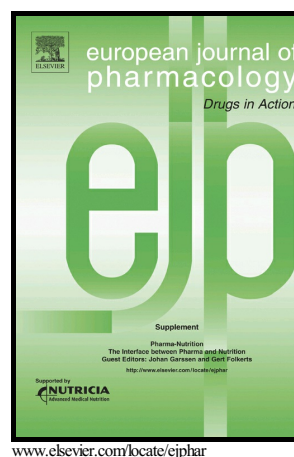


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**Effect of the potent and selective DP1 receptor antagonist,  
asapiprant (S-555739), in animal models of allergic rhinitis  
and allergic asthma**

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**Abbreviations:**

AHR (airway hyper-responsiveness); AUC (area under the curve); BALF (bronchoalveolar lavage fluid); cAMP (cyclic adenosine monophosphate); CRTH2 (chemoattractant receptor homologous molecule expressed on Th2 cells); Cmax (maximum concentration achieved after dosing); IAR (immediate airway response); IC50 (half-maximal inhibitory concentration); Ki (inhibitor constant); LAR (late airway response); LT (leukotriene); OVA (ovalbumin); PC400 (400% increase in lung resistance); PG (prostaglandin); RL (lung resistance); sRaw (specific airway resistance); TX (thromboxane).

**Keywords**

Prostaglandin D<sub>2</sub>,

Synthetic DP<sub>1</sub> receptor antagonist

Allergic rhinitis

asapiprant

Nasal congestion

## **Abstract**

Prostaglandin (PG) D<sub>2</sub> elicits responses through either the DP<sub>1</sub> and/or DP<sub>2</sub> receptor.

Experimental evidence suggests that stimulation of the DP<sub>1</sub> receptor contributes to

allergic responses, such that antagonists are considered to be directed therapies for

allergic diseases. In this study, we demonstrate the activity of a novel synthetic DP<sub>1</sub>

receptor antagonist termed asapiprant (S-555739) for the DP<sub>1</sub> receptor and other

receptors in vitro, and assess the efficacy of asapiprant in several animal models of

allergic diseases. We determined the affinity and selectivity of asapiprant for the DP<sub>1</sub>

receptor in binding assays. In the animal models of allergic rhinitis, changes in nasal

resistance, nasal secretion, and cell infiltration in nasal mucosa were assessed after

antigen challenge with and without asapiprant. Similarly, in the animal models of

asthma, the effect of antigen challenge with and without asapiprant on antigen-induced bronchoconstriction, airway hyper-responsiveness, mucin production, and cell infiltration in lung were assessed. In binding studies, asapiprant exhibited high affinity and selectivity for the DP<sub>1</sub> receptor. Significant suppression of antigen-induced nasal resistance, nasal secretion, and cell infiltration in nasal mucosa was observed with asapiprant treatment. In addition, treatment with asapiprant suppressed antigen-induced asthmatic responses, airway hyper-responsiveness, and cell infiltration and mucin production in lung. These results show that asapiprant is a potent and selective DP<sub>1</sub> receptor antagonist, and exerts suppressive effects in the animal models of allergic diseases. Thus, asapiprant has potential as a novel therapy for allergic airway diseases.

## 1. Introduction

Prostaglandin (PG) D<sub>2</sub> is a chemical mediator produced in large amounts by mast cells in response to allergic stimulation in humans and other mammals (Lewis et al., 1982). Increased PGD<sub>2</sub> levels are observed in nasal lavage fluid after antigen provocation in subjects with allergic rhinitis (Naclerio et al., 1985) and in bronchoalveolar lavage fluid (BALF) in subjects with asthma (Murray et al., 1986). PGD<sub>2</sub> exerts its actions by binding to two specific cell-surface receptors: DP<sub>1</sub> receptor (Boie et al., 1995) and CRTH2/DP<sub>2</sub> receptor (Hirai et al., 2001). Both are seven-transmembrane G protein-coupled receptors, and have important roles in airway inflammation in response to PGD<sub>2</sub> (Schuligoi et al., 2010).

Increase in DP<sub>1</sub> receptor-expressing cells have been observed in the lung tissue from asthma patients (Hirano et al., 2011) and in nasal tissue from patients with allergic rhinitis (Yamamoto et al., 2009). Histochemical and morphological investigations from

lung tissue in asthma patients have shown that most DP<sub>1</sub> receptor-expressing cells are macrophage- and monocyte-like cells (Hirano et al., 2011). In nasal mucosal tissue from allergic rhinitis patients, the DP<sub>1</sub> receptor is expressed in infiltrated inflammatory cells, epithelial goblet cells, serous glands, and vascular endothelium and epithelium (Nantel et al., 2004, Yamamoto et al., 2009). Collectively, these observations support an important role of the DP<sub>1</sub> receptor in allergic inflammation in the upper and lower airways.

We have previously investigated the role of the DP<sub>1</sub> receptor in allergic diseases using a DP<sub>1</sub> receptor antagonist, S-5751 (Tsuru et al., 1997). S-5751 suppressed airway hyper-responsiveness and infiltration of eosinophils into lungs in animal models of asthma using guinea pigs (Mitsumori et al., 2003), rats (Hirano et al., 2011), and sheep (Shichijo et al., 2009). Moreover, in guinea pigs, S-5751 alleviated nasal responses induced by antigens (Arimura et al., 2001). We demonstrated that PGD<sub>2</sub> induced nasal congestion through the dilation of sinusoid vessels (Takahashi et al.,

2012), and combined exposure of PGD<sub>2</sub> with histamine or thromboxane (TX) A<sub>2</sub> mimetics caused nasal congestion in a synergistic fashion, which was suppressed by S-5751 (Yasui et al., 2008). These results suggest that PGD<sub>2</sub> has several roles in allergic responses in upper and lower airways, mediated via the DP<sub>1</sub> receptor.

Recently, we discovered a new DP<sub>1</sub> receptor antagonist termed asapiprant (S-555739), which has better DP<sub>1</sub> receptor antagonist activity and selectivity *in vitro* and better bioavailability *in vivo* compared with S-5751. This compound also shows suppressive effects on allergic airway responses in several animal models. Currently, asapiprant is in Phase III clinical trials as an indication for allergic rhinitis (<http://www.shionogi.co.jp/en/company/index.html>). Here, we report both the *in vitro* and *in vivo* pharmacological profile of asapiprant. The results of these studies demonstrate that asapiprant has potential as a therapy for allergic diseases of the upper and lower airways.

## 2. Materials and methods

### 2.1. Drugs and reagents

S-5751 ((Z)-7-[(1R, 2R, 3S)5S)-2-(5-hydroxybenzo[b]thiophen-3-ylcarbonylamino)-10-norpinan-3-yl]hept-5-enoic

acid) and asapirant (S-555739: [2-(Oxazol-2-yl)-5-(4-{4-[(propan-2-yl)oxy]phenylsulfonyl}piperazine-1-yl)phenoxy]

acetic acid) were synthesized at Shionogi & Co., Ltd (Osaka, Japan). PGD<sub>2</sub>, ovalbumin

(OVA), cyclophosphamide monohydrate, and urethane were obtained from Sigma–

Aldrich (St Louis, MO, USA). PGD<sub>2</sub> was dissolved in ethanol and diluted to appropriate

concentrations using physiological (0.9%) saline. Fexofenadine hydrochloride was

purchased from Nacalai Tesque Inc. (Kyoto, Japan). Pranlukast was obtained from

Funakoshi Co., Ltd (Tokyo, Japan).

## 2.2. Animals

The present study was conducted in accordance with the Declaration of Helsinki and the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the US National Institutes of Health (Bethesda, MD, USA). Male Hartley guinea pigs (300–400 g) were purchased from Japan SLC Inc. (Hamamatsu, Japan). Male brown Norway rats (240–400 g) were obtained from Japan Charles River (Yokohama, Japan). The guinea pigs and rats were housed in an air-conditioned room at 20–26°C with a relative humidity of 30–70%, and were fed standard laboratory chow and given water *ad libitum*. Female sheep (32–46 kg) naturally sensitive to *Ascaris suum* antigen were used. Experiments employing guinea pigs and rats were undertaken following the rules and regulations for the care and use of experimental animals as stipulated by the Animal Use and Care Committee of Shionogi & Co., Ltd. Experiments using sheep were approved

by the Animal Research Committee of Mount Sinai Medical Center (Miami Beach, FL, USA).

### 2.3. Binding assay for DP<sub>1</sub> receptor and other prostanoid receptors

The binding assays of asapiprant and S-5751 to the human DP<sub>1</sub> receptor and TXA<sub>2</sub> receptor (TP) on human platelet membranes were conducted using a previously reported method (Kishino et al., 1991). Binding to the other prostanoid receptors for PGI<sub>2</sub> (IP), PGF<sub>2α</sub> (FP), PGE<sub>2</sub> (EP<sub>1</sub>, EP<sub>2</sub>, EP<sub>3</sub>, and EP<sub>4</sub>), and DP<sub>2</sub> (another receptor for PGD<sub>2</sub>) was evaluated with respective receptor-transfected cells as described elsewhere (Sugimoto et al., 2003; Shichijo et al., 2009).

### 2.4. Cellular function assay for the DP<sub>1</sub> receptor

The functional antagonist activity of asapiprant on the DP<sub>1</sub> receptor was evaluated by examining PGD<sub>2</sub>-induced elevation of cyclic adenosine monophosphate (cAMP) in platelet-rich plasma derived from venous blood (humans, guinea pigs, and sheep), and in rat DP<sub>1</sub>-transfected cells stimulated with PGD<sub>2</sub>, as described elsewhere (Shichijo et al., 2009). The functional antagonist activity of asapiprant on the DP<sub>2</sub> receptor was evaluated by examining PGD<sub>2</sub>-induced shape change of peripheral eosinophils derived from humans and guinea pigs, as reported previously (Heinemann et al., 2003).

## 2.5. Pharmacokinetic study

After the oral administration of asapiprant or S-5751 to rats, guinea pigs, dogs (Kitayama Labes Co., Ltd., Japan), and sheep at 10 mg/kg in suspension with 0.5% methylcellulose solution, the plasma concentrations of the drugs were measured by liquid chromatography/tandem mass spectrometry or high performance liquid

chromatography.

## 2.6. Guinea pig model of allergic rhinitis

Using the method described in our previous paper (Takahashi et al., 2012), we examined the effects of asapirant, S-5751, fexofenadine, and pranlukast on PGD<sub>2</sub>- and/or OVA-induced nasal responses in guinea pigs. In brief, animals pretreated with cyclophosphamide (30 mg/kg, ip) were intraperitoneally sensitized with 1 mg OVA with 10 mg alum diluted in saline on day 0 and intranasally challenged with 1% OVA on day 7. They were challenged again with 2% OVA on day 14 and challenged with 0.5% PGD<sub>2</sub> or 2% OVA on day 21. During the PGD<sub>2</sub> challenge studies, specific airway resistance (sRaw) was measured before (pre-challenge value) and 5 min, 10 min, 30 min, and 1 h after the final intranasal challenge with PGD<sub>2</sub> or saline. The area under the curve (AUC) of sRaw from 0 min to 1 h was quantified and used to assess the

magnitude of PGD<sub>2</sub>-induced nasal resistance. During the OVA challenge studies, sRaw was measured before (pre-challenge value) and 20 min, 2 h, 3 h, 5 h, and 7 h after the final intranasal challenge with OVA or saline. The first peak of sRaw was exhibited at 20 min [immediate airway response (IAR)] and the second peak was at 3–7 h [late airway response (LAR)] after the OVA challenge. AUC of sRaw from 3 h to 7 h was quantified and used to evaluate LAR. Test compounds were suspended in 0.5% methylcellulose solution and administered orally to guinea pigs 1 h before the PGD<sub>2</sub> or OVA challenge on day 21.

To examine the effects of asapiprant on OVA-induced cell infiltration in nasal lavage fluids, the nasal cavity was washed with 5 mL of saline and nasal lavage fluid was collected 5 h after the OVA challenge on day 21. The total number of cells in the nasal lavage fluid was counted with Turk's solution (Kanto Chemical Co., Inc., Osaka, Japan). For the differential cell count, cells were prepared using a Cytospin (Thermo Fisher Scientific Inc., Boston, MA, USA) and were stained with May–Grünwald and

Giemsa solutions (Merck, Darmstadt, Germany). Test compounds were suspended in 0.5% methylcellulose solution and administered orally to guinea pigs 1 h before the OVA challenge on day 21.

To examine the effects of asapiprant, fexofenadine, and pranlukast on nasal secretion, a piece of paper (Kimwipe; Crecis, Tokyo, Japan) was inserted into the left anterior naris after the OVA challenge on day 21. After 20 min, the paper was removed and weighed. Data were expressed as the increase in weight from the pre-challenge value. Test compounds were suspended in 0.5% methylcellulose solution and administered orally to guinea pigs once daily from the second challenge to the last challenge.

## 2.7. Sheep model of allergic rhinitis

Vehicle (for a control response) was administered orally to naturally sensitive sheep

from day 1 to day 4 (Figure 1). Then, 40 puffs of 500  $\mu\text{g/mL}$   $\text{PGD}_2$  (20 puffs/nostril) were challenged into the nose and nasal resistance was measured. After an interval of 3 days, vehicle (for a control response) was administered orally from day 8 to day 11, then the nasal challenge was performed by administering 40 puffs of *Ascaris suum* antigen (82,000 PNU/mL; 20 puffs/nostril) into the nose, and nasal resistance was measured. After these measurements, no manipulations were conducted for 10 days (i.e., a wash-out period). After the wash-out period, asapirant (5 mg/kg) was administered for 4 days (as above), and then the  $\text{PGD}_2$  challenge was performed. After an interval of 3 days, asapirant was administered for 4 days, and then the *Ascaris suum* antigen challenge was performed.

Nasal resistance was measured using a modified mask rhinomanometry method.

The sheep's head was placed in a Plexiglas® hood with attachments for a faceplate containing a pneumotachograph (to measure flow) and two catheter ports (to measure the pressure differential between the nose and mouth). One of the ports was connected

to a polyethylene catheter that was inserted through the nasal passage to the back of the mouth to provide pressure at the nasopharynx. Nasal resistance was measured pre-challenge as well as immediately, 0.5 h, 1 h, and 2 h after the challenge. Data were expressed as the percent increase in nasal resistance from baseline. AUC of the nasal resistance from 0 h to 2 h was quantified and used to assess the magnitude of nasal resistance.

## 2.8. Rat model of asthma

Using a method described previously (Hirano et al., 2011), we examined the effects of asapiprant on airway hyper-responsiveness (AHR), cell infiltration, and mucin production in BALF in rats. In brief, animals were sensitized with 100 µg OVA with 1 mg alum diluted in saline on day 0 and inhaled with 1% OVA on day 12, 19, 26, and 33. The measurements of various asthmatic parameters were performed on day 36. To

assess the magnitude of AHR, AUC was calculated by the trapezoidal rule from the dose–response curve for acetylcholine. The number of inflammatory cells in BALF was quantified as described above (*Guinea pig model of allergic rhinitis*). Mucin levels in BALF were also measured using a method described previously (Hirano et al., 2011). The test compound was suspended in 0.5% methylcellulose solution and administered orally to rats once daily for 3 days (day 33–35).

## 2.9. Sheep model of asthma

A detailed description of the sheep asthma model is presented in previous reports (Abraham, 2008; Shichijo et al., 2009). For these studies, we determined baseline  $PC_{400}$  (i.e., the amount of carbachol required to cause a 400% increase in lung resistance) within 3 days before antigen challenge. On the challenge day, baseline values for lung resistance ( $R_L$ ) were obtained and then the sheep ( $n = 4$ ) were treated with asapirant (3

mg/kg, iv) 30 min before the antigen challenge (*Ascaris suum*).  $R_L$  was measured immediately after antigen challenge.  $R_L$  was then measured hourly from 1 h to 6 h after the challenge and half-hourly from 6.5 h to 8 h after the challenge. One day and 7 days after antigen challenge,  $R_L$  was measured, followed by the determination of the post antigen  $PC_{400}$  to determine if the animals had developed antigen-induced AHR. Because the control responds to the antigen, vehicle was administered instead of asapiprant to the same four sheep before the examinations of asapiprant.

#### 2.10. Statistical analyses

Data are expressed as mean  $\pm$  SEM. Statistical significance was assessed using

Dunnett's test or Welch's *t*-test. A level of  $P < 0.05$  was considered to be significant.

### 3. Results

#### 3.1. Binding and functional activities of asapiprant for prostanoid receptors in vitro

The effect of asapiprant on the binding of [ $^3$ H]-PGD<sub>2</sub> to the membrane fraction of human platelet was examined. The effects of asapiprant on the other prostanoid receptors, TP, IP, FP, EP<sub>1</sub>, EP<sub>2</sub>, EP<sub>3</sub>, and EP<sub>4</sub>, were also examined using the respective receptor-transfected cells and labeled ligands. The inhibition constant (K<sub>i</sub>) values of asapiprant and S-5751 to the DP<sub>1</sub> receptor were 0.44 nM and 2.4 nM, respectively (Table 1). Although asapiprant bound to the EP<sub>2</sub> receptor, its binding to EP<sub>2</sub> was about 300 times less potent than that to the DP<sub>1</sub> receptor (Table 1). High concentrations of asapiprant (10  $\mu$ M) inhibited the binding to 29 receptors by  $\leq 24\%$ , including H<sub>1</sub> receptor and CysLT<sub>1</sub> receptor, and two channels (data not shown). In addition, it showed  $\leq 17\%$  inhibition of ten enzymes (data not shown). In a functional assay, asapiprant

strongly inhibited the cAMP elevation elicited by PGD<sub>2</sub> in human platelets with a half-maximal inhibitory concentration (IC<sub>50</sub>) value of 16 nM, which was about 50-fold more potent than another DP<sub>1</sub> antagonist, S-5751 (840 nM). Moreover, almost complete inhibition was observed at 1 μM asapiprant (data not shown). We also performed Schild analysis using cAMP assay. The increasing concentrations of asapiprant did not cause an attenuation of maximal response to PGD<sub>2</sub>, which was suggestive of surmountable antagonism (data not shown). To evaluate the inter-species difference in the effects of asapiprant on the DP<sub>1</sub> receptor *in vitro*, we conducted the cAMP production assay with platelets (guinea pigs and sheep) or DP<sub>1</sub> receptor-transfected cells (rats). Strong inhibition by asapiprant was observed in the cAMP elevation induced by PGD<sub>2</sub> in guinea pigs, rats, and sheep with IC<sub>50</sub> values (nM) of 61, 74, and 15, respectively (Table 1), which were lower than the IC<sub>50</sub> values for S-5751 (5400, 980, and 130, respectively). No antagonistic activity or cellular functional inhibition of asapiprant to the DP<sub>2</sub> receptor (Table 1) was observed. These results show that asapiprant had markedly

superior potency and selectivity to the DP<sub>1</sub> receptor as compared with S-5751.

### 3.2. Pharmacokinetic profile

After a single oral administration of asapiprant and S-5751 in rats, guinea pigs, dogs, and sheep, the plasma concentrations of these agents were measured over 24 h (Table 2).

In all species, asapiprant had a higher plasma concentration than S-5751.

### 3.3. Effects on PGD<sub>2</sub>-induced nasal resistance in guinea pigs and sheep

PGD<sub>2</sub> is more effective than histamine and bradykinin in producing nasal congestion in humans (Doyle et al., 1990). In the present study, we investigated the effect of PGD<sub>2</sub> on nasal resistance in sensitized animals. Intranasal challenge with 0.5% PGD<sub>2</sub> led to a rapid increase in nasal resistance (sRaw) from 5 min to 60 min in sensitized guinea pigs

(Figure 2A). Oral administration of asapiprant at 1 and 3 mg/kg significantly ( $P < 0.01$ )

suppressed the increase in nasal resistance by 82% and 92%, respectively (Figure 2B).

By contrast, S-5751 showed partial suppression on PGD<sub>2</sub>-induced nasal resistance in guinea pigs at 30 mg/kg by 76% that was inferior to the suppression by asapiprant at 3 mg/kg (Figure 2C).

In sheep that are naturally sensitive to *Ascaris suum* antigen, oral administration of asapiprant at 5 mg/kg suppressed PGD<sub>2</sub>-induced nasal resistance by 86% but with no statistically significant difference from PGD<sub>2</sub>-induced nasal resistance of vehicle-treated animals ( $n = 4$ , Figure 3A).

#### 3.4. Effects on antigen-induced nasal resistance in guinea pigs and sheep

The effect of asapiprant on antigen-induced increase in nasal resistance in guinea pigs was evaluated and compared with the effect of fexofenadine and pranlukast. After an

antigen challenge, an immediate increase of nasal resistance and subsequent persisting nasal resistance were observed until 7hr after challenge (Figure. 4A). When orally administered 1 h before the antigen challenge, asapiprant (3, 10, and 30 mg/kg) suppressed IAR by 52%, 57%, and 96%, and LAR by 67%, 50%, and 79%, respectively (Figure 4B, C). Fexofenadine (20 mg/kg) administered orally in the same manner did not reduce IAR (Figure 4D). Oral administration of pranlukast (10 mg/kg) significantly ( $P < 0.05$ ) suppressed IAR by 51% (Figure 4D). Neither fexofenadine nor pranlukast showed a protective effect on LAR (data not shown). These results demonstrate that asapiprant exerted stronger suppressive effects on antigen-induced nasal resistance than fexofenadine and pranlukast in this model.

In naturally sensitive sheep, intranasal challenge with *Ascaris suum* antigen led to an increase in nasal resistance for 2 h after the challenge (Figure 3B). Orally administered with asapiprant (5 mg/kg) for 4 days, the asapiprant significantly ( $P < 0.01$ ) suppressed antigen-induced nasal resistance by 73% (Figure 3B).

### 3.5. Effects on antigen-induced nasal secretion in guinea pigs

Figure 4E shows the effect of asapiprant, fexofenadine, and pranlukast on antigen-induced nasal secretion in sensitized guinea pigs. In the vehicle-treated group, the amount of nasal secretion was increased by antigen challenge within 20 min. Treatment with 3 and 30 mg/kg of asapiprant significantly ( $P < 0.01$ ) suppressed nasal secretion by 53% and 72%, respectively. Treatment with fexofenadine (20 mg/kg) also significantly ( $P < 0.01$ ) suppressed nasal secretion by 71%, whereas pranlukast (10 mg/kg) showed no protective effect. These results show that the suppressive effect of asapiprant on antigen-induced nasal secretion was comparable with that of fexofenadine.

### 3.6. Effects on antigen-induced cell infiltration in nasal lavage fluids in guinea pigs

In patients with allergic rhinitis, nasal provocation with antigen causes the accumulation of inflammatory cells in the nasal cavity at LAR (Naclerio, 1991; Terada et al., 1994).

Therefore, we examined the effect of asapiprant on the infiltration of inflammatory cells in nasal lavage fluids obtained 5 h after antigen challenge in sensitized guinea pigs. The total number of cells, macrophages, eosinophils, and neutrophils in nasal lavage fluids were significantly ( $P < 0.05$ ) increased after antigen challenge compared with that after saline challenge (Table 3). Treatment with asapiprant significantly ( $P < 0.05$ ) suppressed the number of these inflammatory cells compared with vehicle treatment (Table 3).

### 3.7. Effects on a rat model of asthma

To evaluate the effect of asapiprant on antigen-induced AHR to acetylcholine,

infiltration of inflammatory cells, and mucus secretion in BALF in the rat asthma model, asapirant was administered orally once daily for 3 days after the last challenge (Figure 5). Repeated exposure of antigen induced a significant ( $P < 0.05$ ) increase in AHR, the number of inflammatory cells, and mucin production. Treatment with asapirant at 10 mg/kg significantly ( $P < 0.05$ ) reduced AHR, infiltration of inflammatory cells, and mucin production in BALF, although treatment with asapirant at 0.1 mg/kg did not have a significant effect on any responses.

### 3.8. Effects on sheep model of asthma

The effects of asapirant (3 mg/kg, iv) on antigen-induced bronchoconstriction and AHR in sheep are shown in Figures 6A and B. Antigen challenge in vehicle-treated sheep induced a rapid increase in  $R_L$  (early response) and a delayed increase in  $R_L$  (late response) (Figure 6A). Significant ( $P < 0.05$ ) suppression on the late response, but not

the early response (Figure 6A) was observed with asapiprant. Antigen challenge in vehicle-treated sheep also induced AHR 1 day and 7 days after the antigen challenge, as indicated by the decrease in the PC<sub>400</sub> compared to baseline (Figure 6B). Treatment with asapiprant (3 mg/kg) significantly ( $P < 0.01$ ) blocked antigen-induced AHR both on day 1 and day 7 (Figure 6B).

#### 4. Discussion

The purpose of this study was to clarify the pre-clinical pharmacological characteristics of asapiprant (S-555739), a novel DP<sub>1</sub> receptor antagonist. Our *in vitro* investigations of receptor binding and cellular function showed that asapiprant was a highly potent and selective antagonist for the DP<sub>1</sub> receptor. Moreover, the significant suppression of antigen-induced nasal resistance, nasal secretion, and infiltration of inflammatory cells in nasal cavity fluids was observed with asapiprant treatment in the animal model of allergic rhinitis. In addition, treatment with asapiprant suppressed antigen-induced late asthmatic responses, airway hyper-responsiveness, and cell infiltration and mucin production in bronchoalveolar lavage fluid in the animal model of allergic asthma. The efficacies of asapiprant on the pathology of rhinitis and asthma in the animal model and bioavailability for several species *in vivo* were superior to those of S-5751.

PGD<sub>2</sub> mediates an increase in nasal congestion in humans (Doyle et al., 1990).

When sensitized guinea pigs and sheep were exposed to PGD<sub>2</sub> via the intranasal route, nasal resistance was rapidly increased (Figures 2 and 3A). Therefore, PGD<sub>2</sub> is involved in nasal congestion not only in humans but also in guinea pigs and sheep. The nasal cavity of sheep is longer and more complex than the human nose. However, the structures of the epithelial membrane and the composition of the mucin network in the sheep nasal cavity are similar to those in humans (Soane et al., 2001). Illum (1996) reported that the deposition patterns of compounds on the nasal cavity of sheep were similar to those in humans. Therefore, the examinations of the efficacy of asapiprant on antigen- or PGD<sub>2</sub>-induced nasal responses in sheep would be useful to predict the effects on nasal responses in humans. In the present study, asapiprant almost completely suppressed PGD<sub>2</sub>-induced nasal resistance in sheep (Figures 3A), suggesting that asapiprant has a potent antagonistic activity at the DP<sub>1</sub> receptor *in vivo*. Another DP<sub>1</sub> antagonist S-5751 showed partial suppression on PGD<sub>2</sub>-induced nasal resistance in guinea pigs at 30 mg/kg (Figure 2C), whereas asapiprant showed the almost complete

suppression at 3 mg/kg (Figure 2B, C). Thus, asapiprant has superior potency of antagonist activities for the DP<sub>1</sub> receptor to S-5751, resulting in more potent efficacy in disease models *in vivo*.

In nasal allergen provocation tests in subjects with allergic rhinitis, nasal congestion frequently appears as a biphasic response in which IAR is followed by a LAR (Dvoracek et. al., 1984). We previously exhibited an antigen-induced IAR and LAR in a guinea pig model of allergic rhinitis. As expected, asapiprant significantly inhibited the antigen-induced IAR in the guinea pig model (Figure 4B). Moreover, asapiprant (5 mg/kg) suppressed the antigen-induced rapid increase of nasal resistance (IAR) in sheep (Figure 3B). The increase in nasal resistance in IAR is considered to be caused by vasodilatation and vascular permeability in the nasal mucosa. In particular, given the strong inhibitory effects of  $\alpha$ -adrenergic blockers on nasal congestion observed in humans (Hochban et al., 1999) and guinea pig models of allergic rhinitis (Mizutani, 2003), vasodilatation of the nasal mucosa is a major contributor to IAR. We

previously reported that PGD<sub>2</sub> and DP<sub>1</sub> receptor agonists induced the dilatation of microvessels in the nasal mucosa (Takahashi et al., 2012). Together with these evidences, the results in the present study suggest that asapiprant suppresses the increase in nasal resistance in IAR by suppressing the dilatation of the microvessels.

Nasal hypersecretion is induced by antigen provocation in patients with allergic rhinitis and suppressed by H<sub>1</sub> receptor antagonists (Okubo and Gotoh, 2006). In the present study, antigen challenge induced a significant increase in nasal secretion in the guinea pig model (Figure 4E). As expected, the extent of nasal secretion was decreased significantly by fexofenadine, but not by pranlukast. Nasal provocation with leukotriene D<sub>4</sub> (LTD<sub>4</sub>) increased nasal secretion in a clinical study, but the increase was less than that induced by histamine (Okuda et al., 1988). Interestingly, treatment with asapiprant significantly decreased nasal secretion to an extent comparable to that with fexofenadine. Therefore, asapiprant might control nasal secretion through a different mechanism than these already launched products. The results from the animal models of

allergic rhinitis suggest that the suppressive pathways of asapiprant on nasal resistance and nasal secretion differ from those of cys-LT<sub>1</sub> receptor antagonist and H<sub>1</sub> receptor antagonist. However, further studies such as combination of asapiprant and the launched products in this model are needed to show the differentiation of asapiprant with them.

Epidemiological studies have demonstrated that asthma and allergic rhinitis often co-exist in the same patient (Eriksson et al., 2011). Allergic rhinitis is a risk factor for asthma, preceding the onset of asthma and increasing the risk of developing asthma (Koh and Kim, 2003). Therefore, asthma and allergic rhinitis should be treated concomitantly. In the present study, we evaluated the effect of asapiprant in experimental models of asthma using rats and sheep in addition to the models of allergic rhinitis. Treatment with asapiprant significantly inhibited antigen-induced AHR in rats.

In addition, a single injection of asapiprant suppressed AHR and the late response in sheep, suggesting some contribution of the DP<sub>1</sub> receptor to asthmatic inflammation.

Little et al. (2002) reported that AHR is associated with the accumulation of

inflammatory cells in the lungs, which is characteristic of airway inflammation. In the present study, asapiprant markedly suppressed the infiltration of inflammatory cells in BALF in rats and sheep (Figure 4B; data for sheep not shown). Therefore, asapiprant might suppress AHR through the suppression of the infiltration of inflammatory cells in the lungs. Our previous studies (Hirano et al., 2007) also suggest that antagonism of the DP<sub>1</sub> receptor down-regulates cytokine production by inflammatory cells, which would be one of the mechanisms of action of asapiprant for suppressing AHR and inflammation in asthma.

Excessive production of mucin in the airways weakens lung function and increases the risk of morbidity and mortality in respiratory diseases. Choi et al. (2011) demonstrated that PGD<sub>2</sub> increased the expression of the *MUC5B* gene in a human lung cell line via the DP<sub>1</sub> receptor. The suppression of mucin production by asapiprant observed in the present study suggests that the DP<sub>1</sub> receptor contributes to mucin production in the lung. Therefore, asapiprant may show the inhibitory effect of mucus

secretion in human asthma patients.

Previously, we showed that suppression mechanism of DP<sub>1</sub> receptor antagonist was difference from that of montelukast (a cysteinyl leukotriene receptor-1 antagonist) using asthma model of sheep (Shichijo et al., 2009). Additionally, we observed combinational effect of asapirant with montelukast in asthma model in rat (under preparation of another manuscript). Moreover, we showed in our previous report that anti-inflammation mechanism of DP<sub>1</sub> receptor antagonist was distinct from a glucocorticoid in rat asthma model (Hirano et al., 2011). Therefore, we thought that asapirant is a novel alternative and/or combinational medicine for asthma therapy.

Our finding with asapirant showed a potent novel therapy in allergic inflammation, however, previous trial showed that another DP<sub>1</sub> receptor antagonist (laropirant) did not demonstrate efficacy in asthma and rhinitis (Philip et al., 2009). In our animal experiment, asapirant exerted the same level of suppression with laropirant in antigen-induced nasal resistance in guinea pigs with the lower plasma

concentration levels of compound (plasma concentration levels of ED50; asapiprant 333 ng/mL, laropiprant 7130 ng/mL). These results may suggest that asapiprant would be more potent compound than laropiprant in clinical studies for allergic diseases.

In conclusion, our results show that asapiprant is a DP<sub>1</sub> receptor antagonist with superior potency and specificity compared with S-5751, and that asapiprant exerts suppressive effects on various allergic responses in animal models of allergic rhinitis and asthma. Moreover, it was suggested that the suppressive pathways of asapiprant differ from those of other agents used for the treatment of airway allergic diseases (e.g., antihistamines, antileukotrienes, and glucocorticoids). These results suggest that asapiprant has potential as a novel therapy for allergic airway diseases such as allergic rhinitis and asthma.

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## Figure Legends

Figure 1 Experimental protocol for the sheep model of allergic rhinitis.

Figure 2 Effect of asapiprant on PGD<sub>2</sub>-induced nasal resistance (sRaw) in guinea pigs.

(A) Sensitized guinea pigs were challenged via the intranasal route with 20 µL per nostril of 0.5% PGD<sub>2</sub> and time-dependent changes in nasal resistance were measured using a plethysmograph and (B) expressed as AUC. Administration of asapiprant was conducted orally 1 h before challenge with PGD<sub>2</sub>. Data were expressed as the percent increase from the pre-challenge sRaw value. Values are the mean ± SEM of 12 guinea pigs. AUC, area under the curve; S, saline-challenged control; V, vehicle control. \*\*P < 0.01 vs. saline-challenged control by Welch's t-test, ##P < 0.01 vs. vehicle control by Dunnett's test.

Figure 3 (A) Effect of asapiprant on PGD<sub>2</sub>-induced nasal resistance and (B) antigen-induced nasal resistance in sheep. Oral administration with vehicle or asapiprant (5 mg/kg) was initiated 4 days before challenge with PGD<sub>2</sub> or antigen. Nasal resistance was measured for 2 h. Values are the mean  $\pm$  SEM of 4 sheep. \*\*P < 0.01 vs. vehicle control by Welch's t-test.

Figure 4 Effect of asapiprant (3, 10, 30 mg/kg = 6, 20, 60  $\mu$ mol/kg), fexofenadine (20 mg/kg = 40  $\mu$ mol/kg), and pranlukast (10 mg/kg = 10  $\mu$ mol/kg) on antigen-induced nasal response in a guinea pig model of allergic rhinitis. Sensitized guinea pigs were challenged via the intranasal route with 10  $\mu$ L per nostril of 2% OVA. Effects on time-dependent changes in (A) nasal resistance, (B, D) IAR, and (C) LAR were measured using a plethysmograph. Data were expressed as the percent increase from the pre-challenge sRaw value. LAR was expressed as AUC for 3–7 h. (A–D) Administration of asapiprant, fexofenadine, and pranlukast was conducted orally 1 h

before antigen challenge. (E) Effects on nasal secretion were measured for 20 min after antigen challenge. Oral administration with asapiprant, fexofenadine, and pranlukast was initiated 1 week before antigen challenge. Values are the mean  $\pm$  SEM of 10–12 guinea pigs. AUC, area under the curve; Fex, fexofenadine; IAR, immediate airway response; LAR, late airway response; Pra, pranlukast; S, saline-challenged control; V, vehicle control. \*P < 0.05, \*\*P < 0.01 vs. saline-challenged control by Welch's t-test. #P < 0.05, ##P < 0.01 vs. vehicle control by Dunnett's test.

Figure 5 (A) Effect of asapiprant on antigen-induced AHR, (B) infiltration of inflammatory cells, and (C) mucin production in a rat model of asthma. Administration of asapiprant was conducted orally once daily from the final challenge for 3 days.

Values are the mean  $\pm$  SEM of 7–10 rats. AHR, airway hyper-responsiveness; AUC, area under the curve; S, saline-challenged control; V, vehicle control. \*P < 0.05, \*\*P <

0.01 vs. saline-challenged control by Welch's t-test. #P < 0.05, ##P < 0.01 vs. vehicle control by Dunnett's test.

Figure 6 (A) Effect of 3 mg/kg of asapiprant on the time-course of antigen-induced changes in RL and (B) AHR to inhaled carbachol expressed as PC400 in a sheep model of asthma. Administration of asapiprant was initiated 30 min before challenge with inhaled antigen. AHR was measured 1 day and 7 days after antigen challenge. Values are the mean  $\pm$  SEM of 4 sheep. \*P < 0.05, \*\*P < 0.01 vs. vehicle control by Welch's t-test.

# Tables

**Table 1. Binding affinity of asapiprant to the DP<sub>1</sub> receptor and other prostanoid receptors *in vitro***

| Target           | Species | Preparation  | Assay   | asapiprant          | S-5751 |
|------------------|---------|--------------|---------|---------------------|--------|
| Receptor binding |         |              |         | K <sub>i</sub> (nM) |        |
| DP <sub>1</sub>  | Human   | Platelet     | binding | 0.44                | 2.4    |
| DP <sub>2</sub>  | Human   | Transfectant | binding | >5000               | >5000  |
| TP               | Human   | Platelet     | binding | >4800               | 320    |
| IP               | Human   | Transfectant | binding | >6300               | >6800  |
| FP               | Human   | Transfectant | binding | >3700               | 4700   |
| EP <sub>1</sub>  | Human   | Transfectant | binding | >6600               | 9300   |

|                       |                             |                     |                     |                 |             |
|-----------------------|-----------------------------|---------------------|---------------------|-----------------|-------------|
| <b>EP<sub>2</sub></b> | <b>Human</b>                | <b>Transfectant</b> | <b>binding</b>      | <b>120</b>      | <b>170</b>  |
| <b>EP<sub>3</sub></b> | <b>Human</b>                | <b>Transfectant</b> | <b>binding</b>      | <b>&gt;4000</b> | <b>2600</b> |
| <b>EP<sub>4</sub></b> | <b>Human</b>                | <b>Transfectant</b> | <b>binding</b>      | <b>&gt;2400</b> | <b>90</b>   |
| <b>Function</b>       | <b>IC<sub>50</sub> (nM)</b> |                     |                     |                 |             |
| <b>DP<sub>1</sub></b> | <b>Human</b>                | <b>Platelet</b>     | <b>cAMP</b>         | <b>16</b>       | <b>840</b>  |
|                       | <b>Guinea pig</b>           | <b>Platelet</b>     | <b>cAMP</b>         | <b>61</b>       | <b>5400</b> |
|                       | <b>rat</b>                  | <b>Transfectant</b> | <b>cAMP</b>         | <b>74</b>       | <b>980</b>  |
|                       | <b>sheep</b>                | <b>Platelet</b>     | <b>cAMP</b>         | <b>15</b>       | <b>120</b>  |
| <b>DP<sub>2</sub></b> | <b>Human</b>                | <b>Eosinophile</b>  | <b>shape change</b> | <b>&gt;3000</b> | <b>n.t.</b> |
|                       | <b>Guinea pig</b>           | <b>Eosinophile</b>  | <b>shape change</b> | <b>&gt;3000</b> | <b>n.t.</b> |

n.t., not tested

**Table 2. Pharmacokinetic profile of asapiprant in rats, guinea pigs, dogs, and sheep**

|            | asapiprant |                  |                       | S-5751           |                       |
|------------|------------|------------------|-----------------------|------------------|-----------------------|
|            | Dose       | C <sub>max</sub> | AUC <sub>0-24hr</sub> | C <sub>max</sub> | AUC <sub>0-24hr</sub> |
|            | mg/kg      | µg/mL            | µg • h/mL             | µg/mL            | µg • h/mL             |
| rat        | 10         | 3.6              | 56.8                  | 1.0              | 1.6                   |
| guinea pig | 10         | 1.1              | 10.0                  | 0.6              | 4.1                   |
| dog        | 10         | 19.4             | 446.0                 | 2.7              | 2.9                   |
| sheep      | 10         | 1.6              | 7.9                   | 0.1              | 2.1                   |

**Table 3. Effect of asapiprant on antigen-induced cell infiltration in nasal lavage fluids in a guinea pig model of allergic rhinitis**

| Group      | Dose  |                | Cell Number (x 10 <sup>5</sup> /animal) |               |             |  |  |  |  |  |
|------------|-------|----------------|-----------------------------------------|---------------|-------------|--|--|--|--|--|
|            | mg/kg | Macrophage     | Eosinophil                              | Neutrophil    | Lymphocyte  |  |  |  |  |  |
| Saline     |       | 0.03 ± 0.01 ** | 0.04 ± 0.01 **                          | 0.04 ± 0.04 * | 0.01 ± 0.00 |  |  |  |  |  |
| Vehicle    |       | 3.26 ± 0.85    | 11.55 ± 2.47                            | 1.29 ± 2.05   | 0.69 ± 0.41 |  |  |  |  |  |
| asapiprant | 1     | 1.77 ± 0.71    | 4.32 ± 1.01 ##                          | 0.15 ± 0.53 # | 0.07 ± 0.02 |  |  |  |  |  |
|            | 3     | 0.98 ± 0.47 #  | 4.17 ± 1.50 ##                          | 0.46 ± 0.24 # | 0.08 ± 0.03 |  |  |  |  |  |
|            | 10    | 0.21 ± 0.07 ## | 2.74 ± 0.70 ##                          | 0.03 ± 0.03 # | 0.04 ± 0.02 |  |  |  |  |  |

\* $P < 0.05$ , \*\* $P < 0.01$  vs. vehicle control by the Welch's t-test (n=8).

# $P < 0.05$ , ## $P < 0.01$  vs. vehicle control by Dunnett's test (n = 8).

Fig. 1.

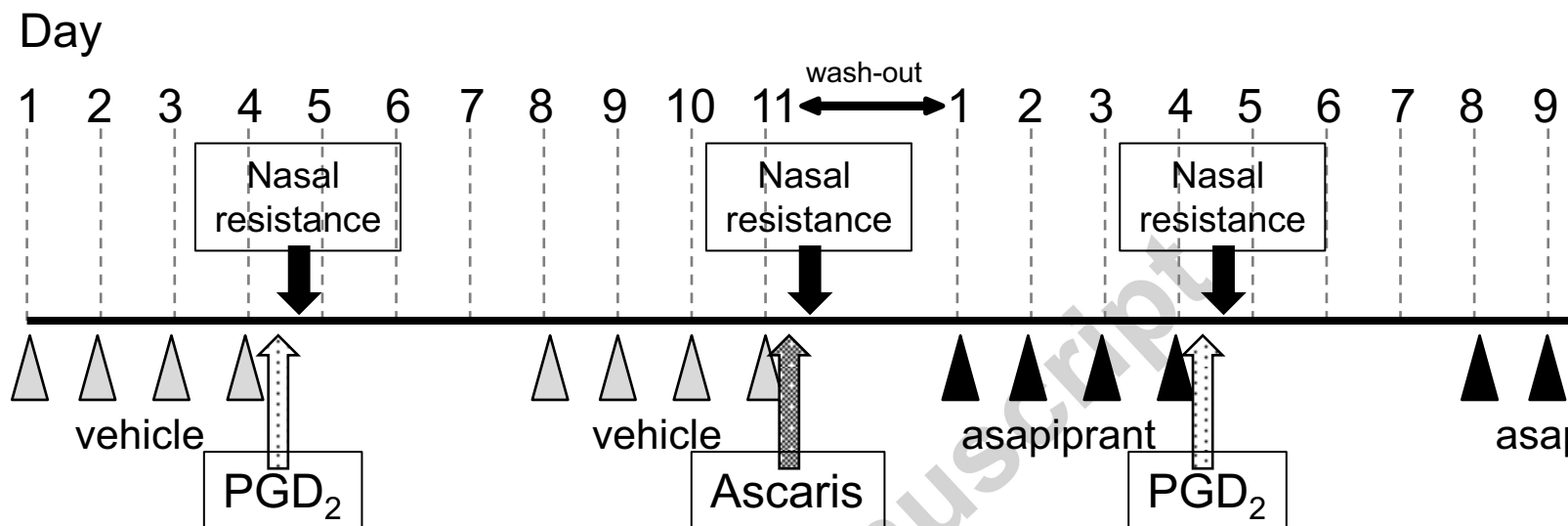


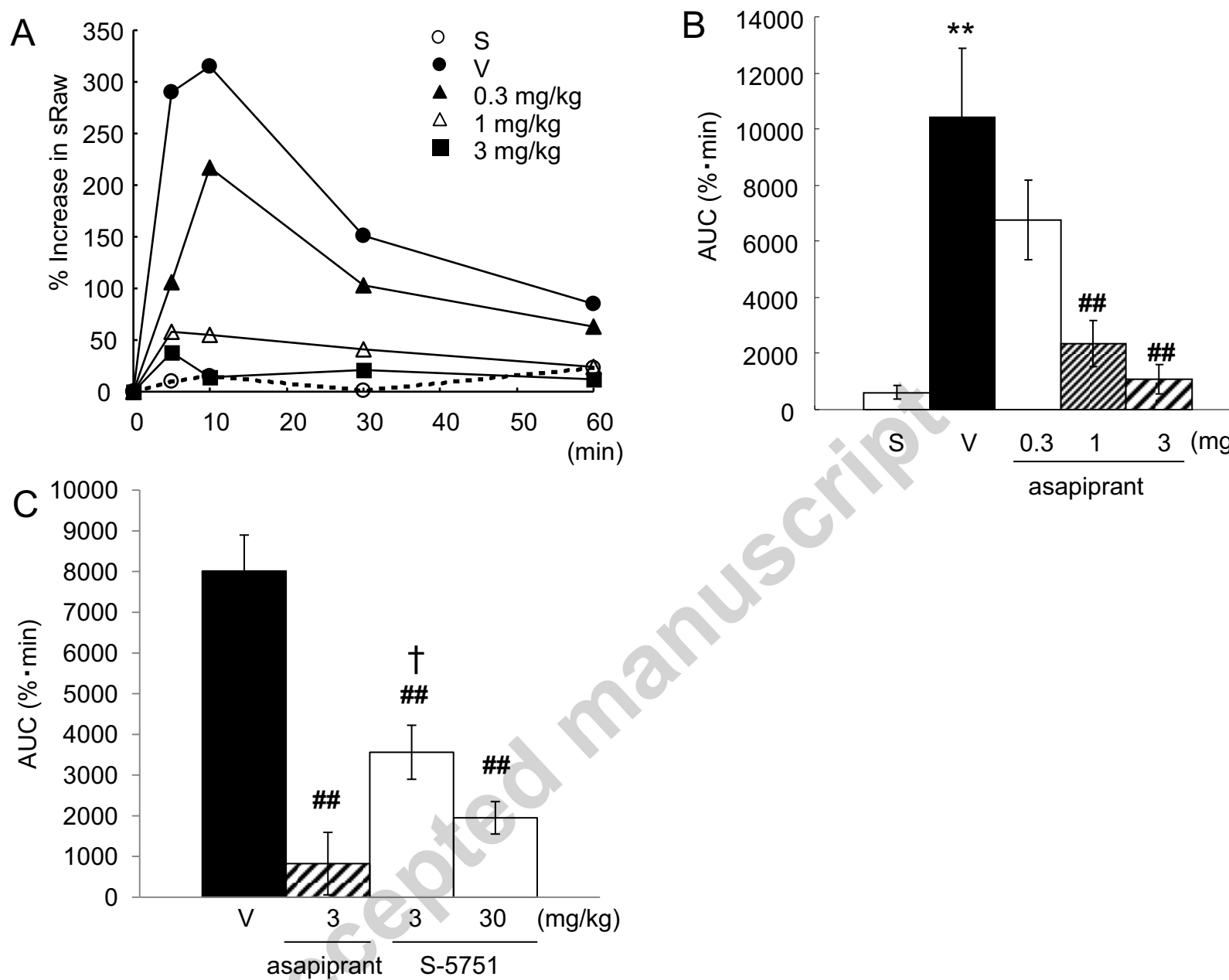
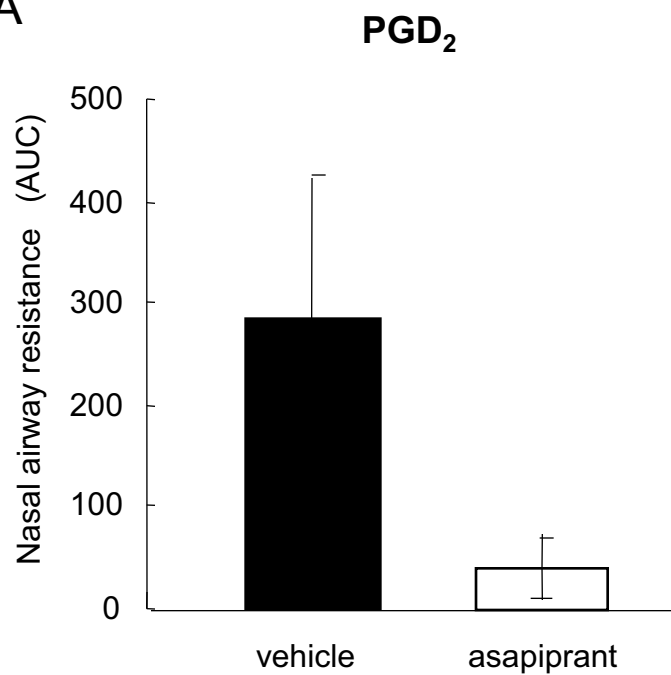
**Fig. 2.**

**Fig. 3.**

**A**



**B**

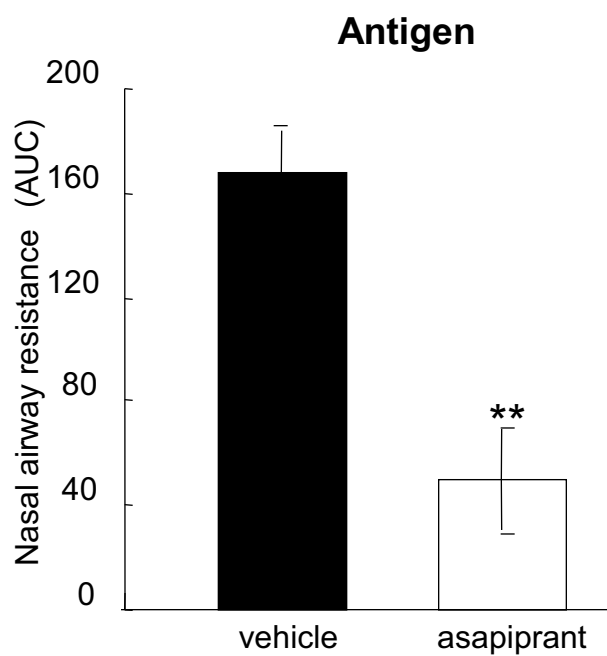


**Fig. 4.**

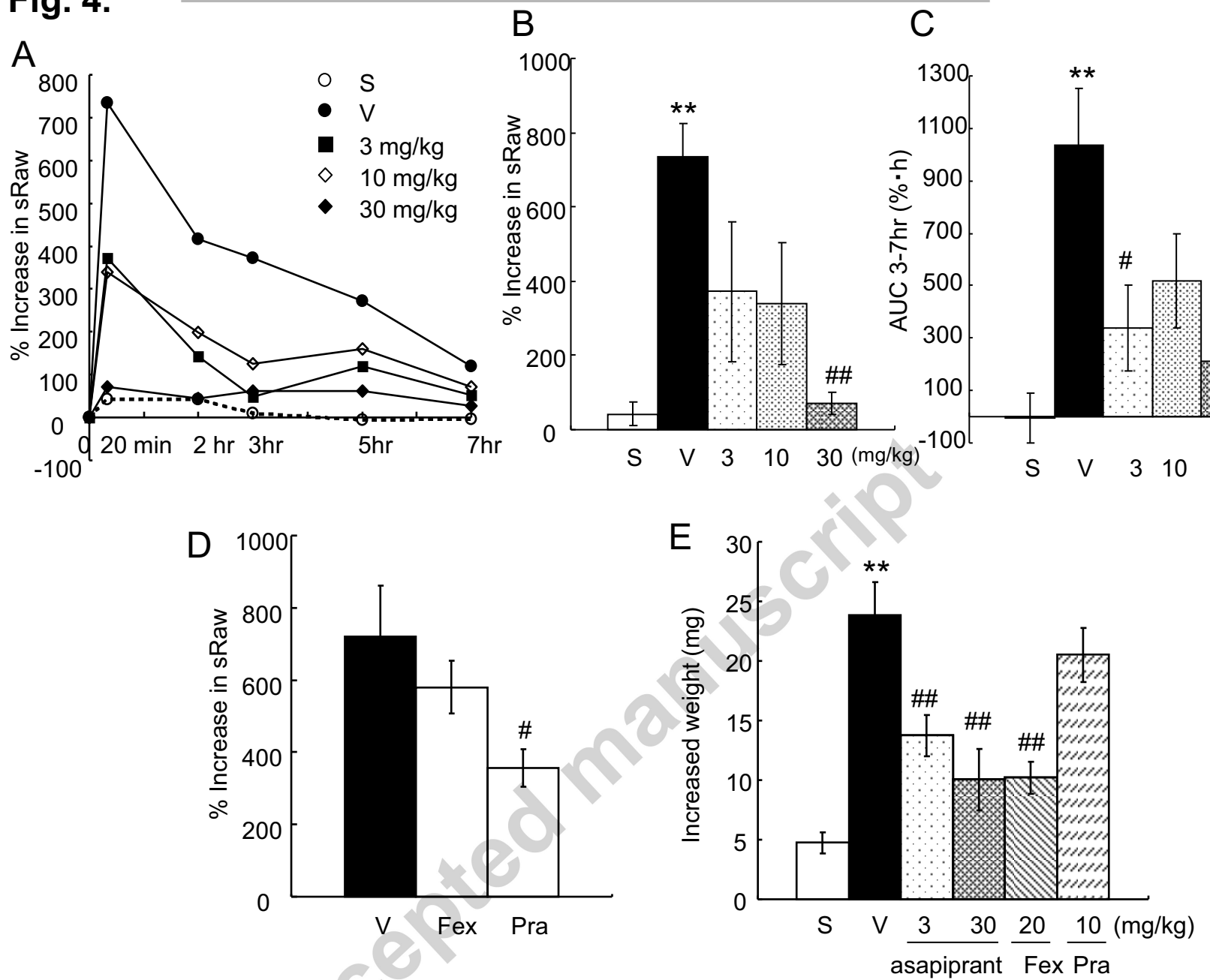


Fig. 5.

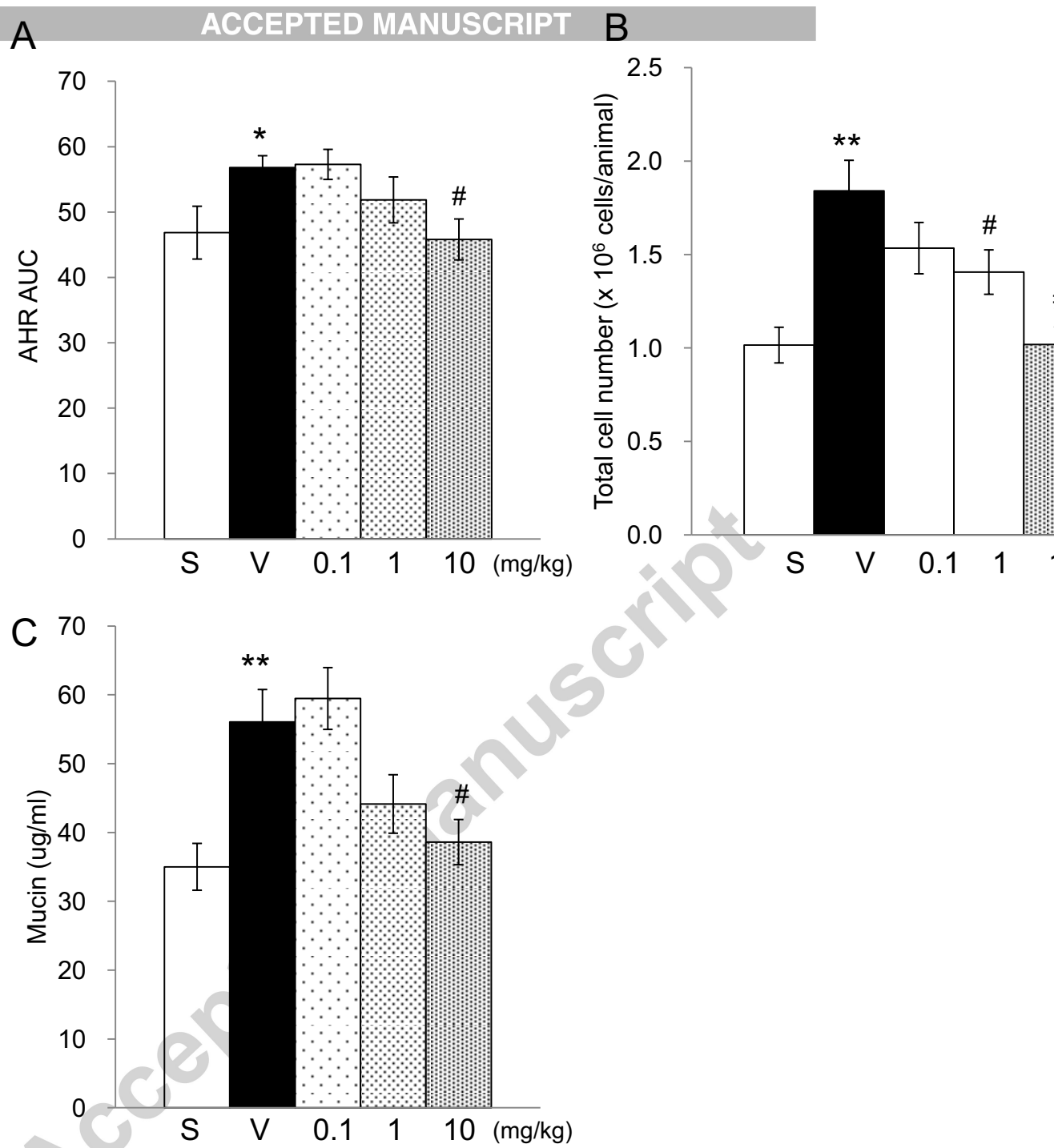


**Fig. 6.**