Suppressive Effects of Y-24180, a Long-Acting Antagonist for Platelet-Activating Factor, on Allergic Pulmonary Eosinophilia in Mice

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ABSTRACT—We studied the effects of (+)-4-(2-chlorophenyl)-2-[2-(4-isobutylphenyl)ethyl]-6,9-dimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepine (Y-24180), a long-acting antagonist for platelet-activating factor (PAF), on antigen-induced eosinophil infiltration and interleukin-5 (IL-5) release in the bronchoalveolar lavage fluid (BALF) of mice. Mice actively sensitized with ovalbumin (OA) were challenged by injecting intratracheally OA 3 times every fourth day. Both the number of eosinophils and level of IL-5 were significantly increased in the BALF 24 hr after the last OA challenge. Either Y-24180 or prednisolone was orally administered once a day for 10 days beginning one day before the first OA challenge. WEB2086, another PAF antagonist, was orally administered once or twice a day for 10 days. Y-24180 (0.3–3 mg/kg) suppressed the eosinophil infiltration in a dose-dependent manner and suppressed the IL-5 release at the highest dosage. Prednisolone (10 mg/kg) significantly suppressed both the eosinophil infiltration and IL-5 release. In contrast, WEB2086 affected neither the eosinophil infiltration nor IL-5 release when administered once a day (10–100 mg/kg/day). This drug never affected the IL-5 release but significantly suppressed eosinophil infiltration even when administered twice a day (30–200 mg/kg/day). These results indicate that the suppressive effect of Y-24180 on allergic pulmonary eosinophilia is due to not only to its long-lasting PAF-antagonism but also due to its suppressive effect on IL-5 release.

Keywords: Y-24180, Platelet-activating factor (PAF)-receptor antagonist, Eosinophil, Interleukin-5

Infiltration of inflammatory cells such as eosinophils and lymphocytes into the airway mucosa and lumen is one of the dominant pathological features seen in bronchial asthma (1–3). Epithelial damage ensues after infiltration of inflammatory cells. Epithelial damage may play an important role in airway hyperresponsiveness in chronic asthma (4). This damage may be induced by major basic protein (MBP), eosinophil cationic protein (ECP) and eosinophil peroxidase (EPO), each derived from eosinophils. Eosinophils migrate into an inflamed region through the activation by various chemoattractants. Platelet-activating factor (PAF) has been shown to be a potent chemoattractant for eosinophils (5). (+)-4-(2-Chlorophenyl)-2-[2-(4-isobutylphenyl)ethyl]-6,9-dimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepine (Y-24180) is a synthesized compound that showed potent PAF antagonism (6–8). This compound suppresses various PAF-induced reactions in vitro and in vivo. For example, Y-24180 suppresses PAF-induced platelet aggregation in rabbit platelet-rich plasma at a concentration of 3.84 nM (IC₅₀) (6), PAF-induced lethality in mice at an oral dose of 0.022 mg/kg (ED₅₀) (7), and the late asthmatic response in guinea pigs at an oral dose of 1 mg/kg (8). Y-24180 also suppresses antigen-induced pulmonary eosinophilia in guinea pigs (8).

Recently, interleukin-5 (IL-5) has been shown to play a crucial role in pulmonary eosinophilia (9–12). Anti-IL-5 monoclonal antibody (anti-IL-5 mAb) suppresses antigen-induced eosinophilia in mice (9–11) and guinea pigs (12). These interesting findings have stimulator researchers to investigate the function of antiasthmatic compounds in IL-5 production and its release.

The aim of this paper is to test the effects of Y-24180 on allergic eosinophilia and IL-5 release in the lungs of ovalbumin (OA)-sensitized mice and to compare the effects with those of WEB2086, another PAF antagonist, and prednisolone.

MATERIALS AND METHODS

Animals
Six-week-old, male BALB/c mice (Charles River
Japan, Kanagawa) were used. The animals were housed at constant temperature (23±2°C) and relative humidity (55±5%) and were allowed free access to food and water.

**Drugs and materials**

Y-24180 and WEB2086 (apafant) were synthesized at our laboratories. Prednisolone was purchased from Nippon UCLAFL K.K. (Tokyo). These compounds were suspended in 0.5% hydroxypropyl methylcellulose as a vehicle. OA (Grade V) and pentobarbital sodium (Nembutal) were purchased from Sigma Chemical Co. (St. Louis, MO, USA) and Abbott Laboratories (North Chicago, IL, USA), respectively.

**Sensitization and challenge**

Sensitization and challenge were carried out according to the method of Nagai et al. (11), with minor modifications. Briefly, mice were actively sensitized with intraperitoneal injection of 10 μg of OA and 1 mg of Al(OH)$_3$ gel dissolved in 0.5 ml of saline twice on the first day and days 12–14. From days 8–11 after the second sensitization, mice were challenged 3 times every fourth day by intratracheal injection of 1 μg of OA dissolved in 50 μl of saline (Fig. 1).

**Evaluation of the effects of drugs**

Y-24180, WEB2086, and prednisolone were orally administered once a day for 10 days beginning one day before the first OA challenge (single daily dose paradigm). Additionally, WEB2086 was orally administered twice a day at intervals of 12 hr for 10 days (twice daily dose paradigm). The paradigm of drug administration was illustrated in relation to OA challenges (Fig. 2).

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**Fig. 1.** Schedule for sensitization and antigen challenge. OA: ovalbumin, i.p.: intraperitoneal injection, i.t.: intratracheal injection.

**Fig. 2.** Schedules for drug treatment. ●: single daily treatment; the drug to be tested was orally given once a day for 10 days before the first OA challenge. ▲: twice daily treatment; the drug was orally given twice a day at intervals of 12 hr for 10 days before the first OA challenge. On the day of OA challenge, these drugs were given 1 hr prior to the respective challenge. BALF: bronchoalveolar lavage fluid.
**Bronchoalveolar lavage**

The animals were sacrificed by an intraperitoneal injection of pentobarbital sodium (160 mg/kg) 24 hr after the last OA challenge. The trachea was cannulated, and the closed airway lumen was washed 4 times with 1 ml of saline containing 0.1% of bovine serum albumin (Grade V, Sigma Chemical Co.) pre-warmed at 37°C. The bronchoalveolar lavage fluid (BALF) was centrifuged at 4°C for 10 min (150 x g) to separate it into the supernatant and cell pellet. The supernatant was used for the measurement of IL-5 level. The cell pellet was suspended in 1 ml of saline. The cell suspension was used for eosinophil counts.

**Measurement of the number of eosinophils in the BALF**

Total cells were calculated by using a Burker-Turk’s counting chamber after the Turk’s staining. Eosinophils, neutrophils, macrophages and lymphocytes were calculated after centrifugation in Cytospin 3™ (Shandon Southern Instruments, Pittsburgh, PA, USA) and staining with May-Giemsa stain.

**Measurement of IL-5 released in the BALF**

The level of IL-5 was measured with an enzyme linked immunosolvent assay kit (mouse IL-5 ELISA system; Amersham International plc, Buckinghamshire, England).
Statistics
Each result is shown as the mean±S.E. The statistical significance was evaluated by the Dunnett method. A value of P<0.05 was considered to be significant.

RESULTS

Effects of drugs on the allergic infiltration of eosinophils in the airway
The effects of single daily treatments with Y-24180, WEB2086 and prednisolone on the OA-induced increase in the number of eosinophils in the BALF are shown in Fig. 3. The number of eosinophils in the BALF of OA-sensitized mice were significantly increased 24 hr after the last OA challenge. Y-24180 significantly suppressed the eosinophil infiltration by 35%, 61% and 81% at doses of 0.3, 1 and 3 mg/kg, respectively. Prednisolone strongly suppressed the infiltration by 91% at a dose of 10 mg/kg. In contrast, WEB2086 was ineffective even at the highest dose of 100 mg/kg. The effects of twice daily treatment with WEB2086 on the OA-induced increase in the number of eosinophils in the BALF are shown in Fig. 4. This paradigm of treatment (30–200 mg/kg/day) suppressed the eosinophil infiltration by around 60%. Thus, Y-24180 seemed to be as effective as prednisolone and to have a considerably greater efficacy on this allergic eosinophilia.

**Fig. 5.** Effects of single daily treatment with Y-24180, WEB2086 or prednisolone on IL-5 release in the BALF of OA-sensitized mice 24 hr after the last OA challenge. IL-5 was measured by ELISA. Each column represents the mean±S.E. of 14–16 animals; *P<0.05, **P<0.01, significantly different from the OA control. Values in parentheses indicate % inhibition.

**Fig. 6.** Effects of twice daily treatment with WEB2086 on IL-5 release in the BALF of OA-sensitized mice 24 hr after the last OA challenge. IL-5 was measured by ELISA. Each column represents the mean±S.E. of 8 animals; **P<0.01, significantly different from the OA control. Values in parentheses indicate % inhibition.
than WEB2086.

**Effects of drugs on the allergic release of IL-5 in the airway**

The level of IL-5 in the BALF of the OA control mice were significantly increased after the last OA challenge (Fig. 5). Single daily treatment with the highest dose of Y-24180 (3 mg/kg) or with prednisolone (10 mg/kg) significantly suppressed the allergic release of IL-5 by 51% and 54%, respectively (Fig. 5). However, neither single nor twice daily treatment with WEB2086 was effective against the allergic release of IL-5 (Figs. 5 and 6).

**DISCUSSION**

In this paper, the effects of Y-24180 on allergic pulmonary eosinophilia and IL-5 release were studied by analyzing the BALF obtained from OA-sensitized mice. Eosinophils are the predominant type of leukocytes found at the locus of inflammation in allergic diseases. In addition, they may contribute to airway hyperresponsiveness by releasing cytotoxic proteins such as MBP, ECP and EPO (4) or lipid mediators including leukotriene (LT) C4 (13) and PAF (14). In this study, we found a large number of eosinophils accumulated in the BALF of OA-sensitized mice 24 hr after the last OA challenge (Figs. 3 and 4).

Single daily treatment with Y-24180 (0.3–3 mg/kg) significantly suppressed the allergic infiltration of eosinophils in the airway (Fig. 3). However, similar treatment with WEB2086 was ineffective even at the highest dose of 100 mg/kg. In mice, PAF-induced mortality was suppressed by Y-24180 and WEB2086 with ED50 values of 0.022 and 1.42 mg/kg, respectively (7). This indicates that Y-24180 is approximately 60 times more potent than WEB2086. Therefore, the single daily dose of WEB2086 used in the present study would be sufficient for its PAF-antagonism. The inhibition of PAF-induced bronchoconstriction in guinea pigs by WEB2086 rapidly diminishes at 3 hr after its administration (15). In contrast, the inhibition by Y-24180 seems to last more than 12 hr (15). Thus, the short duration of WEB2086 may probably explain the lack of effectiveness in the single daily treatment. In fact, WEB2086 was found to be effective in suppressing the airway infiltration of eosinophils by twice daily treatment at the lowest dose of 15 mg/kg (Fig. 4). The result suggests that PAF plays an important role in the allergic pulmonary eosinophilia. Single daily treatment with prednisolone strongly suppressed the eosinophil infiltration (Fig. 3). Since several studies have shown that steroids suppress allergic eosinophilia (16–18), our results are consistent with these observations.

Recently, it has been known that IL-5 plays an important role in the development of eosinophilia (9–12). Single daily treatment with the highest dose of Y-24180 (3 mg/kg) significantly suppressed the allergic release of IL-5 in the airway, but single or twice daily treatment of WEB2086 never suppressed it (Figs. 5 and 6). Thus, the suppression of IL-5 release by Y-24180 seems not to be associated with PAF antagonism. In our model, the highest dose of Y-24180 also suppressed the allergic increase in lymphocytes in the BALF (data not shown). The suppression of IL-5 release thus may be due to a reduced emigration of IL-5 producing cells such as helper T lymphocytes into the airway. Prednisolone significantly suppressed the allergic release of IL-5. The suppression of eosinophil emigration by prednisolone would be probably due to the suppression of the IL-5 production of helper T lymphocytes (19). Therefore, it may be important to examine whether Y-24180 directly suppress the IL-5 production by helper T lymphocytes.

Single daily treatment of Y-24180 (3 mg/kg) suppressed the airway infiltration of eosinophils more effectively compared with twice daily treatment of WEB2086 (Figs. 3 and 4). Thus, the strong suppression of eosinophil emigration by Y-24180 should be due to not only PAF-antagonism but also the suppression of IL-5 release.

In conclusion, single daily treatment with Y-24180 (0.3–3 mg/kg) as well as twice daily treatment with WEB2086 (30–200 mg/kg/day) significantly suppressed the eosinophil infiltration in the airway of OA-sensitized mice. These effects seem to be due to long-lasting PAF-antagonism. Moreover, the highest dose of Y-24180 more strongly suppressed the eosinophil infiltration than WEB2086. This additional effect may be due to the suppression of IL-5 release.

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