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Inhibitory effect of F-1322 on allergic eosinophil infiltration in airways

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Abstract

The effects of F-1322 (*N*-[2-[4-(benzhydryloxy)piperidino]ethyl]-3-hydroxy-5-(3-pyridylmethoxy)-2-naphthamide) on antigen-induced eosinophil infiltration and interleukin-5 production in the airways and on in vitro eosinophil migration were investigated. F-1322 (10–30 mg/kg, p.o.) inhibited antigen-induced eosinophil infiltration and interleukin-5 production in the airways of sensitized mice in a dose-dependent manner. Furthermore, F-1322 (0.1–10 μM) prevented the in vitro migration of eosinophils from guinea-pigs and humans induced by recombinant human interleukin-5, platelet-activating factor, and leukotriene B_4 in a concentration-dependent manner. These results indicate that F-1322 has an inhibitory effect on allergic eosinophil infiltration of the airways by preventing both eosinophil migration and interleukin-5 production. These pharmacological profiles suggest that F-1322 will be a useful therapeutic for allergic diseases, especially asthma. © 1998 Elsevier Science B.V.

Keywords: F-1322; Eosinophil migration; Interleukin-5 production

1. Introduction

In bronchial asthma, chronic inflammation of the airways is an important feature that underlies the bronchoconstriction and bronchial hyperresponsiveness of the disease (Frigas and Gleich, 1986; Holgate et al., 1987). The mechanism of the development of bronchial hyperresponsiveness is not clear, although it is clinically well characterized. Although the relationship between airway eosinophilia and hyperresponsiveness is controversial (Gibson et al., 1989), a close link between them in asthmatic patients has been suggested. Activated eosinophils are considered to play an important role in the pathogenesis of asthma. In fact, a significant correlation has been found clinically between the severity of asthma and the number of eosinophils in the airways, and corticosteroid treatment of clinical symptoms in asthmatic patients is accompanied by a decrease in the number of eosinophils in the blood (Horn et al., 1975). Nakajima et al. (1992) have reported that antigen-induced eosinophil infiltration of mouse airways is inhibited by anti-interleukin-5 antibody. Therefore, breaking off the interaction between interleukin-5 and eosinophils and/or preventing the migration of eosinophils will result in inhibition of eosinophil infiltration into the tissue and thus will contribute to curing allergic diseases including asthma.

F-1322 (*N*-[2-[4-(benzhydryloxy)piperidino]ethyl]-3-hydroxy-5-(3-pyridylmethoxy)-2-naphthamide) is a newly developed compound which potently inhibits both thromboxane A_2 synthetase (IC_{50} value: 1.7×10^{-8} M) and 5-lipoxygenase activities (IC_{50} value: 9×10^{-7} M) and possesses histamine antagonist activity (pD'_2 value: 7.18) (Lee et al., 1995). In order to assess the therapeutic benefits of F-1322 in asthma, we investigated the effect of F-1322 on antigen-induced eosinophil infiltration into the mouse trachea and interleukin-5 levels in mouse bronchoalveolar lavage fluid and on the in vitro migration of eosinophils from guinea-pigs and humans.

2. Materials and methods

2.1. Reagents

Ovalbumin and bovine serum albumin were purchased from Sigma Chemical (St. Louis, MO). Platelet-activating factor (PAF) (Cascade, Reading, Berkshire), leukotriene

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B_4 (Cayman Chemical, Ann Arbor, MI), and recombinant human interleukin-5 (R and D Systems, Minneapolis, MN) were used. RPMI1640 (Nissui Pharmaceutical, Tokyo) was supplemented with 2 mM L-glutamine, 27 mM HEPES, and 2 g/l sodium bicarbonate.

2.2. Drugs

F-1322 (Lee et al., 1995) was synthesized by Pharmaceuticals Research Laboratories of Fujirebio (Tokyo). Dexamethasone was purchased from Wako (Osaka). F-1322 and dexamethasone were administered orally as a suspension of 5% arabic gum solution. For the in vitro experiment, F-1322 was first dissolved in dimethylsulfoxide (DMSO) and polyoxyethylene hydrogenated castor oil 60 in ethanol and thereafter diluted with RPMI1640 containing 0.4% bovine serum albumin (final concentration of DMSO and polyoxyethylene hydrogenated castor oil 60 in ethanol was less than 0.01%, respectively).

2.3. Antigen-induced eosinophil infiltration into mouse trachea

Eosinophil infiltration into the trachea was induced by the inhalation of antigen in sensitized mice according to the methods described by Nakajima et al. (1992). Briefly, 6-week-old female BALB/c mice (Japan SLC, Sizuoka) were immunized intraperitoneally twice with 1 μg of ovalbumin in 4 mg of aluminum hydroxide with a 2-week interval between injections. Fourteen days after the second immunization, the sensitized mice were individually placed in a chamber and challenged by inhalation of aerosolized ovalbumin (100 mg/ml dissolved in saline) or saline for 20 min, delivered by an ultrasonic nebulizer (DeVilbiss, Somerset, PA). Twenty-four hours after inhalation, the mice were anesthetized by an intraperitoneal injection of pentobarbital sodium (100 mg/kg) and the tracheas were excised. After the tracheas were fixed in 10% formalin, the specimens were embedded in paraffin, sectioned into 3-μm thick slices, and stained with Luna solution. The number of eosinophils in the submucosal tissue of the trachea was counted, using a light microscope at a magnification of $\times$400, and expressed as the number of eosinophils per length of the basement membrane of the trachea. F-1322 (10–30 mg/kg), dexamethasone (10 mg/kg) or vehicle (5% arabic gum alone) was administered orally once a day for 5 days before the inhaled antigen challenge.

2.4. Antigen-induced interleukin-5 production in mouse bronchoalveolar lavage fluid

Mice were immunized and challenged with ovalbumin by the same procedure described above. Twenty-four hours after the inhaled antigen challenge, the mice were anesthetized with pentobarbital and the tracheas were cannulated with a disposable catheter (3 Fr. size; Atom, Tokyo). The airway lumen was washed 3 times with phosphate buffered saline without Ca^{2+} and Mg^{2+} (PBS(−)). The bronchoalveolar lavage fluid was pooled in a 1.5 ml plastic tube and then centrifuged at $400 \times g$ for 10 min at 4°C. The supernatant was collected and the amount of interleukin-5 was measured with a mouse interleukin-5 enzyme-linked immunosorbent assay kit (Endogen, Cambridge, MA). F-1322, dexamethasone or vehicle was administered orally 2 h before the antigen challenge.

2.5. Purification of guinea-pig eosinophils

One milligram of polymyxin B sulfate (Wako) was injected intraperitoneally into 4-week-old male Hartley guinea pigs (Japan SLC) weekly for 8 weeks. One week after the last injection, the peritoneal cavity was lavaged with PBS(−) supplemented with 200 IU heparin sodium (Wako), and the lavage fluids were then centrifuged at $400 \times g$ for 10 min at 4°C. The sediment was resuspended in 1.080 g/ml Percoll (Pharmacia Biotech, AB, Uppsala) and layered onto discontinuous gradients of Percoll (1.090, 1.095, and 1.120 g/ml). After centrifugation at $1200 \times g$ for 20 min at 20°C, the eosinophils were recovered from the 1.095/1.120 g/ml interface and washed twice with PBS(−) by centrifugation. The eosinophil numbers were adjusted to 4.4×10^6/ml in RPMI1640 containing 0.4% bovine serum albumin. The cell purity and viability were determined by using Diff-Quick stain and the Trypan blue exclusion test, respectively. Eosinophil preparations with a purity and viability greater than 95% were used for the experiment.

2.6. Purification of human blood eosinophils

Heparinized venous blood was obtained from healthy donors. The granulocyte fraction including eosinophils was separated by a combination of dextran sedimentation and centrifugation through Ficoll-Paque (specific gravity = 1.077 g/ml: Pharmacia Biotech) (Ochiai et al., 1991). Thereafter, eosinophils were isolated by immunomagnetic negative selection, using anti-CD16 immunomagnetic beads (Advanced Magunetics, Cambridge, MA) (Hansel et al., 1989). The eosinophil numbers were adjusted to 2.2×10^6/ml in RPMI1640 containing 0.4% bovine serum albumin. The cell purity and viability were determined by using Diff-Quick stain and the Trypan blue exclusion test, respectively. Eosinophil preparations with a purity and viability greater than 95% were used for the experiment.

2.7. Eosinophil migration assay

The eosinophil migration assay was performed according to the modified Boyden chamber technique, using a 48-well microchemotaxis chamber (Neuro Probe, Cabin John, MD) as previously described (Kay, 1970; Coëffier et al., 1991). In brief, PAF, leukotriene B_4 or recombinant human interleukin-5 was diluted in RPMI1640 containing 0.05% bovine serum albumin and dispensed into the lower wells. The eosinophils were treated with each test compound or vehicle for 5 min at 37°C (final cell concentra-

tion: 4×10^6/ml and 2.2×10^6/ml for guinea pigs and humans, respectively) and were then added to the upper chamber, which was separated from the lower well by a nitrocellulose filter (3 μm pore size) (Sartorius AG, Gettingen). Then, migration was induced by chemoattractants at 37°C in a humidified atmosphere of 95% air and 5% CO_2 for 2 h. After the incubation, the filters were fixed with a solution containing 50% saturated $HgCl_2$ and 50% ethanol. Thereafter, they were stained with Carrazi's hematoxylin solution and with chromotrope 2R. The number of cells that had migrated 60 μm or more was counted in 10 high-power fields with a light microscope at a magnification of $\times$400.

2.8. Statistics

Data are summarized as means $\pm$ S.E. The statistical analysis of the results was performed by analysis of variance, using Dunnett's multiple comparison test (SAS system ver. 6; SAS institute Japan, Tokyo), and P values < 0.05 were considered significant. The IC_{50} values were estimated by linear regression analysis of the concentration–response curves.

3. Results

3.1. Effect on antigen-induced eosinophil infiltration into mouse trachea

In the control group (vehicle administered), eosinophil infiltration into the trachea of sensitized mice was induced by antigen inhalation at 24 h (6.3 ± 0.6 eosinophils/mm, mean $\pm$ S.E., $n = 6$), but not by saline challenge.

F-1322 inhibited the antigen-induced eosinophil infiltration in the trachea, the percent inhibition being 52.5% at a dose of 30 mg/kg p.o. ($n = 5$, $P < 0.05$) (Fig. 1A). Dexamethasone also significantly inhibited eosinophil infiltration by 80.7% ($n = 5$, $P < 0.01$) (Fig. 1A).

3.2. Effect on antigen-induced interleukin-5 production in bronchoalveolar lavage fluid

In the control group, the amount of interleukin-5 produced in bronchoalveolar lavage fluid of sensitized mice 24 h after saline inhalation was lower than the detectable limit (< 5.0 pg/ml). Twenty-four hours after the antigen challenge, the amount of interleukin-5 was 157.5 ± 15.2 pg/ml ($n = 10$).

F-1322 inhibited the antigen-induced interleukin-5 production in bronchoalveolar lavage fluid of sensitized mice in a dose-dependent manner. The percent inhibition was 45.4% and 56.4% ($n = 10$, $P < 0.05$) at doses of 10 and 30 mg/kg p.o., respectively (Fig. 1B). Dexamethasone also significantly inhibited interleukin-5 production by 87.0% ($n = 10$, $P < 0.01$) (Fig. 1B).

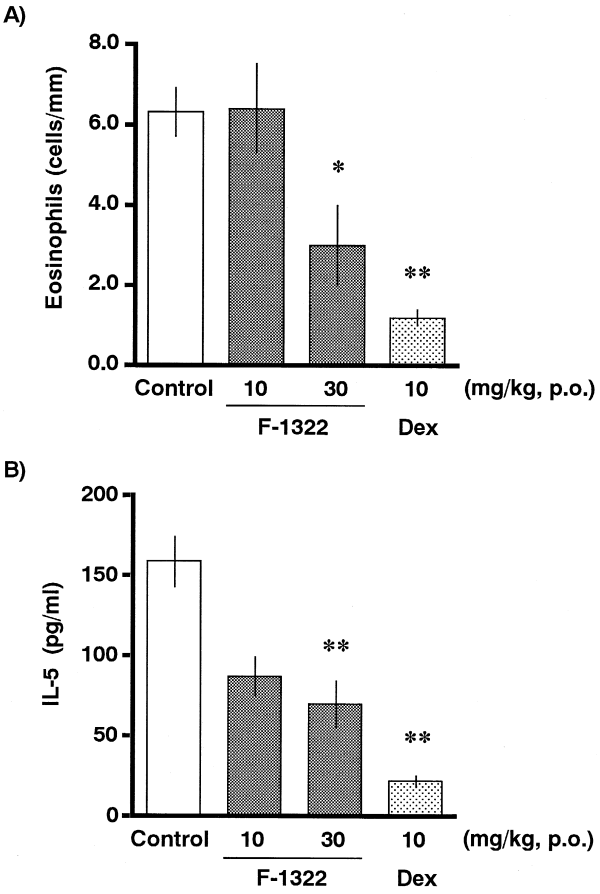

Fig. 1. Effects of F-1322 on antigen-induced eosinophil infiltration into the mouse trachea (A) and on antigen-induced interleukin-5 production in mouse bronchoalveolar lavage fluid (B). (A) F-1322 was administered orally once a day for 5 days. The antigen challenge was performed 2 h after the last administration, and eosinophil infiltration into the trachea was examined at 24 h. Dex; dexamethasone. (B) Drugs were administered 2 h before the antigen challenge, and interleukin-5 production in bronchoalveolar lavage fluid was examined at 24 h. Each column represents the mean $\pm$ S.E. for 5 to 10 animals. $^{*}P < 0.05$, $^{**}P < 0.01$, significantly different from the mean value of the control.

3.3. Effect on in vitro guinea-pig eosinophil migration

The in vitro migration of guinea-pig eosinophils significantly increased in response to PAF at 10^{-8} M (221.7 ± 18.9 cells/10 high-power fields, $n = 5$), leukotriene B_4 at 10^{-9} M (156.3 ± 20.0, $n = 3$), and recombinant human interleukin-5 at 300 ng/ml (222.5 ± 6.0, $n = 3$). The basal migration was 11.1 ± 6.9 cells/10 high-power fields, 11.3 ± 1.9, and 26.8 ± 0.3, respectively.

As shown in Fig. 2, F-1322 prevented the eosinophil migration in response to these three chemoattractants in a concentration-dependent manner. At 10 μM of F-1322, the inhibitory effect on eosinophil migration in response to PAF, leukotriene B_4 and recombinant human interleukin-5 was 81.1% ($n = 5$), 95.7% ($n = 3$), and 93.6% ($n = 3$),

respectively, and the IC_{50} values were 5.0, 2.9, and 3.6 μM, respectively.

3.4. Effect on in vitro human eosinophil migration

The in vitro migration of human eosinophils increased in response to PAF at 10^{-8} M (293.7 ± 6.5 cells/10 high-power fields, $n = 3$) and recombinant human interleukin-5 at 300 ng/ml (179.3 ± 43.9, $n = 3$). The basal migration was 23.0 ± 7.6 cells/10 high-power fields and 7.0 ± 1.7, respectively.

As shown in Fig. 3, F-1322 prevented the eosinophil migration in response to these two chemoattractants in a concentration-dependent manner. At 10 μM of F-1322, the inhibitory effect on eosinophil migration in response to PAF and recombinant human interleukin-5 was 75.5% ($n = 3$) and 81.1% ($n = 3$), respectively, and the IC_{50} values were 0.48 and 0.76 μM, respectively.

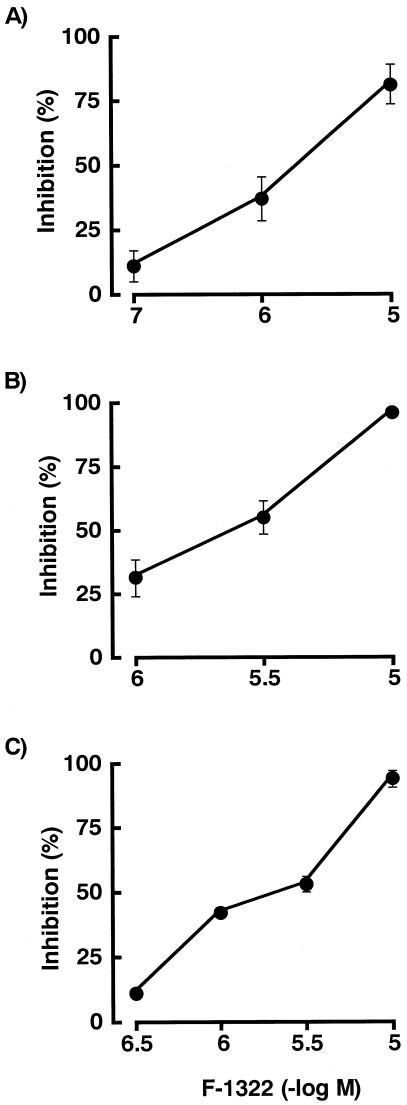

Fig. 2. Effect of F-1322 on the in vitro migration of guinea-pig eosinophils induced by PAF (10^{-8} M) (A), leukotriene B_4 (10^{-9} M) (B) or recombinant human interleukin-5 (300 ng/ml) (C). The guinea-pig eosinophils were incubated with F-1322 (0.1–10 μM) for 5 min at 37°C. Then, the eosinophil migration assay was performed as described in Section 2.

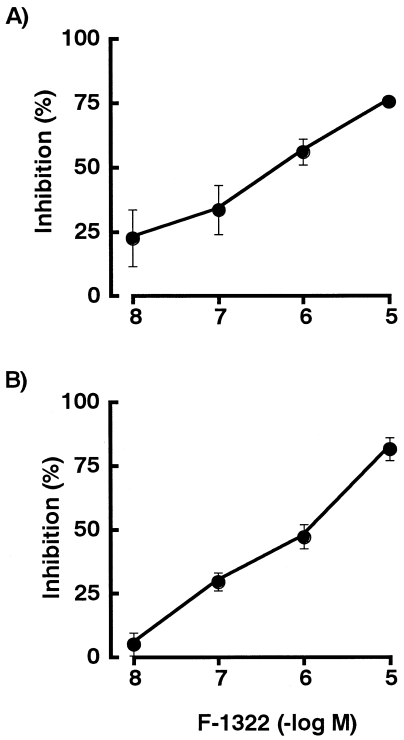

Fig. 3. Effect of F-1322 on the in vitro migration of human eosinophils induced by PAF (10^{-8} M) (A) or recombinant human interleukin-5 (300 ng/ml) (B). The human eosinophils were incubated with F-1322 (0.01–10 μM) for 5 min at 37°C. Then, the eosinophil migration assay was performed as described in Section 2.

4. Discussion

It is well established that airway hyperreactivity and pulmonary eosinophil accumulation are essential characteristics of the pathology of asthma (Frigas and Gleich, 1986; Holgate et al., 1987). Recently, it has been shown that interleukin-5 acts not only as a chemoattractant, but also as an activator of eosinophils, because interleukin-5 regulates eosinophil proliferation and differentiation, enhances the response to other chemoattractants, and prolongs their survival (Yamaguchi et al., 1988a,b; Coëffier et al., 1991).

Nakajima et al. (1992) developed a murine model of antigen-induced eosinophil accumulation in the airways and this eosinophil recruitment was mediated by interleukin-5. In the present study, F-1322 prevented antigen-induced eosinophil infiltration into the mouse trachea (Fig. 1A). F-1322 also significantly decreased antigen-induced interleukin-5 production in mouse bronchoalveolar lavage fluid (Fig. 1B). Dexamethasone, which was used as a

reference compound, also inhibited both antigen-induced eosinophil infiltration and interleukin-5 production in sensitized mice more potently than did F-1322 (Fig. 1A and B). These results suggested that the inhibitory effect of F-1322 on antigen-induced eosinophil infiltration may be attributable to prevention of interleukin-5 production. Further research is planned to study the mechanisms of the inhibitory effect on interleukin-5 production.

We also performed eosinophil migration assays, using guinea-pig and human eosinophils, to examine the effect of F-1322. In both guinea-pig and human preparations, F-1322 prevented in vitro eosinophil migration in a concentration-dependent manner (Figs. 2 and 3). Therefore, the inhibitory effect of F-1322 on eosinophil migration may also be responsible for the inhibition of antigen-induced eosinophil infiltration in the mouse airways.

Thromboxane A_2 synthetase inhibitors such as CS-518 (sodium 2-(1-imidazolylmethyl)-4,5-dihydrobenzo[b] thiophene-6-carboxylate) increase the production of prostaglandins and inhibit eosinophil chemotaxis induced by PAF (Itoh et al., 1993). It has also been shown that the elevated intracellular cAMP levels induced by prostaglandins prevent eosinophil migration (Kaneko et al., 1995). It has also been reported that 5-lipoxygenase blockade inhibits eosinophil chemotaxis in guinea-pigs (Muñoz et al., 1997). Since F-1322 has potent inhibitory effects on both thromboxane A_2 synthetase and 5-lipoxygenase (Lee et al., 1995), the inhibitory effect of F-1322 on eosinophil migration may be partly explained by blockade of these enzymes. However, it has been shown that high concentrations of prostaglandin E_2 and cAMP inhibit interleukin-2 production, but not interleukin-5 production (Betz and Fox, 1991). Thus, further investigations are needed to clarify the inhibitory mechanisms on eosinophil migration and interleukin-5 production.

In summary, we have shown that F-1322 prevents antigen-induced eosinophil infiltration into the airways and that this effect may be attributable to the inhibition of both eosinophil migration and interleukin-5 production. These pharmacological profiles suggest that F-1322 will be a useful therapeutic for allergic diseases, especially asthma.

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