

Therapeutic Potential of ASP3258, a Selective Phosphodiesterase 4 Inhibitor, on Chronic Eosinophilic Airway Inflammation

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Key Words

Phosphodiesterase 4 · Brown Norway rat · Eosinophil · Emesis · Vascular injury · Fibrinogen

Abstract

We investigated and compared the pharmacological effects of a PDE4 inhibitor ASP3258 (3-[4-(3-chlorophenyl)-1-ethyl-7-methyl-2-oxo-1,2-dihydro-1,8-naphthyridin-3-yl] propanoic acid), with those of roflumilast, the most clinically advanced PDE4 inhibitor known. ASP3258 inhibited human PDE4A, 4B, 4C, and 4D with respective IC₅₀ values of 0.036, 0.050, 0.45, and 0.035 nmol/l, all approximately 3–6 times more potent than roflumilast. ASP3258 inhibited LPS-induced TNF- α production and PHA-induced IL-5 production in human whole blood cells with respective IC₅₀ values of 110 and 100 nmol/l, both approximately 10 times less potent than roflumilast. Repeatedly administered ASP3258 and roflumilast both suppressed chronic airway eosinophilia induced by repeated exposure to ovalbumin in Brown Norway rats with respective ED₅₀ values of 0.092 and 0.17 mg/kg. We also evaluated the toxicological profiles of ASP3258. Although PDE4 inhibitors induce emesis by mimicking the pharmacological action of an α_2 -adrenoceptor antagonist, repeated administration of ASP3258 (3 mg/kg) had no such inhibitory effect on rats anesthetized with α_2 -adrenoceptor

agonist. PDE4 inhibitors are also known to induce vascular injury in rats. Although repeatedly administered ASP3258 (3 and 10 mg/kg) significantly increased plasma fibrinogen, a biomarker for toxicity, 1 mg/kg of ASP3258 did not. These results suggest that ASP3258 is an attractive PDE4 inhibitor for treating chronic eosinophilic airway inflammation due to asthma.

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Introduction

Asthma is a chronic inflammatory disorder of the airway induced by various initiating factors. During its development, the airway is infiltrated by inflammatory cells like eosinophils, mast cells, and CD4⁺ T lymphocytes, which produce inflammatory mediators that subsequently exacerbate and perpetuate the inflammatory state [1, 2]. This chronic inflammation, which is associated with airway hyperresponsiveness (AHR) and typically leads to airflow obstruction, is an important component of the progression of asthma [3]. Although glucocorticoids are presently the most effective anti-inflammatory therapy available for many inflammatory diseases, including asthma, some patients show poor or even no response [4]. Therefore, novel anti-inflammatory agents with different mechanisms of action need to be identified.

Cyclic nucleotide phosphodiesterases (PDEs) are enzymes which metabolize cAMP and cGMP into 5'-AMP and 5'-GMP, respectively. At least 11 isozymes of PDE (PDE1–11) have been identified thus far, and PDE4, the major isozyme in leukocytes, consists of four subtypes: PDE4A, 4B, 4C, and 4D. Except for PDE4C, these subtypes are distributed in a number of cell types that are considered important in the pathogenesis of inflammatory airway diseases [5]. Studies in genetically modified mice have revealed that PDE4B ablation suppresses antigen-induced eosinophilia and asthmatic responses [6] and that PDE4D ablation may be responsible for the occurrence of emesis [7]. PDE4 inhibitors are believed to be one such alternative anti-inflammatory agent, as these compounds exhibit a range of anti-inflammatory effects such as suppressing inflammatory mediator production and inhibiting leukocyte infiltration into inflamed tissues by a mechanism different from that employed by glucocorticoids [8, 9]. However, while clinical trials of PDE4 inhibitors have shown the expected efficacy in asthma patients [10], these compounds are known to induce emesis in a number of animal species, including humans, and vascular injury mainly in rats but also in dogs and monkeys, suggesting that there is also a risk for humans [11–18].

ASP3258 (3-[4-(3-chlorophenyl)-1-ethyl-7-methyl-2-oxo-1,2-dihydro-1,8-naphthyridin-3-yl] propanoic acid) is a novel and highly selective PDE4 inhibitor derived from naphthyridin [19] that lacks the 3,4-dialkoxyphenyl group found in roflumilast [20] and cilomilast [21], the most clinically advanced PDE4 inhibitors known. Our previous studies have shown that ASP3258 exerts a wide range of anti-inflammatory [19, 22, 23] and antiasthmatic effects [24] with low emetic potential in several animal models of acute airway inflammation in which symptoms were induced by one-time antigen exposure. For instance, ASP3258 inhibited ovalbumin (OVA)-induced production of interleukin (IL)-4, tumor necrosis factor (TNF)- α , and cysteinyl leukotrienes and leukocyte infiltration into the airways of rats, as well as also inhibiting OVA-induced bronchoconstriction and AHR, two characteristic symptoms of asthma, in sensitized guinea pigs [19, 24]. However, given that asthma is a chronic airway inflammation, its effects on airway inflammation need to be investigated using a chronic disease model.

To evaluate the therapeutic potential of ASP3258, we examined the inhibitory effects of ASP3258 on human PDE4 enzymes, cytokine production in whole blood cells, and OVA-induced chronic airway leukocyte infiltration in rats and compared our findings with those achieved

with roflumilast. In addition, possible adverse effects of repeatedly administered ASP3258, such as emetic activity and vascular injury, were also evaluated.

Materials and Methods

Animals

Brown Norway (BN) and F344/N rats were purchased from Charles River Japan (Kanagawa, Japan) and SLC (Shizuoka, Japan), respectively. All experiments were carried out in accordance with regulations of the Animal Ethics Committee of Astellas Pharma Inc.

Chemicals

ASP3258 and roflumilast were synthesized by Astellas Pharma Inc. (Tsukuba, Japan), while prednisolone was purchased from Nacalai Tesque Inc. (Kyoto, Japan). These compounds were suspended in 0.5% methylcellulose (Shin-Etsu Chemical, Tokyo, Japan) and orally administered to the animals. OVA (albumin, chicken egg, grade V), 3-isobutyl-1-methylxanthine, adenosine 3',5'-cyclic monophosphate sodium salt, and lipopolysaccharide (LPS; *Escherichia coli*, 0127:B8) were purchased from Sigma-Aldrich (St. Louis, Mo., USA). [5',8-³H]adenosine 3',5'-cyclic phosphate, ammonium salt, and polylysine-coated yttrium silicate scintillation proximity assay beads were purchased from Amersham Pharmacia Biotech (Piscataway, N.J., USA). Aluminum hydroxide gel was prepared using aluminum sulfate and sodium hydroxide. Phytohemagglutinin (PHA; DIFCO, Detroit, Mich., USA), RPMI 1640 medium (GIBCO BRL, Grand Island, N.Y., USA), inactivated *Bordetella pertussis* (Wako Pure Chemical Industries, Ltd., Osaka, Japan), xylazine (Celactal 2% injection; Bayer Yakuin, Osaka, Japan), ketamine (Ketalar 50; Sankyo, Tokyo, Japan), and heparin (heparin for intravenous injection, Shimizu Pharmaceutical Co., Ltd., Shizuoka, Japan) were also purchased.

PDE4 Subtype Assay

Recombinant human PDE4A, 4B, 4C, and 4D were prepared as previously described [25]. The inhibitory effects of compounds on each PDE4 subtype were measured as previously reported [19].

Cytokine Production in Human Whole Blood Cells

Blood was collected from healthy volunteers via heparinized syringes in accordance with regulations of the Research Ethics Committee of Astellas Pharma Inc. Whole blood (20 μ l) was diluted with RPMI 1640 medium and preincubated with each test compound for 30 min at 37°C in 5% CO₂ before stimulation by 3 μ g/ml LPS for TNF- α production or by 10 μ g/ml PHA for IL-5 production. The total volume of the reaction solution was set at 200 μ l/well. Twenty hours after stimulation, the concentrations of each cytokine in the culture supernatant were determined using a human TNF- α or IL-5 enzyme-linked immunosorbent assay (ELISA) system (Amersham Pharmacia Biotech).

Table 1. IC₅₀ values of ASP3258 and roflumilast for recombinant human PDE4 subtypes

Compound	IC ₅₀ value, nmol/l			
	PDE4A	PDE4B	PDE4C	PDE4D
ASP3258	0.036 (0.022–0.059)	0.050 (0.029–0.088)	0.45 (0.19–1.0)	0.035 (0.016–0.078)
Roflumilast	0.21 (0.14–0.31)	0.15 (0.13–0.18)	1.7 (0.97–2.9)	0.13 (0.10–0.17)

IC₅₀ values were calculated by logistic regression using SAS. Results are presented as the mean of 4 independent experiments. Values in parentheses represent 95% confidence intervals.

OVA-Induced Airway Eosinophilia in Sensitized BN Rats

Inhibitory effects of compounds on OVA-induced pulmonary eosinophilia were determined as previously described [26] with minor modifications. Briefly, female BN rats were sensitized by intraperitoneal injection of OVA (1 mg) containing aluminum hydroxide gel (alum, 20 mg) and *B. pertussis* (6×10^9 cells) in 1 ml of PBS on day 0. Sensitized rats were exposed to an aerosol of 1% (w/v) OVA for 30 min with an ultrasonic nebulizer NE-U12 (Omuron, Kyoto, Japan) every third day from days 3 to 21 (7 times). Test compounds were orally administered once daily from days 1 to 21. On the day of antigen exposure, these compounds were administered 1 h before the start of antigen inhalation. Twenty-four hours after the last exposure, bronchoalveolar lavage fluid was obtained and the number of eosinophils was counted.

OVA-Induced AHR in Sensitized BN Rats

AHR was measured as previously described [27] with some modifications. Briefly, male BN rats (7 weeks) were sensitized by intraperitoneal injections of 1 mg OVA containing 20 mg of alum in 1 ml of saline solution for 3 consecutive days. Three weeks later, the animals were exposed to an aerosol of 1% OVA (w/v) with an ultrasonic nebulizer NE-U12 (Omuron, Kyoto, Japan) for 15 min. This exposure was repeated a total of 3 times every third day. ASP3258 was orally administered once daily during the OVA exposure period (7 successive days). On the day of OVA exposure, ASP3258 was administered 1 h before the start of inhalation. Forty-eight hours after the last exposure, animals were anesthetized with pentobarbital (100 mg/kg, i.p.), and artificial respiration was applied. The animals were exposed to aerosolized saline (baseline) followed by increasing concentrations of acetylcholine (1×10^{-5} to 1×10^{-1} mol/l) using an ultrasonic nebulizer via the trachea cannula. The maximum airway resistance after each exposure to acetylcholine was measured using a respiratory function analyzer BioSystem XA (Buxco). The log of the acetylcholine concentration required to produce an airway resistance increase equal to 150% of the baseline value was calculated and represented as PC150 as an index of AHR.

Duration of Anesthesia

ASP3258 (3 mg/kg) was orally administered to rats once daily for 10 successive days. Thirty minutes after the last ad-

ministration, the rats were anesthetized by hindlimb intramuscular injection of xylazine (10 mg/kg) and ketamine (25 mg/kg). The interval between the loss and recovery of the righting reflex was defined as the duration of anesthesia.

Fibrinogen Concentration in Blood

Test compounds were orally administered to rats once daily for 5 successive days. Thirty minutes after the last administration, blood was collected and plasma was prepared. The fibrinogen concentration in the plasma was determined using a commercial assay kit (Roche Diagnostics).

Data Analysis

All statistical analyses were conducted using the SAS system (SAS Institute Inc., Cary, N.C., USA). Data are expressed as the mean with 95% confidence limits or mean $\pm$ standard error. For in vitro studies, the IC₅₀ value in each independent experiment, calculated via logistic regression analysis using the nonlinear least squares method, was used to calculate the mean and 95% confidence limits. For in vivo studies, the ED₅₀ value was calculated using linear regression. Statistical significance of differences between group means was determined using Student's *t* test or Dunnett's multiple range test. Values with *p* < 0.05 were considered significant.

Results

Inhibition of PDE4 Subtypes

ASP3258 was seen to inhibit human recombinant PDE4A, 4B, 4C, and 4D concentration-dependently with IC₅₀ values of 0.036, 0.050, 0.45, and 0.035, respectively (table 1). Roflumilast similarly inhibited with IC₅₀ values of 0.21, 0.15, 1.7, and 0.13, respectively, values which are almost identical to previous studies [28, 29]. ASP3258 inhibited PDE4A, 4B, and 4D with similar IC₅₀ values, and the inhibition was approximately 10 times less potent for PDE4C (table 1). ASP3258 potency ranged from approximately 3 to 6 times that of roflumilast.

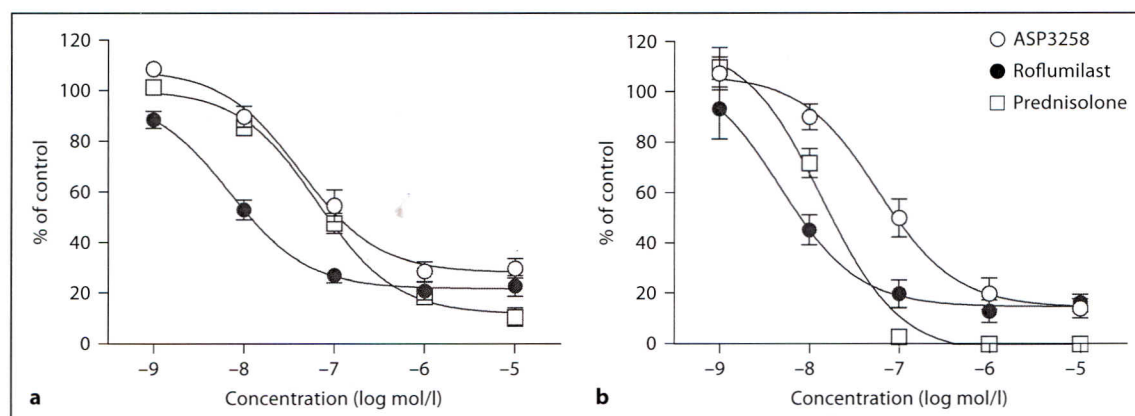


Fig. 1. Effects of ASP3258, roflumilast, and prednisolone on LPS-induced TNF- α (a) and PHA-induced IL-5 (b) production in human whole blood cells. Human whole blood diluted in culture medium was preincubated for 30 min at 37°C in the absence or presence of ASP3258, roflumilast, and prednisolone before stimulation with 3 μ g/ml LPS for TNF- α production or with 10 μ g/ml PHA for IL-5 production. Twenty hours after the stimulation, each cytokine concentration in the culture supernatant was determined using ELISA. Data are expressed as mean $\pm$ SE of 4 independent experiments.

Inhibition of TNF- α Production in Human Whole Blood Cells

TNF- α is believed to be involved in the development of chronic inflammatory diseases, while the inhibition of its signaling is thought to have therapeutic potential in various chronic inflammatory diseases [1]. We found that LPS-stimulated human whole blood cells produced TNF- α at a concentration of 907 ± 167 pg/ml. Under LPS-nonstimulated conditions, TNF- α production was below the detection limit of the ELISA (<51.2 pg/ml). ASP3258, roflumilast, and prednisolone all inhibited LPS-induced TNF- α production in a concentration-dependent manner with IC_{50} values of 110, 11, and 92 nmol/l, respectively (fig. 1; table 2). Like above, the results for roflumilast were almost identical to those of a previous study using a similar system [30]. Although ASP3258 was approximately 10 times less effective than roflumilast in inhibiting LPS-induced TNF- α production, it did show similar potency to prednisolone.

Inhibition of IL-5 Production in Human Whole Blood Cells

IL-5 plays an essential role in orchestrating the eosinophilic inflammation of asthma [31], a notion supported by findings of an anti-IL-5 mAb reducing the number of severe asthma exacerbations in patients with refractory eosinophilic asthma [32]. We assessed the functional activity of ASP3258 by measuring the PHA-stimulated IL-5 production in human whole blood cells. PHA-stimulated human whole blood cells produced IL-5 at a concentra-

Table 2. IC_{50} values of ASP3258, roflumilast, and prednisolone for cytokine production in human whole blood cells

Compound	IC_{50} value, nmol/l	
	LPS-induced TNF- α production	PHA-induced IL-5 production
ASP3258	110 (40–320)	100 (31–350)
Roflumilast	11 (6.3–21)	6.5 (2.2–19)
Prednisolone	92 (51–170)	15 (9.1–25)

IC_{50} values were calculated by logistic regression using SAS. Results are presented as the mean (95% confidence interval) of 4 independent experiments.

tion of 725 ± 99.1 pg/ml. Under PHA-nonstimulated conditions, IL-5 production was below the detection limit of the ELISA (<31.3 pg/ml). We also found that ASP3258, roflumilast, and prednisolone all inhibited PHA-induced IL-5 production in a concentration-dependent manner, with respective IC_{50} values of 100, 6.5, and 15 nmol/l (fig. 1; table 2), demonstrating that for IL-5, ASP3258 was approximately 15 and 7 times less potent than roflumilast and prednisolone, respectively.

OVA-Induced Eosinophil Infiltration in the Airways of Sensitized BN Rats

To assess the anti-inflammatory effects of ASP3258 and roflumilast, leukocyte infiltration into the airways

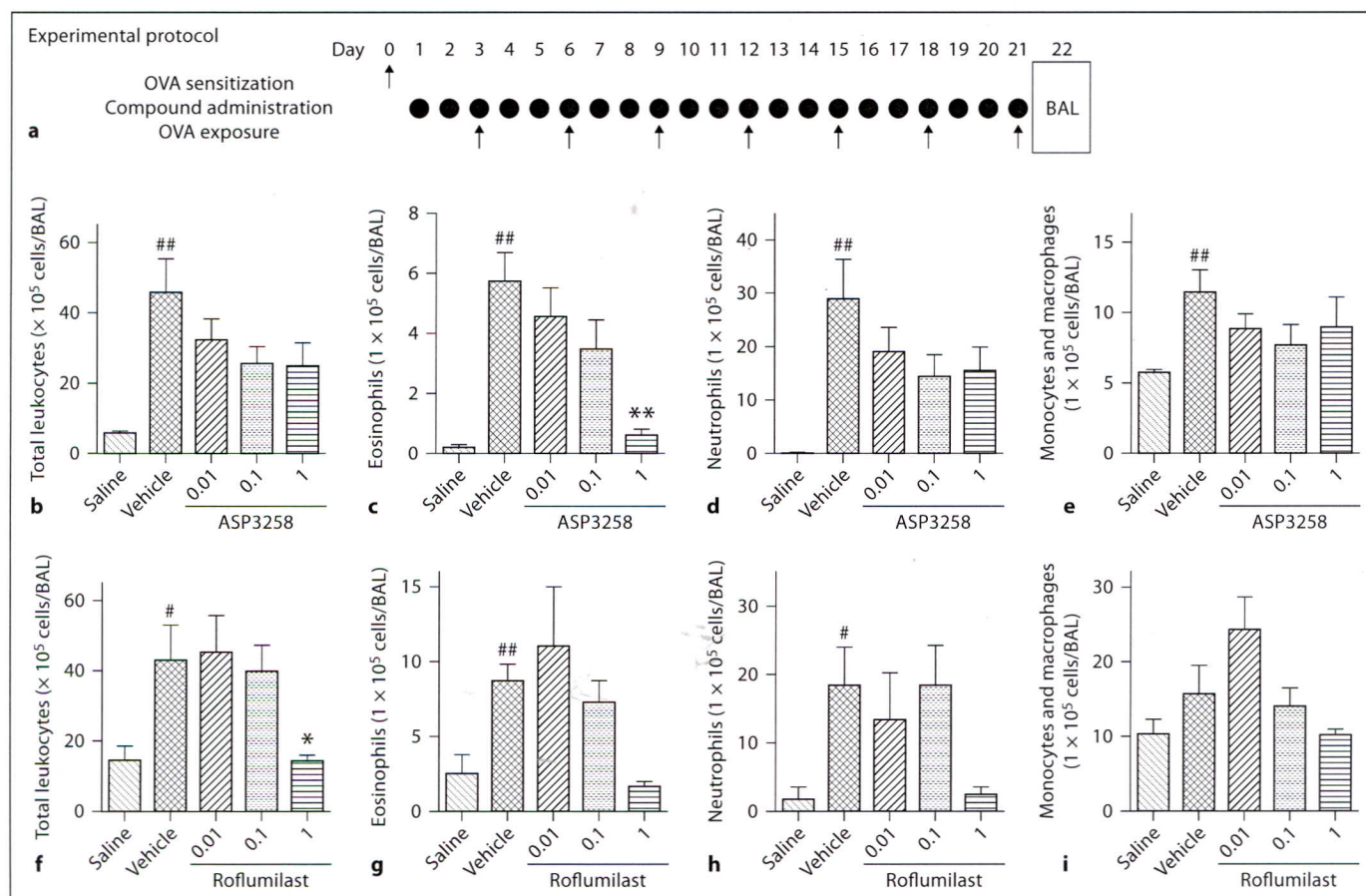


Fig. 2. Effects of ASP3258 and roflumilast on OVA-induced airway eosinophilia in BN rats. BAL = Bronchoalveolar lavage. **a** The experimental protocol. OVA-induced leukocyte infiltration was established as described in Materials and Methods. **b–i** Data are expressed as mean $\pm$ SE of 9–10 animals. # $p < 0.05$, ## $p < 0.01$, significant difference between saline and vehicle (Student's *t* test); * $p < 0.05$, ** $p < 0.01$, significant difference from vehicle (Dunnett's multiple range test).

of OVA-exposed BN rats was investigated. After repeated exposure to an antigen, a significant increase in total leukocyte number in bronchoalveolar lavage fluid was observed (fig. 2b, f). Cells observed in bronchoalveolar lavage fluid included eosinophils, neutrophils, monocytes, and macrophages. Compared with the saline group, the numbers of eosinophils and neutrophils in vehicle groups significantly increased in all cases (fig. 2c, d, g, h). Repeated administration (21 successive days) of ASP3258 and roflumilast dose-dependently inhibited the airway eosinophilia with respective ED_{50} values (and 95% confidence intervals) of 0.092 mg/kg (0.024–0.33) and 0.17 mg/kg (0.036–2.0) (fig. 2c, g; table 3), demonstrating that ASP3258 was approximately 2 times more potent than roflumilast at inhibiting eosinophil infiltration. Roflumilast (1 mg/kg) also showed almost complete inhibition of airway neutrophilia (fig. 2h).

Table 3. Effects of ASP3258 and roflumilast on OVA-induced eosinophil infiltration in the airways

Compound	ED_{50} , mg/kg, p.o.
ASP3258	0.092 (0.024–0.33)
Roflumilast	0.17 (0.036–2.0)

Eosinophil infiltration in the saline and vehicle groups was defined as 100 and 0% inhibition, respectively. ED_{50} values were calculated from the inhibition percentage using linear regression. Values in parentheses represent 95% confidence intervals.

OVA-Induced AHR in Sensitized BN Rats

AHR is a typical and pivotal condition that occurs in conjunction with bronchial asthma. Repeated exposure of sensitized BN rats to OVA solution resulted in an in-

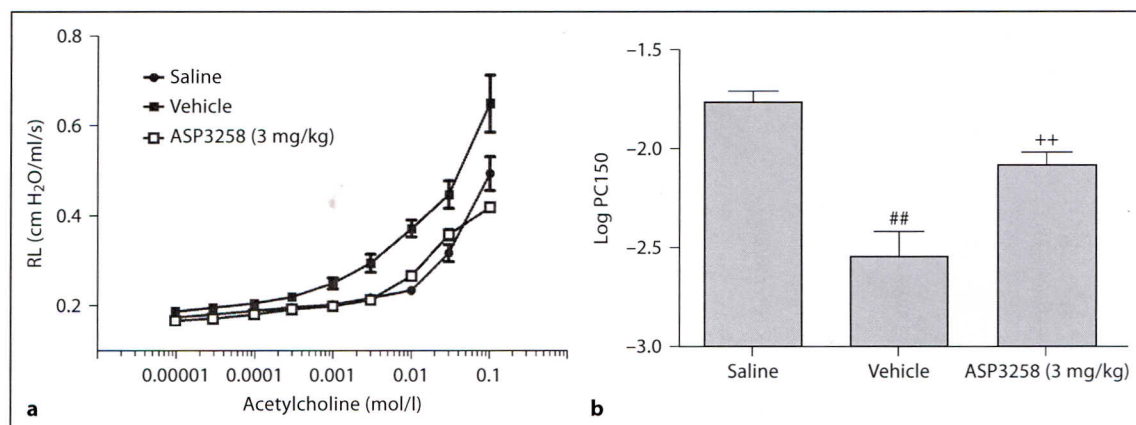


Fig. 3. Effects of ASP3258 on OVA-induced AHR in sensitized BN rats. Results are expressed as airway reactivity to acetylcholine (**a**) and the PC150 value of acetylcholine (**b**). Data are shown as the mean $\pm$ SE of the results from 7 to 8 animals. ## $p < 0.01$, significantly different from saline group (Student's *t* test); ++ $p < 0.01$ compared to vehicle (Student's *t* test). RL = Lung resistance.

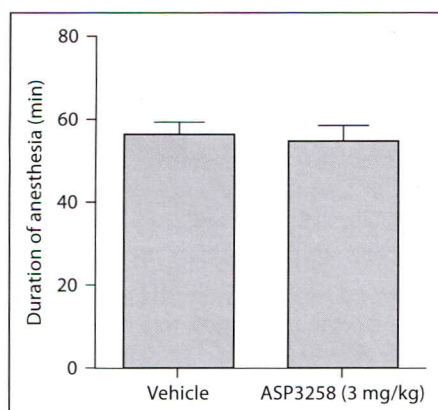


Fig. 4. Effect of ASP3258 on the duration of anesthesia induced by the combination of xylazine and ketamine in rats. ASP3258 (3 mg/kg) was orally administered to rats once daily for 10 successive days. Thirty minutes after the final administration, the rats were anesthetized with a combination of xylazine (10 mg/kg) and ketamine (25 mg/kg). The duration of anesthesia was defined as the interval between loss and recovery of the righting reflex. Data are expressed as the mean $\pm$ SE of 9–10 animals.

crease in airway reactivity to acetylcholine and a decrease in the PC150 value compared to the saline group (fig. 3). Orally administered ASP3258 (3 mg/kg) almost completely inhibited the reactivity to acetylcholine and significantly increased the PC150 value (fig. 3).

Effect on Duration of Anesthesia

PDE4 inhibitors induce emesis by mimicking the pharmacological actions of α_2 -adrenoceptor antagonists

[33, 34]. To assess potential emetic activity in rats lacking a vomiting reflex, the duration of anesthesia induced by xylazine (α_2 -adrenoceptor agonist) was measured after repeated administration of ASP3258 (once daily for 10 successive days). The duration of anesthesia for the vehicle group was 56.4 ± 3.0 min. A dose of 3 mg/kg of ASP3258, which corresponds to about 30 times the ED₅₀ value in a chronic eosinophilia model, was found not to affect the duration (fig. 4).

Fibrinogen Concentration in Blood

PDE4 inhibitors generally induce vascular injury during preclinical toxicology tests, an observation found mainly in rats but also in dogs and monkeys [15–18]. Although the mechanisms of the injury are not well understood and there are no specific biomarkers for its indications, the injury does correlate with marked increases in several serum proteins, including acute phase proteins [16, 35, 36]. Dagues et al. [18] reported that when the selective PDE4 inhibitor CI-1044 is orally administered to rats for 3 successive days, the plasma fibrinogen level significantly increases in a time-dependent manner after 24 h, and vascular fibrinoid necrosis is observed after 48 h. Here, we investigated and compared ASP3258 and roflumilast side effects by repeatedly (5 successive days) administering the two separately in rats and measuring plasma fibrinogen levels. Although ASP3258 (3 and 10 mg/kg) and roflumilast (1, 3, and 10 mg/kg) significantly increased the fibrinogen level, 1 mg/kg ASP3258, which is 10 times the ED₅₀ value, did not (fig. 5).

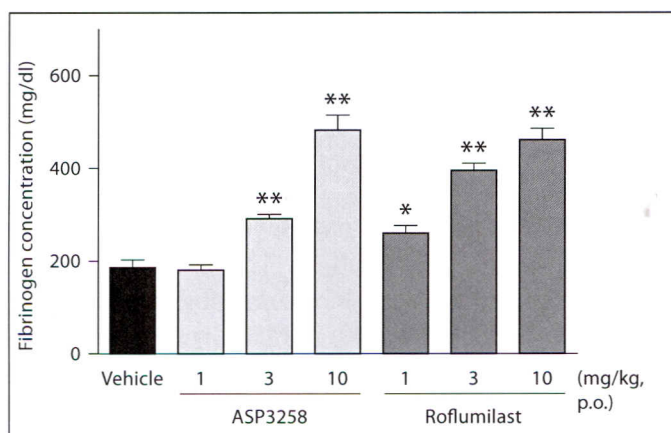


Fig. 5. Effects of PDE4 inhibitors on plasma fibrinogen concentration in rats. Test compounds were orally administered to rats once daily for 5 successive days. On the last day, blood samples were collected and the plasma was obtained. Fibrinogen levels were determined using a commercial kit. Data are expressed as mean $\pm$ SE of 4–5 animals. * $p < 0.05$, ** $p < 0.01$, significant difference from vehicle (Dunnett's multiple range test).

Discussion

ASP3258 is a potent and selective PDE4 inhibitor that has been found to exert a wide range of anti-inflammatory and antiasthmatic effects with low emetic potential in single administration animal studies [19, 24]. Here, we investigated its pharmacological and side effects when repeatedly administered to assess ASP3258's therapeutic potential on asthma. Given its efficacy in potently inhibiting antigen-induced chronic airway eosinophilia with low emetic- and vascular injury-inducing activity, we believe ASP3258 has great potential for use in treating chronic eosinophilic airway inflammation due to asthma.

Although ASP3258 inhibited PDE4 enzymes approximately 3–6 times more potently than roflumilast did (table 1), its inhibitory effect on cytokine production from human whole blood cells was 10 times weaker. This discrepancy in efficacy may be due in part to ASP3258's extremely high binding rate to human plasma protein (99.84%; 0.16% as the existing rates of free drug), which is greater than that of roflumilast (98.9%; 1.1% as the existing rates of free drug) [37], as, in general, pharmacological effects are exerted by free drugs [38], and ASP3258's existing rate of free drug was almost 7 times lower than that of roflumilast. As ASP3258 also showed a high binding rate to rat plasma protein (99.50%), we prepared a rat model of OVA-induced chronic airway inflammation to investigate the effects of ASP3258.

Seven OVA exposures to sensitized BN rats at 3-day intervals mainly brought about airway eosinophilia and neutrophilia. ASP3258 and roflumilast showed obvious and more potent suppressive activities on airway eosinophilia (ASP3258: $ED_{50} = 0.092$ mg/kg and roflumilast: $ED_{50} = 0.17$ mg/kg) in the chronic model (fig. 2) than in the acute model following a single administration (ASP3258: $ED_{50} = 0.81$ mg/kg and roflumilast: $ED_{50} = 0.46$ mg/kg) [24]. ASP3258 plasma concentration is clearly not the cause of this potent activity in the chronic model, since pharmacokinetics profiles for repeated administration (1 mg/kg, p.o., 14 successive days) in rats showed no clear difference from those for single administration (data not shown). Although the precise reasons for this discrepancy are unknown, like glucocorticoids, which show their full efficacy against asthma 3 or 4 months after starting administration [39, 40], PDE4 inhibitors may also fully exert their anti-inflammatory effects only via chronic administration. Airway eosinophilia is one of the most characteristic pathogenesis of asthma and correlates with its severity [41]. Interestingly, the PDE4 inhibitor RO-20-1724 reduced both AHR and airway eosinophilia and seemed to have no effect on airway neutrophilia in a mouse model of virus-induced asthma [42]. In fact, ASP3258 inhibited both AHR (fig. 3) and airway eosinophilia to a greater degree than neutrophilia in sensitized BN rats (fig. 2). IgE is also considered to play a key role in pathogenesis of asthma [43]. In our chronic model, total and OVA-specific plasma IgE levels increased in the vehicle group compared to the saline group, although ASP3258 (0.01–1 mg/kg) had no effect on the levels (data not shown). These results are consistent with the findings that PDE4B ablation suppresses antigen-induced eosinophilia and AHR but not antigen-specific plasma IgE levels [6]. We therefore suspect that ASP3258 may exert its antiasthmatic effects mainly through PDE4B inhibition.

Owing to their wide range of anti-inflammatory effects, PDE4 inhibitors may possess equal efficacy to glucocorticoids or may at least be a preferred option in glucocorticoid-resistant patients. However, their clinical efficacy is limited by emesis [44, 45]. Several studies have reported that inhibiting α_2 -adrenoceptor agonist-induced anesthesia is an effective way to estimate this emetic effect in rodents lacking a vomiting reflex [33, 34]. We previously reported using single oral administration that ASP3258 (10 mg/kg), but not ASP3258 (3 mg/kg), inhibited α_2 -adrenoceptor agonist-induced anesthesia in rats [19]. In the present study, repeatedly administered ASP3258 (3 mg/kg for 10 successive

days), which corresponds to 30 times the ED₅₀ value in a chronic eosinophilia model, showed no inhibitory effect on the anesthesia. Further, in vivo observations in cynomolgus monkeys revealed that repeated oral administration of ASP3258 (3, 10, and 30 mg/kg for 7 successive days) did not induce emesis. These results suggest that repeated administration of ASP3258 does not strengthen emetic activity. Studies using knockout mice have revealed that PDE4B ablation suppresses antigen-induced eosinophilia and asthmatic responses [6] and PDE4D ablation may be responsible for the occurrence of emesis [7]. However, ASP3258 and roflumilast inhibited PDE4B and PDE4D with similar IC₅₀ values (table 1), suggesting that the low emetic potential for ASP3258 is not due to PDE4B selectivity. One alternative idea is that PDE4 inhibitors induce emesis by acting on the central nervous system [34]. We previously reported that when ¹⁴C-labeled ASP3258 was orally administered to rats at 1 mg/kg, the ratios of ASP3258 concentration in the tissue to that in the plasma were 0.15 (lung), 1.5 (heart), 8.1 (liver), 0.55 (kidney), and 0.028 (brain). Thus the favorable profile of ASP3258 may be attributable to its low central nervous system permeability, which seems to be maintained during the repeated administration period.

PDE4 inhibitors generally induce vascular injury and other unwanted side effects in experimental animals that compromise clinical study design [15, 18, 35, 46–49]. Although the mechanism producing vascular injury is poorly understood, it appears to correlate with increases in blood acute phase protein levels, such as fibrinogen [18, 35, 36]. Although ASP3258 (3 and 10 mg/kg) and

roflumilast (1, 3, and 10 mg/kg) significantly increased the fibrinogen level, 1 mg/kg of ASP3258 did not (fig. 5). In a 13-week Good Laboratory Practice toxicity study of ASP3258 in rats, vascular injury was observed at doses of 3 mg/kg or more, but not at 1 mg/kg. Because these fibrinogen-increasing doses (5 successive days of administration) correlated well with the vascular injury doses during chronic administration, plasma fibrinogen could be an early and sensitive biomarker for vascular injury induced by ASP3258 at least in rats. Given that ASP3258 (1 mg/kg), which corresponds to 10 times the ED₅₀ value in a chronic eosinophilia model, showed no vascular injury, ASP3258 may offer an adequate safety margin to vascular toxicity in rats.

In conclusion, ASP3258 shows a potent inhibitory effect on human PDE4 and cytokine production in human whole blood cells. Further, the compound shows potent inhibitory effects on OVA-induced chronic airway eosinophilia with low emetic activity and vascular injury-inducing activity in rats. Taken together, these results suggest that ASP3258 is an attractive PDE4 inhibitor for treating chronic airway eosinophilic inflammation due to asthma.

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