Anti-asthmatic effect of ASP3258, a novel phosphodiesterase 4 inhibitor

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Abstract

ASP3258 is a potent and selective PDE4 inhibitor and exerts a wide-range of anti-inflammatory effects with low emetic potential, a major adverse effect of PDE4 inhibitors. Here, we investigated the anti-asthmatic potency of ASP3258 as compared with those of two representative PDE4 inhibitors: roflumilast and cilomilast. Orally administered ASP3258, roflumilast, and cilomilast all inhibited ovalbumin (OVA)-induced eosinophil infiltration into the airway of sensitized Brown Norway rats with ED50 values of 0.81, 0.46, and 4.4 mg/kg, respectively. Histological examination also revealed a decreasing trend in inflammatory cell infiltration into the lungs following ASP3258 administration. In vitro investigation of bronchodiarylative activities showed that these compounds (10⁻⁸–10⁻⁶ M) concentration-dependently inhibited OVA-induced contraction of trachea isolated from sensitized guinea pigs but had no effect on spasmon-precontracted tracheal tension prepared from non-sensitized guinea pigs up to 10⁻⁶ M. In vivo experiments using sensitized guinea pigs showed that these orally administered compounds inhibited OVA-induced increases in airway resistance with ED50 values of 2.2, 0.35, and 12 mg/kg, respectively. Further, orally administered ASP3258 (0.1 and 1 mg/kg), roflumilast (0.1 and 1 mg/kg), and cilomilast (10 mg/kg) significantly suppressed airway hyperresponsiveness caused by OVA exposure. ASP3258's potent inhibition of antigen-induced bronchoconstriction and airway hyperresponsiveness, two characteristic symptoms of bronchial asthma, suggests that this compound will be useful in treating asthma.

1. Introduction

Asthma is a chronic inflammatory disorder of the airway induced by various initiating factors such as environmental allergens. The airway is infiltrated by inflammatory cells—including eosinophils, mast cells, and CD4⁺ T lymphocytes—which produce inflammatory mediators such as cytokines, chemokines, and leukotrienes, subsequently exacerbating and perpetuating the inflammatory state [12]. This chronic inflammation is associated with airway hyperresponsiveness (AHR), which leads to recurrent episodes of wheezing, breathlessness, chest tightness, and coughing typically associated with airflow obstruction [3]. At the very least, inflammation is clearly an important component of the development and progression of asthma. Although glucocorticoids are presently the most effective anti-inflammatory therapy available for many inflammatory diseases, including asthma, some patients with these diseases show a poor or absent response even to high doses of glucocorticoids [4]. Therefore, to aid these poorly responsive patients, research is currently underway to identify novel anti-inflammatory agents with mechanisms of action differing from those of glucocorticoids.

Phosphodiesterase (PDE) 4 is the major PDE isozyme in leukocytes. Previous studies have demonstrated that PDE4 inhibitors exhibit a variety of anti-inflammatory effects, including suppression of inflammatory mediators such as tumor necrosis factor (TNF)-α, interleukins, and leukotrienes, as well as leukocyte infiltration into inflamed tissues [5,6]. As such, PDE4 inhibitors have been considered as alternative anti-inflammatory agents to glucocorticoids. However, while clinical trials have confirmed the proposed efficacy of PDE4 inhibitors in treating asthma [7], emesis was reported as a dose-limiting side effect, and therefore research has been focused on developing PDE4 inhibitors with lower emetic activity than that found with presently available compounds [8–10].

ASP3258 is a novel, potent (IC₅₀ = 0.28 nM), and highly selective PDE4 inhibitor derived from naphthyridin [11] with a structure lacking the 3,4-dialkoxyphenyl group found in roflumilast [12] and cilomilast [13]. A previous study found that orally administered ASP3258 inhibited ovalbumin (OVA)-induced production of interleukin (IL)-4, TNF-α, and cysteinyl leukotrienes (cys-LTs), as well as leukocyte infiltration into the airways of sensitized Brown Norway (BN) rats [11], and also inhibited cigarette smoke exposure-induced pulmonary accumulation of mononuclear cells and neutrophils in guinea pigs [14]. Here, we investigated the anti-asthmatic effects of ASP3258 in several experimental models in which symptoms were elicited by antigen inhalation, including antigen-induced eosinophil infiltration into lungs in BN rats and AHR in guinea pigs. The compound’s effects on antigen-induced airway contraction in vivo and isolated trachea from sensitized guinea pigs were also studied. In this manner, we confirmed the anti-asthmatic effects of ASP3258 and compared them...
with those of structurally different PDE4 inhibitors, such as roflumilast and cilomilast.

2. Materials and methods

2.1. Animals

Female BN rats and male Hartley guinea pigs were purchased from Charles River Japan (Kanagawa, Japan) and Japan SLC (Shizuoka, Japan). All animal experimental procedures were approved by the corporate Animal Ethical Committee.

2.2. Chemicals

ASP3258, roflumilast, and cilomilast were synthesized by Astellas Pharma Inc. (Ibaraki, Japan), and 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 animals. OVA (albumin, chicken egg, grade V), carbachol, histamine, pyrilamine, gallamine, and urethane were purchased from Sigma-Aldrich (St. Louis, MO, USA). Leukotriene D₄ (Cayman Chemical Co., Arbor, MI, USA) and acetylcholine chloride (Daiichi Pharmaceutical, Tokyo, Japan) were also used.

2.3. OVA-induced eosinophil infiltration in the airways of sensitized BN rats

OVA-induced eosinophil infiltration was determined as described previously [15] with minor modifications. Briefly, 4-week-old female BN rats were sensitized by intraperitoneal injections of OVA (1 mg) and Alum (20 mg) for 3 consecutive days. Three weeks after the sensitization, the rats were exposed to an aerosol of 1% (w/v) OVA for 20 min with an ultrasonic nebulizer (NE-U12; Omron Corporation, Tokyo, Japan). Test compounds or vehicle (0.5% methylcellulose) were orally administered 1 h before the start of OVA-exposure. Twenty-four hours after exposure, bronchoalveolar lavage (BAL) fluid was obtained, and the number of eosinophils was counted.

2.4. Histological evaluation

To avoid possible traumatic damage due to BAL, histological assessment of the lung tissue was performed in separate animals. Animals to be evaluated were sacrificed 24 h after antigen exposure, after which the lungs were removed and fixed in 10% neutral-buffered formalin, embedded in paraffin, cut into 2-μm sections, and stained with hematoxylin and eosin. The sections were analyzed in a blind fashion, and the degrees of observed pathological changes including infiltration of eosinophils, lymphocytes, and macrophages into the peribronchiolar/perivascular regions or the alveolus, were scored as follows: 0, none; 1, slight; 2, mild; 3, moderate; and 4, severe.

2.5. OVA-induced contractions in tracheal tissue isolated from sensitized guinea pigs

Male Hartley guinea pigs (7 weeks) were sensitized by intraperitoneal injection of OVA (5 μg) containing Alum (1 mg). The animals were again immunized with the same procedure 7 days later. Thirty-one to thirty-eight days after the first sensitization, the animals were sacrificed, and the tracheas were removed. Each trachea was longitudinally cut so that the smooth muscle regions were caught between cartilages, and then divided into four strips. The tracheal preparation was vertically suspended in an organ bath maintained at 37 °C filled with 10 mL of modified Krebs bicarbonate buffer solution (118.4 mM NaCl, 4.7 mM KCl, 1.2 mM KH₂PO₄, 1.2 mM MgSO₄, 2.5 mM CaCl₂, 25 mM NaHCO₃, 10 mM glucose) aerated with a mixture of 95% O₂ and 5% CO₂. One end of the strip was tied at the bottom of the bath and the other was connected to a force transducer (TB-611, Nihon Kohden, Tokyo, Japan) to record isometric tension on a polygraph system (AP-621G, Nihon Kohden, Tokyo, Japan), and then equilibrated under a resting tension of 1 g for over 120 min. After an equilibration period, the strip was pretreated with vehicle or test drug (10⁻⁸–10⁻⁴ M) for 30 min, and then stimulated by cumulative addition of OVA (10⁻⁸–10⁻² mg/mL). After the response at each concentration reached a plateau, the next concentration of OVA was added. Finally, the strip was exposed to 10⁻⁴ M carbachol to obtain the maximum contraction. Results were expressed as the percentage of maximum contraction induced by carbachol.

2.6. Spasmmogen-induced contraction in tracheal tissue isolated from non-sensitized guinea pigs

Tracheal muscle strips were prepared from non-sensitized guinea pigs and suspended in the tissue bath as described above. Each strip was allowed to equilibrate for over 120 min, and then was preliminarily contracted with 3 × 10⁻⁶ M carbachol twice. After washing...
and an additional equilibration period, the strips were contracted with carbachol (10⁻⁶ M), histamine (3×10⁻⁵ M) or leukotriene D₄ (3×10⁻⁶ M). Once the contractile response reached a plateau, vehicle or increasing concentrations of test drug (10⁻⁷, 10⁻⁶, and 10⁻⁵ M) were cumulatively added at 20-min intervals. After final addition of the test drug, 10⁻⁵ M isoproterenol was added to define the maximum relaxation of each strip. Data were obtained as a decrease in the tension of the strip below the plateau level.

2.7. OVA-induced bronchoconstriction in sensitized guinea pigs

OVA-induced bronchoconstriction was measured as previously described [16], with some modification. Guinea pigs (4 weeks) were sensitized by three intraperitoneal injections of 5 µg OVA containing 1 mg of Alum every 2 weeks. One week after the last sensitization, the animals were anesthetized with urethane (1.2 g/kg, i.p.). A tracheal cannula was inserted, and the animal was ventilated at a rate of 60 strokes/min and 10 mL/kg body weight per cycle using a constant volume respirator.

Model 683 (Harvard; South Natick, MA, USA). Airway resistance was measured with a respiratory function measuring apparatus BioSystem XA (Buxco Electronics, Inc., Sharon, CT, USA) connected to the tracheal cannula. The animals were pretreated with gallamine (1 mg/kg, i.v.) and pyrilamine (2 mg/kg, i.v.) at 10 and 2 min prior to antigen challenge. After intravenous injection of OVA (0.6 mg/kg), airway resistance was measured continuously for 15 min. The effects of compounds were evaluated using the percentage change in airway resistance from the pre-challenge value. The area under the time–response curve (AUC) for the 15 min of observation was used as an index. The test compound was orally administered 1 h before antigen challenge.

2.8. OVA-induced AHR

AHR was measured as previously described [17], with some modification. Briefly, guinea pigs (4 weeks) were sensitized by intraperitoneal injections of 10 µg OVA containing 10 mg of Alum. One week later, the animals were exposed to an aerosol of 0.5% OVA (w/v) for 5 min. This was repeated a total of three times at one-week intervals as a booster. To avoid anaphylactic shock, the animals were pretreated with pyrilamine (10 mg/kg, i.p.) 30 min before each OVA booster. One week after the final booster, the animals were challenged with aerosolized 0.5% OVA (w/v) for 30 min. AHR was assessed as the change in airway reactivity to acetylcholine 24 h after OVA challenge.

Animals were anesthetized with urethane (1.2 g/kg, i.p.), and artificial respiration was carried out as described above. Spontaneous respiration was stopped by administering gallamine (1 mg/kg, i.v.). The animals were exposed to aerosolized saline (baseline) followed by increasing concentrations of acetylcholine (1, 2, 4, 8, 16, and 32 mg/mL) using an ultrasonic nebulizer via the trachea cannula. Each concentration was delivered over two strokes of the respirator with 3-min intervals. 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 200% of the baseline value was calculated and represented as PC200 as an index of AHR.

Fig. 2. Quantitative assessment of histological scores in the lung from saline, vehicle, and ASP3258 (3 mg/kg, p.o.)-treated BN rats. Histological signs observed in peribronchiolar/perivascular areas and alveolar areas were scored. (A) Infiltration of eosinophils into the peribronchiolar/perivascular areas. (B) Infiltration of lymphocytes into the peribronchiolar/perivascular areas. (C) Infiltration of eosinophils into the alveoli, and (D) infiltration of macrophages into the alveoli. Each symbol represents an individual animal’s data point. Horizontal lines indicate medians. **p<0.01, *p<0.05, significantly different from saline group (Wilcoxon’s rank sum test), #p<0.05, significantly different from vehicle group (Wilcoxon’s rank sum test).
2.9. Statistical analysis

All statistical analyses were conducted using the SAS system (SAS Institute Inc., Cary, NC, USA). Data were expressed as means ± standard error. The percent inhibition values were calculated for unstimulated and control stimulated levels. The doses causing 50% inhibition were calculated using linear regression. The statistical significance of differences between groups was determined using Student’s t-test, Dunnett’s multiple range test, or Wilcoxon rank sum test. Values with $p<0.05$ were considered significant.

3. Results

3.1. OVA-induced eosinophil infiltration in the airways of sensitized BN rats

To assess the anti-asthmatic effect of ASP3258, eosinophil infiltration into the airways of OVA-exposed BN rats was investigated (Fig. 1). The eosinophil infiltration into the airways was significantly increased after exposure to antigen. ASP3258 dose-dependently inhibited the antigen-induced eosinophil infiltration with significant inhibition at doses of 1, 3, and 10 mg/kg. Similarly, roflumilast (significant at 1 and 3 mg/kg) and cilomilast also dose-dependently inhibited eosinophil infiltration. The ED$_{50}$ values (and 95% confidence intervals) of ASP3258, roflumilast, and cilomilast were 0.81 (0.36–1.5), 0.46 (0.34–1.8), and 4.4 (2.8–8.7) mg/kg, respectively (Table 1).

3.2. OVA-induced histological changes in the lung of sensitized BN rats

To better understand the anti-asthmatic effect of ASP3258 at the pathological level, OVA-induced histological changes in the lungs of sensitized BN rats were investigated (Figs. 2 and 3). Results showed significant infiltration of eosinophils into peribronchiolar/perivascular areas and alveoli, lymphocytes into peribronchiolar/perivascular areas, and macrophages into alveoli in vehicle control animals. ASP3258 (3 mg/kg) not only significantly reduced infiltration of lymphocytes into peribronchiolar/perivascular areas, but also induced decreasing trends in histological scores for not only eosinophil infiltration into peribronchiolar/perivascular areas and alveoli but also macrophage infiltration into alveoli.

![Fig. 3. Representative micrographs of lung tissue stained with hematoxylin and eosin. (A, D) Saline-exposed, (B, E) OVA-exposed, and (C, F) OVA-exposed and ASP3258 (3 mg/kg)-treated animal lungs, respectively. Scale bars represent 100 μm.](image)

![Fig. 4. Effects of ASP3258 (A), roflumilast (B), and cilomilast (C) on OVA-induced contraction in tracheal muscle strips isolated from OVA-sensitized guinea pigs. Tracheal strips prepared from OVA-sensitized animal were pretreated with vehicle ( ), 10$^{-8}$ M ( ), 10$^{-7}$ M ( ), or 10$^{-6}$ M ( ) of test compounds for 30 min, and then stimulated by cumulative addition of OVA (10$^{-5}$–10$^{-2}$ mg/mL) to record isometric tension. Strips were then exposed to 10$^{-4}$ M carbachol to obtain maximum contraction. Results are expressed as the percent of the maximum contraction and are given as the mean ± SE of four individual determinations.](image)
Data for OVA (10⁻¹⁰ M) contraction in tracheal muscle strips isolated from sensitized guinea pigs. * Effects of ASP3258, roflumilast, cilomilast, and prednisolone on OVA-induced bronchoconstriction in sensitized guinea pigs. Contraction intensity was at maximum 90% of that evoked by 10⁻² M carbachol. Pretreatment with ASP3258 inhibited the OVA-induced contraction (Fig. 4). The cumulative addition of OVA (10⁻¹⁰ to 10⁻⁸ M) induced concentration-dependent contractions in the tracheal strips prepared from OVA-sensitized guinea pigs. Contraction intensity was at maximum 90%–100% of that evoked by 10⁻⁴ M carbachol. Pretreatment with ASP3258 inhibited the OVA-induced contraction in a concentration-dependent manner, with percent responses to maximum OVA concentration (10⁻⁴ M) of 95% (vehicle), 87% (10⁻⁸ M), 79% (10⁻⁷ M, p < 0.05 vs. vehicle), and 64% (10⁻⁶ M, p < 0.01 vs. vehicle), respectively (Table 3). Roflumilast and cilomilast also inhibited OVA-induced tracheal contraction (Fig. 4 and Table 3). None of these compounds affected basal tone (data not shown). In contrast to the findings in tracheal strips prepared from OVA-sensitized guinea pigs, neither ASP3258, roflumilast, nor cilomilast exerted any significant relaxant effect on carbachol-, histamine-, or leukotriene D₄-precontracted tracheal strips prepared from non-sensitized guinea pigs, up to 10⁻⁶ M (Fig. 5).

### 3.4. OVA-induced bronchoconstriction

When OVA-sensitized guinea pigs were intravenously administered OVA, an increase in airway resistance occurred that continued for more than 15 min (Fig. 6). Orally administered ASP3258, roflumilast, and cilomilast dose-dependently inhibited this antigen-induced increase in the airway resistance, with ED₅₀ values (95% confidence intervals) of 2.2 (0.15 to 5.4), 0.35 (0.15 to 1.0), and 12 (7.5 to 25) mg/kg, respectively (Table 2 and Fig. 6). Prednisolone (30 mg/kg) showed no significant inhibition. None of these compounds affected basal airway resistance (data not shown).

### 3.5. OVA-induced AHR

AHR is a typical and pivotal condition that occurs in conjunction with bronchial asthma. Repeated exposure of sensitized guinea pigs to OVA solution resulted in an increase in airway reactivity to acetylcholine and a decrease in the PC20 value compared to the saline group (Fig. 7). Orally administered ASP3258 dose-dependently inhibited the reactivity to acetylcholine and significantly increased PC20 values at 0.1 and 1 mg/kg. Roflumilast (0.01–1 mg/kg) and cilomilast (0.1–10 mg/kg) also dose-dependently inhibited the hyperreactiveness to acetylcholine and increased the PC20 values significantly at 0.1 and 1 mg/kg for roflumilast and 10 mg/kg for cilomilast (Fig. 7).

### 4. Discussion

ASP3258, a potent and selective PDE4 inhibitor, exerts a wide-range of anti-inflammatory effects with minimal side effects [11]. Here, we investigated the anti-asthmatic effects of ASP3258 in several experimental models, including antigen-induced eosinophil infiltration into the airway. AHR and airway contraction. Given its efficacy in potently inhibiting these characteristic pathogenesis and symptoms of bronchial asthma, we believe that ASP3258 represents an attractive agent for treating asthma. Eosinophil infiltration into the airway is one of the most characteristic pathogenesis of asthma and correlates with disease severity [18]. Therefore, we investigated the effect of these PDE4 inhibitors on OVA-induced airway eosinophil infiltration in sensitized BN rats, as this

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**Table 2**

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<tr>
<th>Compound</th>
<th>ED₅₀ (mg/kg, p.o.)</th>
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<tbody>
<tr>
<td>ASP3258</td>
<td>2.2 (0.15–5.4)</td>
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<tr>
<td>Roflumilast</td>
<td>0.35 (0.15–1.0)</td>
</tr>
<tr>
<td>Cilomilast</td>
<td>12 (7.5–25)</td>
</tr>
<tr>
<td>Prednisolone</td>
<td>23 (6.0)</td>
</tr>
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The areas under the time–response curve (AUC) of OVA-induced bronchoconstriction in the saline and vehicle groups were defined as 100% and 0% inhibition, respectively. ED₅₀ values were calculated from % inhibition using linear regression. Values in parentheses represent 95% confidence intervals.

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**Table 3**

<table>
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<tr>
<th>Compound</th>
<th>% of maximum contraction (mean ± SE)</th>
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<tbody>
<tr>
<td></td>
<td>Vehicle</td>
</tr>
<tr>
<td>ASP3258</td>
<td>95 ± 1.2</td>
</tr>
<tr>
<td>Roflumilast</td>
<td>97 ± 1.1</td>
</tr>
<tr>
<td>Cilomilast</td>
<td>98 ± 0.2</td>
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Tracheal strips were stimulated by cumulative addition of OVA (10⁻⁸ to 10⁻⁶ M). Contraction induced by 10⁻⁴ M carbachol was defined as maximum contraction. Data for OVA (10⁻⁸ mg/mL) are expressed as the percent of the maximum contraction and are given as the mean ± SE of four individual determinations. *p<0.05, **p<0.01, compared to vehicle (Dunnett’s multiple range test).

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Fig. 5. Effects of ASP3258, roflumilast, and cilomilast on carbachol-, histamine-, and leukotriene D₄-precontracted tension in tracheal muscle strips isolated from non-sensitized guinea pigs. Tracheal strips prepared from non-sensitized animals were contracted with 10⁻⁶ M of carbachol (A), 3 × 10⁻⁵ M of histamine (B), or 3 × 10⁻⁶ M of leukotriene D₄ (C). After the contractile response reached a plateau, vehicle (■) or increasing concentrations of ASP3258 (○), roflumilast (●), or cilomilast (▲) were cumulatively added. After addition of the above compounds, 10⁻⁶ M iso-proterenol was added to define the maximum relaxation of each strip. Results are expressed as the percent of the maximum relaxation and are given as the mean ± SE of four individual determinations.

model has been used extensively to evaluate compounds for anti-asthmatic activity. Results showed that the inhibitory effect of ASP3258 was similar to that of roflumilast and approximately 5-fold stronger than that of cilomilast (Fig. 1 and Table 1). Consistent with BAL cell analyses, histological examination also revealed ASP3258’s decreasing trend in infiltration of inflammatory cells, including eosinophils (Figs. 2 and 3). These results are in accordance with previously reported data using sensitized BN rats [11,19,20]. Despite the wide-range of anti-inflammatory effects that rank PDE4 inhibitors as attractive potential replacements for glucocorticoids, their efficacy is limited by emesis, the main side effect [21,22]. Inhibition of α2-adrenoceptor agonist-induced anesthesia has been reported effective in estimating the emetic effect of PDE4 inhibitors in rodents lacking a vomiting reflex [23,24]. Therefore, we estimated the degree of dissociation between emetic activity and anti-asthmatic activity using a single route of administration in a single species. ASP3258 induced no emetic effect in rats at 3 mg/kg but did at 10 mg/kg, corresponding to 12 times the ED50 value. When the same comparison was conducted for roflumilast and cilomilast, a significant emetic effect was observed at doses 6.5- and 0.68-fold higher than the ED50 values, respectively (Table 1). Taken together, these results indicate that ASP3258 possesses a wider safety margin than roflumilast or cilomilast.

Human bronchial asthma is characterized by early acute bronchoconstriction, followed by a late-phase bronchoconstriction response,

Fig. 7. Effects of ASP3258 (A), roflumilast (B), and cilomilast (C) on OVA-induced AHR in sensitized guinea pigs. Results are expressed as airway reactivity to acetylcholine (left) and the PC200 value of acetylcholine (right). Data are shown as the mean ± SE of the results from 8 to 12 animals. *p<0.05, **p<0.01, significantly different from saline group (Student’s t-test). 

Fig. 6. Effects of ASP3258 (A), roflumilast (B), and prednisolone (D) on increased lung resistance (RL) induced by OVA challenge in sensitized guinea pigs. Results are expressed as the time course of increased airway resistance for 15 min after OVA challenge (left) and as the AUC (right). Test compounds were orally administered 1 h before antigen challenge. Data represent the mean ± SE of the results from 10 animals. *p<0.01 significantly different from saline group (Student’s t-test). *p<0.05, **p<0.01, compared to vehicle (Dunnett’s multiple range test).

AHR, and airway eosinophilic inflammation. Effects of these compounds on improving respiratory function were found to be effective in sensitized guinea pigs given their similarity to humans in airway anatomy and response to inflammatory mediators [25], ASP3258, roliflumast, and cilomilast all inhibited OVA-induced increase in airway resistance (Fig. 6 and Table 2); the values of roliflumast and cilomilast in particular were quite similar to those reported in a previous study using a similar system [13,26]. To investigate the mechanism of action, the bronchodilator effects of these compounds were assessed. In vitro investigation showed that these compounds inhibited OVA-induced contraction of trachea isolated from sensitized guinea pigs but had no effect on spasmogen-precontracted tracheal tension prepared from non-sensitized guinea pigs (Figs. 4 and 5). Our previous report showed that these compounds inhibited production of inflammatory mediators, including cys-LTs, in the airway of OVA-sensitized BN rats [11]. These results suggested that PDE4 inhibitors inhibited OVA-induced tracheal contraction by inhibiting mediator release rather than via direct relaxation of tracheal smooth muscle. In contrast, glucocorticoids have no inhibitory effect on the release of chemical mediators such as histamine and leukotriene from mast cells [27]. Our present finding that prednisolone did not inhibit bronchoconstriction obviously even at a high dose (Fig. 6 and Table 2) is presumed to be due to lack of any inhibition of release of these chemical mediators. Given that glucocorticoids are considered ineffective in treating immediate-type airway obstruction, which involves chemical mediators [27,28], PDE4 inhibitors may be useful in such treatment by suppressing the antigen-induced immediate asthmatic reaction.

Increased AHR is the main symptom and one of the best clinical indices of the severity of bronchial asthma [29]. As one of the goals of asthma treatment is prevention of AHR, the ability of novel compounds to inhibit AHR is often assessed in preclinical models to determine drug efficacy. The compounds examined in the present study inhibited AHR, and the effects of ASP3258 in particular were similar to those of roliflumast and seemed to be more than 10-fold stronger than those of cilomilast (Fig. 7). These results for roliflumast and cilomilast were almost identical to those reported in a previous study using similar systems [30,31]. The glucocorticoid prednisolone has also been found to inhibit AHR in the same guinea pig model used in the present study [17]. While details regarding the precise mechanism causing AHR are unclear, damage to the airway epithelium caused by airway inflammation appears to be involved [32]. Eosinophil infiltration into the airway is often observed in patients with bronchial asthma, and strong evidence has been found that eosinophil cationic protein (ECP) and major basic protein (MBP) derived from eosinophils participate in the pathogenesis of AHR [18,33,34]. IL-4 and TNF-α are also known to be inflammatory mediators involved in airway inflammation and AHR in asthma [35–38], ASP3258, roliflumast, and cilomilast are known to exert anti-inflammatory effects, such as markedly inhibiting antigen-induced airway inflammation of leukocytes, including eosinophils, and production of inflammatory mediators such as IL-4 and TNF-α in sensitized BN rats [11]. Such anti-inflammatory effects are considered possible mechanisms of these compounds’ inhibitory effects on AHR.

ASP3258’s wide safety margin between anti-asthmatic effect and emetic effect and its potent inhibitory effects on antigen-induced acute bronchoconstriction and AHR, two characteristic symptoms of bronchial asthma, suggest it to be an attractive agent for use in treating asthma with mechanisms of action differing from those of glucocorticoids.

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References


