (Flower et al., 1976), it is plausible that PGD$_2$ and histamine synergistically produce the antigen-induced plasma exudation in the early phase. Despite the comparable inhibition of the plasma exudation to that observed by DP receptor antagonists, terfenadine had little effect on the increase in intranasal pressure in anesthetized guinea pig model, suggesting that DP receptor antagonists inhibit nasal blockage via not only suppression of plasma exudation in nasal mucosa but also inhibition of increased blood volume in capacitance vessels resulting from vasodilation (Atkinson and Kaliner, 1995). To define the precise mechanism underlying the suppressive effect of DP receptor antagonist against nasal blockage, further investigation is needed. DP receptor antagonists were also effective in alleviating sneezing occurred in the early phase. However, sneezing is thought to be exclusively caused by a reflex initiated via H$_1$ receptor and PGD$_2$ is not an elicitor of sneezing (Doyle et al., 1990; Naclerio, 1991). Therefore, PGD$_2$ might contribute to the onset of allergen-induced sneezing via some indirect actions.

Next, we assessed the late nasal response by measuring the amount of Evans blue dye that had leaked into the nasal cavity (Naclerio, 1991). Therefore, PGD$_2$ might contribute to the onset of allergen-induced sneezing via some indirect actions.

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TABLE 3
Effect of S-5751 and BW A868C on antigen-induced increase in the number of inflammatory cells in nasal lavage fluid in guinea pig allergic rhinitis model

Nasal lavage was performed 5 h after the last antigen challenge. S-5751 was orally administered 1 h before antigen challenge and BW A868C was intravenously given 10 min before challenge. In the study for investigating the inhibitory action of post-treatment with S-5751 on inflammatory cell infiltration, S-5751 was administered 2 h after the antigen challenge.

<table>
<thead>
<tr>
<th>Challenge</th>
<th>Compound</th>
<th>Dose</th>
<th>n</th>
<th>Total Cells</th>
<th>Eosinophils</th>
</tr>
</thead>
<tbody>
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<td>Pretreatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
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<tr>
<td>Antigen</td>
<td>S-5751</td>
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<td></td>
<td>60.2 ± 9.0##</td>
<td>38.4 ± 6.6##</td>
</tr>
<tr>
<td></td>
<td>S-5751</td>
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<td></td>
<td>36.9 ± 6.7*</td>
<td>22.0 ± 4.7</td>
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<tr>
<td></td>
<td>S-5751</td>
<td>10</td>
<td></td>
<td>24.9 ± 5.5**</td>
<td>14.0 ± 3.6**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td></td>
<td>17.5 ± 3.7**</td>
<td>9.8 ± 2.0##</td>
</tr>
<tr>
<td>Saline</td>
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<td></td>
<td>6.8 ± 1.2</td>
<td>1.1 ± 0.2</td>
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<tr>
<td>Antigen</td>
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<td></td>
<td>22.4 ± 4.5#</td>
<td>13.4 ± 3.5#</td>
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<tr>
<td></td>
<td>BW A868C</td>
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<td>9.1 ± 2.2##</td>
<td>3.8 ± 1.0*</td>
</tr>
<tr>
<td>Post-treatment</td>
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<td></td>
</tr>
<tr>
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<td>Vehicle</td>
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<td>4.1 ± 1.0</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
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<td>54.5 ± 11.0##</td>
<td>34.1 ± 7.4##</td>
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<tr>
<td></td>
<td>S-5751</td>
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<td>27.7 ± 4.0*</td>
<td>15.8 ± 2.4**</td>
</tr>
<tr>
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<td></td>
<td>3</td>
<td></td>
<td>19.1 ± 2.5**</td>
<td>8.1 ± 1.5**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8</td>
<td></td>
<td>12.1 ± 2.9**</td>
<td>5.7 ± 1.6##</td>
</tr>
</tbody>
</table>

Statistical significance. # $P < 0.05$, ## $P < 0.01$ versus saline challenge by Student’s t test.
* $P < 0.05$, ** $P < 0.01$ versus vehicle control by Dunnett’s t test.

TABLE 4
Effects of S-5751 and S-1452 on antigen-induced increase in the number of inflammatory cells in bronchoalveolar lavage fluid in guinea pig asthma model

Bronchoalveolar lavage performed 24 h after the last antigen challenge, respectively. S-5751 and S-1452 were orally administered 1 and 2 h before antigen challenge, respectively.

<table>
<thead>
<tr>
<th>Challenge</th>
<th>Compound</th>
<th>Dose</th>
<th>n</th>
<th>Total Cells</th>
<th>Eosinophils</th>
<th>Macrophages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
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<td>3.2 ± 0.5</td>
<td>1.5 ± 0.4</td>
<td>1.6 ± 0.2</td>
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<tr>
<td>Antigen</td>
<td>S-5751</td>
<td>5</td>
<td></td>
<td>17.1 ± 2.2##</td>
<td>5.0 ± 0.6##</td>
<td>10.3 ± 1.5##</td>
</tr>
<tr>
<td></td>
<td>S-5751</td>
<td>10</td>
<td></td>
<td>9.7 ± 2.3*</td>
<td>2.9 ± 0.7*</td>
<td>5.6 ± 1.4*</td>
</tr>
<tr>
<td></td>
<td>S-5751</td>
<td>8</td>
<td></td>
<td>3.0 ± 0.3</td>
<td>0.5 ± 0.1</td>
<td>2.1 ± 0.2</td>
</tr>
<tr>
<td>Saline</td>
<td>Vehicle</td>
<td>6</td>
<td></td>
<td>12.1 ± 1.9##</td>
<td>4.2 ± 0.8##</td>
<td>5.1 ± 0.7##</td>
</tr>
<tr>
<td>Antigen</td>
<td>S-1452</td>
<td>10</td>
<td></td>
<td>13.8 ± 1.7</td>
<td>4.9 ± 0.7</td>
<td>5.7 ± 0.9</td>
</tr>
</tbody>
</table>

Statistical significance. # $P < 0.01$ versus saline challenge by Student’s t test.
* $P < 0.05$ versus vehicle control by Dunnett’s t test.
and ketotifen on the PGD2-enhanced histamine-induced plasma exudation into the conjunctiva in nonsensitized animals and inhibitory effect of S-5751 applied topically to the eyes of nonsensitized conscious guinea pigs in a vehicle control by Dunnett’s test. Statistical significance: *P < 0.05, **P < 0.01 versus vehicle control by Dunnett’s test. Naclerio et al. (1983, 1985). However, Mu-

fic role in the development of allergic symptoms and inflammatory cell infiltration, suggesting that PGD2 contributes to inflammatory cell migration in later stages of accumulation. In addition, S-5751 significantly inhibited the increase in the number of eosinophils and macrophages in BAL fluids after antigen exposure in asthma model, whereas S-1452 showed no inhibition at 10 mg/kg, which is sufficient dose to exert antiasthmatic actions (Arimura et al., 1992, 1994). These findings suggest that PGD2 plays a critical role in inflammatory cell infiltration into upper and lower airways. Since exogenously applied PGD2 can cause eosinophil accumulation (Emery et al., 1989; Woodward et al., 1996) and antigen-induced infiltration into lung is reduced in DP receptor-deficient mice compared with that in wild-type mice (Matsuoka et al., 2000), suggesting that PGD2-induced cell migration appears to be mediated via DP receptor. However, Hirai et al. (2001) recently demonstrated that CRTH2, a chemoattractant receptor, but not DP receptor, mediates PGD2-dependent migration of blood eosinophils. Therefore, further studies are required to clarify whether the inhibitory action of S-5751 against inflammatory cell migration is due to blocking CRTH2 or DP receptor.

We also evaluated the effect of DP, TP, and H1 receptor antagonists on allergen-induced plasma exudation in the conjunctiva of sensitized guinea pigs. S-5751 and BW A868C as well as ketotifen (H1 receptor antagonist) markedly suppressed the increase in vascular permeability induced by topical allergen, but S-1452 showed no significant inhibition. Although PGD2 is not the only mediator involved in the development of allergic conjunctivitis, S-5751 and BW A868C also almost completely suppressed the allergen-induced conjunctivitis in this model, favoring the possibility that PGD2 also acts as a mediator for enhancing plasma exudation caused by other inflammatory mediators via an increase in blood flow. To ascertain this possibility, we investigated the synergistic effect of PGD2 and histamine in inducing vascular permeability. The mixture of histamine and PGD2 at concentrations that do not individually induce plasma exudation produced a significant increase in microvascular permeability, and this plasma exudation was completely suppressed by either DP receptor antagonist or antihistamine, indicating that PGD2 could enhance the increase in vascular permeability as well as directly produce plasma exudation.

In conclusion, we have demonstrated that PGD2 plays a critical role in the development of allergic symptoms and mucosa during 4 to 5 h after antigen challenge. Pretreatment with S-5751 and BW A868C dramatically inhibited the plasma exudation, whereas pretreatment with terfenadine did not affect even at a dose sufficient to inhibit early nasal responses. Interestingly, even when orally administered 2 h after antigen challenge, S-5751 markedly inhibited plasma exudation occurring in late phase, suggesting that the plasma exudation in the late phase is mediated by PGD2 produced in the late phase. This seems to be inconsistent with previous findings that PGD2 generation occurred only in the early phase (Naclerio et al., 1983, 1985). However, Murakami et al. (1995) have demonstrated that mast cells derived from mouse bone marrow cause delayed PGD2 genera-

Fig. 4. A, effects of S-5751, BW A868C (BW), S-1452, and ketotifen (Keto) on antigen-induced plasma exudation into the conjunctiva of guinea pigs. Ovalbumin solution (2.5%) was administered topically to the eyes of actively sensitized conscious guinea pigs in a 10-μl volume/eye. B, synergistic effect of histamine and PGD2 on microvascular permeability in the conjunctiva in nonsensitized animals and inhibitory effect of S-5751 and ketotifen on the PGD2-enhanced histamine-induced plasma exudation. Histamine (0.001%), PGD2 (0.01%), or their combination was applied topically to the eyes of nonsensitized conscious guinea pigs in a 10-μl volume/eye. Plasma exudation was assessed by measuring the amount of Evans blue dye leaked into the both conjunctiva for 30 min after stimulation. S-5751, S-1452, and ketotifen were orally administered 1, 2, and 1 h before the antigen challenge, respectively, and BW A868C was intravenously given 10 min before challenge. Data represent the mean ± S.E.M. of 5 to 10 animals. Sa, saline-exposed control; V, vehicle; H, histamine. Statistical significance: *P < 0.05, **P < 0.01 versus vehicle control by Dunnett’s test.

In conclusion, we have demonstrated that PGD2 plays a critical role in the development of allergic symptoms and
inflammation triggered by mast cell activation in guinea pigs. Considering the excellent efficacy of S-5751 in guinea pig model of allergic diseases, DP receptor antagonists could be new candidates for medication to treat patients with allergic diseases, and S-5751, the first DP receptor antagonist developed as a drug, is expected to display therapeutic value in clinical studies in humans.

Acknowledgments

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dimethylbicyclo[3.1.1]heptane derivatives: orally active, potent, and selective pros-

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