Effect of a Novel Anti-Inflammatory Compound, YM976, on Antigen-Induced Eosinophil Infiltration into the Lungs in Rats, Mice, and Ferrets

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ABSTRACT

We evaluated the effects of YM976, a selective inhibitor of phosphodiesterase type 4, on antigen-induced eosinophil infiltration into the lungs in rats, mice, and ferrets. In rats, YM976 inhibited the accumulation of eosinophils at an oral ED50 value of 1.7 mg/kg, and in C57Black/6 mice, exhibited a dose-dependent inhibition at an ED50 value of 5.8 mg/kg. In the same dose range in the same mouse model, YM976 suppressed interleukin-5 production. We then compared the inhibitory effect of chronic administration with that of single administration in another rat model of eosinophilia induced by repeated antigen exposure. YM976 administered chronically offered more potent inhibition (ED50 = 0.32 mg/kg p.o.) than a single dose (1.4 mg/kg p.o.). These results indicated that chronic administration is more effective in antigen-induced eosinophilia than a single administration. Emetogenicity is known to be a major adverse effect of phosphodiesterase type 4 inhibitors. We compared the anti-inflammatory activity of YM976 with its emetic activity in ferrets, in which it dose dependently suppressed eosinophil infiltration at an ED50 value of 1.2 mg/kg, but induced no emesis at 10 mg/kg. This suggested that the compound exhibits a considerable dissociation between its anti-inflammatory and emetic effects. In summary, YM976 inhibited eosinophil infiltration in a dose-dependent manner in rats, mice, and ferrets. In ferrets, it suppressed antigen-induced eosinophil infiltration without emesis. Additionally, we demonstrated that the inhibitory effect on eosinophil infiltration was increased by chronic administration. In conclusion, YM976 is a promising drug for the treatment of diseases involving eosinophil activity, such as asthma.

Bronchial asthma is a chronic inflammatory disease of the airways. Nonspecific airway hyperreactivity, inflammatory cell infiltration, and airway edema are the main features of bronchial asthma. Eosinophils are predominant among the inflammatory cells infiltrating the airways, and play a critical role in the pathogenesis of bronchial asthma. Thus, one therapeutic strategy for bronchial asthma would be to target the mechanisms involved in the accumulation and activation of eosinophils in the airways.

Glucocorticosteroids are currently the most effective drugs available for treating the airway inflammation of asthma. Corticosteroid treatment reduces the number and activity of infiltrating inflammatory cells, particularly eosinophils, and damps microvascular leakage and other signs of inflammatory response (Barnes and Pedersen, 1993). Glucocorticosteroids, however, do not inhibit the release of mast cell mediators, have no direct bronchodilating activity, and induce significant systemic adverse effects when given for prolonged periods. Although inhaled corticosteroids greatly reduce the side effects, it has been reported that the problem is still observed in long-term trials in children, even with low doses (Tinkelman et al., 1993). Therefore, a safe alternative antiasthmatic agent to corticosteroids is needed in the management of bronchial asthma.

A selective PDE4 inhibitor is expected to be a novel antiasthmatic agent (Dyke and Montana, 1999) because the elevation of intracellular cAMP, through the inhibition of PDE4 activity, induces the down-regulation of the activities of inflammatory cells such as eosinophils (Dent et al., 1991; Souness et al., 1995), neutrophils (Schudt et al., 1991), and lymphocytes (Essayan DM, 1997). Unfortunately, rolipram, a prototypic PDE4 inhibitor, was reported in a clinical trial to cause nausea and vomiting (Zeller et al., 1984). Thus, novel PDE4 inhibitors with little or no emetic effect are required as novel antiasthmatic agents. We previously reported that YM976 [4-(3-chlorophenyl)-1,7-diethylpyrido[2,3-d]pyrimidin-2(1H)-one] is a promising drug for the treatment of diseases involving eosinophil activity, such as asthma.
din-2(1H)-one] was a strong and selective PDE4 inhibitor having a different structure from rolipram, and that oral administration of YM976 inhibited neutrophil infiltration induced by carrageenan (Aoki et al., 2000). These results suggest that YM976 is a promising novel therapeutic agent for inflammatory diseases.

Several in vitro studies have shown that the continuous increase of intracellular cAMP content, such as prolonged β-adrenoceptor stimulation, up-regulates PDE4 activity (Torphy et al., 1992, 1995). These reports suggested that chronic administration of PDE4 inhibitors might decrease the anti-inflammatory effects by elevating PDE activity.

In this study, we examined the inhibitory effects of YM976, rolipram, and prednisolone on antigen-induced eosinophil infiltration into the lungs in rats and mice. The inhibitory mechanisms for eosinophil infiltration of YM976 were also elucidated in mice. In another rat model, we evaluated its effects on eosinophil infiltration during chronic administration. Additionally, we endeavored to explain the dissociation of antiasthmatic effects from emesis in ferrets. Antigen-induced eosinophilia models in ferrets, however, have never been reported. Thus, we established a ferret eosinophilia model and used it to evaluate the anti-inflammatory effect of YM976 at doses that caused no emesis in the same species.

Materials and Methods

Animals. Brown Norway (BN) rats and ferrets were purchased from Charles River Japan (Atsugi, Japan). C57Black/6 mice were purchased from SLC (Hamamatsu, Japan). All animals were maintained in ordinary animal cages on a constant 12-h light/dark cycle, with food and water available ad libitum. The rats and mice were housed in groups of 6 and 10 per cage, respectively, and ferrets were housed one or two to a cage.

Drugs and Chemicals. YM976 and (+)-rolipram were synthesized by the Department of Chemistry, Yamanouchi Pharmaceutical Co., Ltd. (Tsukuba, Japan). Prednisolone was purchased from Nacalai Tesque (Kyoto, Japan). These drugs were suspended in 0.5% methylcellulose (MC) solution and were orally administered in a volume of 3 ml/kg. The control groups were treated with the relevant vehicle.

The chemicals used were as follows: diethylether and chloroform, purchased from Kanto Chemicals (Tokyo, Japan); ovalbumin grade V or VI (OA), EDTA, Hanks’ balanced salt solution (HBSS) without calcium chloride or magnesium sulfate, and phenylmethylsulfonyl fluoride, all obtained from Sigma Chemical Co. (St. Louis, MO); Al(OH)3 (Imject Alum), from Pierce (Rockford, IL); HEPES, from Life Technologies (Rockville, MD); Bordetella pertussis from Wako Pure Chemical Industries, Ltd. (Osaka, Japan); heparin, from Shimizu Seiyaku (Shimizu, Japan); pentobarbital sodium, from Nacalai Tesque (Kyoto, Japan); Diff-Quik, from International Reagents Corp. (Kobe, Japan); and MC, from Shin-Etsu Chemical Co. (Tokyo, Japan).

Cell Infiltration Induced by Antigen in BN Rats. Female BN rats 4 to 6 weeks old were sensitized by intraperitoneal injections of OA (1 mg) and Al(OH)3 (20 mg) in 1 ml of saline solution three times for 3 consecutive days. Three weeks after the sensitization, the rats were exposed to an aerosol of 1% (w/v) OA for 15 min. Test compounds were administered orally 30 min before and 3 h after every exposure. Twenty-four hours after the final exposure, the mice were anesthetized by an intraperitoneal injection of pentobarbital sodium (50 mg/kg) and the tracheas was cannulated. Lungs were lavaged three times with 1-ml aliquots of HBSS without calcium or magnesium, but supplemented with 0.05 mM EDTA. BALf was centrifuged (500g, for 10 min), and the cell pellet was resuspended in 0.2 ml of HBSS.

Measurement of Pulmonary IL-5 Content in Mice. Mice were sensitized and challenged as described above. Test compounds were orally administered 30 min before and 3 h after each exposure. Eight hours after the final exposure, the mice were anesthetized by an intraperitoneal injection of pentobarbital sodium (50 mg/kg). The lungs were isolated and immediately frozen in liquid N2. The samples were homogenized in 2 ml of HEPES buffer containing EDTA and phenylmethylsulfonyl fluoride and kept on ice. The homogenates were centrifuged at 1500g for 10 min and 50,000g for 20 min, and the supernatants were recovered. A mouse IL-5 enzyme-linked immunosorbent assay kit (Amersham Pharmacia Biotech, Buckinghamshire, UK) was used to measure the amount of IL-5 in the supernatant.

Eosinophilia Model Induced by Repeated Antigen Exposure in BN Rats. A lung eosinophilia model receiving repeated antigen exposures in rats was established by modifying the method reported by Haczku et al. (1994). The experimental protocol is shown in Fig. 1. BN rats weighing 100 to 120 g (n = 62) were sensitized by an intraperitoneal injection of 1 mg of OA, 20 mg of Alum and Bordetella pertussis (6 × 109 organisms). In OA-control and YM976-treated (single and chronic) groups (n = 56), the exposure to 1% (w/v) OA aerosol (for 10 min) was started 3 days after the intraperitoneal sensitization and was repeated every third day up to a total of seven exposures. Animals in the saline group (n = 6) received saline aerosol as a negative control. Twenty-four hours after the final exposure, the animals were anesthetized with an intraperitoneal injection of pentobarbital sodium (50 mg/kg). To examine the effect on the number of eosinophils in the airways, the lungs were lavaged with 2-ml aliquots of 1 unit/ml heparin-containing saline five times through a polyethylene syringe introduced through the tracheotomy.

The animals were treated as follows: all animals were given Compound administration (once a day) 〇: 0.5% MC 〇 : YM976 Saline,OA YM976 single YM976 chronic

1 mg OA+20 mg alum + Bordetella pertussis, i.p. BAL

Fig. 1. Experimental protocol of chronic infiltration of eosinophils into the lungs induced by repeated inhalation of OA in BN rats. BN rats were sensitized by OA, alum, and B. pertussis. In OA-control and YM976-treated groups, the exposure to 1% OA aerosol was started 3 days after the sensitization and repeated every third day with total of seven exposures. Animals in saline group received saline aerosol. The lungs of all animals were lavaged with saline 24 h after the final exposure to measure the number of eosinophils in the airways. YM976 or vehicle (0.5% MC) was orally administered once a day in the manner indicated in this figure.
YM976 or vehicle (0.5% MC) orally from the first to 20th day once daily, and on the 21st day received YM976 or vehicle (0.5%) 30 min before the final exposure to antigen. Animals in the saline group (n = 6) and the OA group (n = 8) were given vehicle from the first to the 21st day. In the YM976-sensitized group (n = 24), YM976 was administered on the 21st day. In the YM976-chronic group (n = 24), the animals were orally given YM976 from day 1 to 21. The doses of YM976 tested were 0.1, 1, and 10 mg/kg.

**Emetogenic Effects in Conscious Ferrets.** The emetogenic activity of YM976 was examined in ferrets fasted overnight. YM976 was suspended in 0.5% MC and was orally administered in volumes of 3 ml/kg. The number of emetic episodes was recorded for 24 h in each period as follows: from the time of administration to 30 min after administration, and from 1 to 2 h, from 2 to 4 h, from 4 to 8 h, and from 8 to 24 h after administration. The emetogenic effects of test compounds were expressed in terms of incidence of emesis.

**Eosinophilia Model Induced by Antigen in Ferrets.** Male ferrets aged 12 to 14 weeks were sensitized with intraperitoneal injections of OA (20 μg) and Al(OH)₃ (4 mg) on days 1, 8, and 15. Seven days after the final injection (day 22), animals were further sensitized by inhalation of 0.5% (w/v) OA for 5 min/day on days 22 to 26 (five times). Then, on day 29, the animals were challenged by 5% OA inhalation for 30 min. Animals in the saline-control group were challenged by saline inhalation. Five minutes before the each inhalation, pyrilamine, an antihistamine agent, was intraperitoneally administered at a dose of 4 mg/kg to prevent death by anaphylaxis. Twenty-four hours after the challenge (day 30), the ferrets were sacrificed by cutting the cervical blood vessels under chloroform anesthesia. The trachea was cannulated and the lungs were lavaged three times with 50 ml of saline supplemented with heparin (1 unit/ml). BALF was centrifuged (500g for 10 min), and the cell pellet was resuspended in 2 ml of saline.

**Measurement of Total Cell Number and Identification of Cell Differentiation.** Total cell number was counted using a cell counter, Celltac-Quik. Cells were identified and differentiated into eosinophils, neutrophils, and mononuclear cells by the standard morphologic techniques, 300 cells being counted at a magnification of 400×, and the absolute number and percentage of each cell type were determined.

**Data Analysis.** Data were expressed as the mean ± S.E. of eight to nine animals. Differences were considered significant when P < .05. Two-tailed Student’s t test was used for the comparison of the differences between groups. For the difference between the OA-control group and each treatment group (Dunnett’s multiple range test), *P < .05, **P < .01, and ***P < .001. For the difference between the saline and OA groups (Student’s t test), *P < .05, **P < .01, and ***P < .001.

**TABLE 1**

<table>
<thead>
<tr>
<th>Compound</th>
<th>n</th>
<th>ED₅₀</th>
</tr>
</thead>
<tbody>
<tr>
<td>YM976</td>
<td>8–9</td>
<td>1.7 (0.94–6.2)</td>
</tr>
<tr>
<td>Rolipram</td>
<td>8–9</td>
<td>1.2 (0.70–1.8)</td>
</tr>
<tr>
<td>Prednisolone</td>
<td>9</td>
<td>0.67 (0.21–3.6)</td>
</tr>
</tbody>
</table>

**Results**

**Effects of YM976, Rolipram, and Prednisolone on Cell Infiltration Induced by Antigen in BN Rats.** The effects of YM976, rolipram, and prednisolone on cell infiltration were evaluated in sensitized BN rats. In the control animals, the total number of cells infiltrating into the lungs was 1.6 × 10⁶ cells/lung, and the proportions of eosinophils, mononuclear cells, and neutrophils were 62, 27, and 11%, respectively. Thus, it was found that eosinophils were predominant. Orally administered YM976 elicited dose-dependent inhibition of the accumulation of eosinophils in the lungs at an ED₅₀ of 1.7 (95% confidence limits = 0.94–6.2) mg/kg. Rolipram and prednisolone also dose dependently inhibited the eosinophil infiltration into the lungs, at oral ED₅₀ values of 1.2 (0.70–1.8) mg/kg and 0.67 (0.21–3.6) mg/kg, respectively (Fig. 2; Table 1).

**Fig. 2.** Effects of YM976, rolipram, and prednisolone on antigen-induced airway eosinophil infiltration in BN rats. The sensitized rats were exposed to OA aerosol for 15 min, and 24 h later, their lungs were lavaged with heparinized saline five times under anesthesia. Each compound was orally administered 30 min before the exposure. Data are expressed as the mean ± S.E. of eight to nine animals (six to seven animals were used for each group). *P < .05, **P < .01, and ***P < .001 for the difference between the saline and OA groups (Student’s t test). For the difference between the OA group and each treatment group (Dunnett’s multiple range test), *P < .05, **P < .01, and ***P < .001.

**Fig. 3.** Effects of YM976 and prednisolone on antigen-induced eosinophil infiltration into the lungs in C57Black/6 mice. Compounds were orally administered 30 min before and 3 h after every exposure. Data are expressed as the mean ± S.E. of eight to nine animals (saline-control: six animals). *P < .01 for the difference between the saline and OA groups (Student’s t test). For the difference between the OA-control group and each compound treatment group (Dunnett’s multiple range test), *P < .01 and ***P < .001.

**Fig. 4.** Effects of YM976, rolipram, and prednisolone on cell infiltration induced by antigen in Mice. Exposure of the sensitized mice to OA resulted in significant eosinophil infiltration (saline group, 0.2 × 10⁶ cells; OA group, 5.1 × 10⁶ cells) into the lungs. And the new infiltration of mononuclear cells and
neutrophils (<10%) was not caused by the antigen exposure (data not shown). YM976 at oral doses of 1, 3, 10, and 30 mg/kg induced dose-dependent inhibition of eosinophil infiltration (Fig. 3) with an ED$_{50}$ value of 3.6 (1.6–6.5) mg/kg p.o. Prednisolone at oral doses of 1, 3, 10, and 30 mg/kg also suppressed the eosinophil infiltration. The ED$_{50}$ value was 0.70 (0.15–1.5) mg/kg (Table 2).

**Effects of YM976 and Prednisolone on IL-5 Production in the Lungs Induced by Antigen in Mice.** To elucidate the mechanisms involved in the inhibition of eosinophil infiltration by YM976, the effect of YM976 on IL-5 production was examined using the same mouse model as was described above. Eight hours after the final exposure, we measured the amount of IL-5 in the lungs. The amount of IL-5 was considerably higher in mice that inhaled OA than in those that inhaled saline (Fig. 4). YM976 reduced the amount of IL-5 by 0.70 (0.15–1.5) mg/kg (Table 2). Prednisolone also decreased the lung IL-5 content with an ED$_{50}$ value of 0.66 (0.30–1.1) mg/kg p.o.

**Effect of Chronic Administration of YM976 on Eosinophilia in BN Rats.** We assessed the effect of chronic administration of YM976 on another rat model of eosinophilia induced by repeated OA exposure. In this model, repeated exposures to antigen induced a significantly greater eosinophil infiltration into the lungs than saline exposure (4.0 $\times$ 10$^5$ cells/lung versus 0.50 $\times$ 10$^5$ cells/lung, Fig. 5). Using this eosinophilia model, we evaluated the effect of chronic administration of YM976 in comparison with that of a single administration. On chronic administration, YM976 at oral doses of more than 1 mg/kg significantly inhibited the eosinophil infiltration, whereas only a 10-mg/kg dose given as a single administration elicited significant inhibitory effect. The inhibitory ratios of YM976 on chronic 21-day administration of 0.1, 1, and 10 mg/kg were 26, 66, and 102%, respectively (ED$_{50}$ = 0.32 mg/kg), and those on a single administration of the same doses were 12, 37, and 65%, respectively (ED$_{50}$ = 1.4 mg/kg). In neither case did the treatment with YM976 affect body weight gain at any time during the experimental period (data not shown).

**Emetogenicity of YM976 in Ferrets.** The results are shown in Table 3. Ferrets receiving only vehicle showed no emesis. YM976 did not elicit emesis up to 10 mg/kg p.o., but did do so within 30 min after administration at doses of 30 mg/kg and more. At 100 mg/kg, the incidence of emetogenicity was 50%.

**Effect of YM976 on Cell Infiltration Induced by Antigen in Ferrets.** We established a ferret model for lung eosinophilia induced by antigen inhalation. A total of seven exposures to OA of the ferrets sensitized by intraperitoneal injection of antigen produced a significantly greater infiltration of eosinophils into the lungs than when ferrets were exposed to saline (Fig. 6). Twenty-four hours after the final exposure, 17 $\times$ 10$^4$ eosinophils were found in the lungs in the OA-control group, as opposed to 5.8 $\times$ 10$^4$ cells/lung in the saline-control group. At this time, no cell types except the eosinophil were changed by antigen challenge (data not shown). YM976 at oral doses of 1, 3, and 10 mg/kg showed a dose-dependent suppression of eosinophil accumulation in the lung at an ED$_{50}$ of 1.2 (0.0074–2.6) mg/kg.

**TABLE 2**

<table>
<thead>
<tr>
<th>Compound</th>
<th>n</th>
<th>Eosinophil Infiltration</th>
<th>n</th>
<th>IL-5 Production</th>
</tr>
</thead>
<tbody>
<tr>
<td>YM976</td>
<td>9–10</td>
<td>3.6 (1.6–6.5)</td>
<td>10</td>
<td>5.8 (3.6–9.6)</td>
</tr>
<tr>
<td>Prednisolone</td>
<td>10</td>
<td>0.70 (0.15–1.5)</td>
<td>10</td>
<td>0.66 (0.30–1.1)</td>
</tr>
</tbody>
</table>
Table 3

Time course and incidence of emetogenicity of YM976 in ferrets
YM976 (3 to 100 mg/kg) was administered orally, in a suspension with 0.5% MC, to normal male ferrets at a volume of 3 ml/kg. Ferrets showing emesis were observed for 24 h after the administration. The data express the number of ferrets showing emesis in one period. The E/T expresses the incidence of the number of animals showing emesis to the total number of test animals.

<table>
<thead>
<tr>
<th>Dose (mg/kg, p.o.)</th>
<th>n</th>
<th>Time after Administration (h)</th>
<th>Incidence E/T</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0–0.5</td>
<td>1–2</td>
</tr>
<tr>
<td>Vehicle</td>
<td>8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>30</td>
<td>6</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>100</td>
<td>4</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

**Discussion**

The number of eosinophils was markedly higher in asthmatic patients than in healthy subjects (Kirby et al., 1987), and was correlated with the severity of asthma symptoms (Kay, 1985; Wardlaw et al., 1988; Barnes, 1989). Bronchial asthma is characterized by a respiratory inflammation in which pathogenesis eosinophils play a predominant role. Therefore, compounds capable of decreasing the pulmonary infiltration of eosinophils may have potential as novel anti-asthmatic agents. PDE4 inhibitors are reported to suppress eosinophil function in vitro (Soussen et al., 1995) and eosinophil infiltration in vivo (Underwood et al., 1993). It is also reported that the PDE4 inhibitors suppress the induction of cytokines such as IL-1β and tumor necrosis factor-α (Molnar Kimber et al., 1993), and the expression of the adhesion molecules that are required for eosinophil activation and chemotaxis (Pober et al., 1993). Indeed, the PDE4 inhibitor CDP-840 was reported to inhibit allergen-induced late responses in asthmatic subjects (Harbinson et al., 1997).

We first evaluated the effect of YM976 on OA-induced eosinophilia in rats and mice to assess the potential of YM976 as an anti-asthmatic agent. Results showed that YM976 dose-dependently decreased eosinophil infiltration into the lungs. Rolipram also suppressed eosinophilia in a dose-dependent manner in mice. A number of reports on the inhibitory mechanisms of PDE4 inhibitors against eosinophilia have been published (Cohan et al., 1996; Blease et al., 1998; Ohta and Yamashita, 1999). PDE4 inhibitors are considered to inhibit eosinophil activation by down-regulating not only chemotactic/activating factors for eosinophils such as chemokines (Banner et al., 1996; Santamaria et al., 1997) but also by increasing microvascular permeability (Adamson et al., 1998) and the expression of selectin and integrin adhesion molecules on either the endothelium or the eosinophil itself (Blease et al., 1998). YM976 and rolipram inhibited eosinophil infiltration in the same inhibitory potency as prednisolone in rats (Fig. 2), suggesting that PDE4 inhibitors might become alternative drugs to prednisolone.

IL-5 is mainly involved in the motility of eosinophils and is an attractive target for the treatment of asthma. PDE4 inhibitors are reported to suppress IL-5 production (Foissier et al., 1996). Accordingly, we examined the effect of YM976 on IL-5 production using the same mouse eosinophilia model. It was reported that the IL-5 level peaked at 8 h after OA exposure, and a soluble IL-5 receptor inhibited the eosinophilia observed 24 h after the exposure (Yamaguchi et al., 1994). Thus, we evaluated the IL-5 level at 8 h after the exposure. As Fig. 4 indicates, YM976 suppressed IL-5 production in the lungs in the same range of doses at which it inhibited eosinophilia. This result indicates that the suppressive effect of YM976 on eosinophilia contributes at least partially to IL-5 reduction. In recent clinical studies, however, neutralization of IL-5 resulted in the suppression of eosinophilia but failed to show effects on early asthmatic response, late asthmatic response, and airway hyperreactivity. The improvement in asthma symptoms by PDE4 inhibitors may not be solely due to IL-5 inhibition, and these effects may be due to the activation of residual eosinophils. Indeed, the finding that YM976 suppressed formyl-methionine-leucyl-phenylalanine-induced leukotriene C4/D4/E4 release from human eosinophils with an IC50 value of 2 nM (M. Aoki, unpublished data) indicates that YM976 also inhibits eosinophil activation.

Because it is reported that the continuous increase in cAMP content can induce the elevation of PDE4 activity (Torphy et al., 1992; Manning et al., 1996), it is theoretically possible that the chronic administration of PDE 4 inhibitors might reduce anti-inflammatory activity. We therefore prepared an eosinophilia model induced by repeated antigen exposure to observe the effect of chronic administration of YM976. Seven OA exposures to sensitized BN rats at 3-day
accumulation than a single administration. In ferret experiments, pulmonary eosinophil infiltration was observed, and the inhibitory ratio in chronic administration was greater than that after a single administration. Although the reason why the repeated administration potentiated the inhibitory activity is unknown, the following is a possible explanation. Airway inflammation may be aggravated by repeated antigen exposure, finally reaching a chronic state in which more complicated pathological changes and drug resistance can frequently be observed. Chronic administration of YM976 may inhibit each antigen-induced inflammatory event, and result in the prevention of a decline to chronic inflammation. Thus, a single dose of the compound before the final antigen exposure is slightly weaker than repeated doses in the suppression of eosinophilia in the airways. A second explanation is that chronic administration of YM976 may inhibit eosinophilopoiesis and tissue eosinophil survival. These results suggest that chronic administration of a PDE4 inhibitor may not reduce its anti-inflammatory activity in vivo and may ameliorate chronic airway inflammation in asthmatic patients.

It has been reported that a major adverse effect of PDE4 inhibitors is the induction of emesis (Soussen and Rao, 1997), and this may limit the therapeutic potential of these agents. Thus, a PDE4 inhibitor with little or no emetogenicity has been sought. To clarify the dissociation of YM976 in the treatment of asthma, we evaluated its inhibitory effect on eosinophil infiltration in ferrets. Because no ferret models, however, had been reported in studies of antigen-induced eosinophil infiltration into the lungs, we established an OA-induced eosinophilia model in ferrets. Repeated exposures of the animals to OA induced a considerable accumulation of eosinophils in the lungs. In this model, YM976 inhibited eosinophil accumulation in a dose-dependent manner at an ED50 of 1.2 mg/kg. However, although YM976 caused no emesis up to 10 mg/kg, at 30 mg/kg, it did induce emesis in 33% of the animals. These results indicate a divergence between the antieosinophilic effect of YM976 and its emetic effect. Although the precise mechanism remains to be determined, there are three hypotheses for reduced emetogenicity, namely, affinity for high-affinity rolipram binding sites, PDE4 subtype selectivity, and brain penetration. First, YM976 may have the low affinity for high-affinity rolipram binding sites, which are related to emetogenicity (Duplantier et al., 1996). Second, YM976 may show different selectivity for PDE4 subtypes (A–D). However, the relationship between subtypes and their functions and activities is not clear. Finally, if the emetogenicity of PDE4 inhibitors is related to the central nervous system, the emetogenicity may contribute to brain penetration of the compound. YM976 may have poor brain penetration.

In summary, YM976, a novel PDE4 inhibitor, suppressed antigen-induced eosinophil infiltration into the lungs in mice and rats as potently as rolipram and prednisolone. YM976 also suppressed IL-5 production in the sensitized mouse lung. In another rat model, we showed that the chronic administration of YM976 more potently inhibited eosinophil accumulation than a single administration. In ferret experiments, YM976 strongly inhibited eosinophil infiltration into the lungs at the doses that did not cause emesis. We conclude that YM976 is an effective PDE4 inhibitor. This compound may be considered a promising new drug for use in the treatment of bronchial asthma and is a possible alternative to corticosteroids.

Acknowledgments

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