Effect of Y-24180, a Platelet-Activating Factor-Receptor Antagonist, on the Antigen-Induced Airway Microvascular Leakage in Guinea Pigs

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Received June 28, 1996 Accepted August 7, 1996

ABSTRACT—The inhibitory effect of Y-24180 ((±)-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) on platelet-activating factor (PAF)- or antigen-induced airway microvascular leakage was studied in guinea pigs by oral administration. The tissue content of extravasated Evans blue dye was used as an index of plasma exudation in the trachea, main bronchi, central intrapulmonary airways and peripheral intrapulmonary airways. In all of these tissues, Y-24180 potently inhibited the leakage induced by PAF. The ED50 value of Y-24180 determined in each of the tissues was approximately 0.02 mg/kg, demonstrating that the inhibitory potency of Y-24180 is 4-6 times that of WEB 2086, another PAF antagonist. Even at a dose of 10 mg/kg, however, Y-24180 showed no inhibitory effect on the leakage induced by leukotriene (LT) D4, histamine or bradykinin. In the antigen-induced model of guinea pigs sensitized with aerosolized ovalbumin, Y-24180 (0.1-10 mg/kg) and WEB 2086 (1-100 mg/kg) potently inhibited the microvascular leakage in all of the examined airway tissues except for the trachea. At 1-100 mg/kg, however, both ONO-1078, an LT-receptor antagonist, and OKY-046, a thromboxane A2 synthetase inhibitor, prevented partially but not significantly the antigen-induced leakage. These results provide evidence that endogenous PAF partially mediates the antigen-induced airway microvascular leakage in guinea pigs.

Keywords: Y-24180, Platelet-activating factor-receptor antagonist, Airway microvascular leakage, Antigen

Platelet-activating factor (PAF) is one of the most potent inflammatory mediators showing a wide variety of biological actions including airway microvascular leakage (1, 2), bronchoconstriction (3, 4), airway hyperresponsiveness (5, 6) and chemoattraction and activation of inflammatory cells such as neutrophils and eosinophils (7, 8). As a result, these findings have raised the possibility that PAF may play an important role in asthma (9, 10). Thus, PAF-receptor antagonists should be novel therapeutic agents for asthma.

The airway hyperresponsiveness is one of the most representative features of asthma (11), and its pathogenesis is linked to the airway inflammation (12). Thus, an increase of the airway microvascular leakage is a key feature of inflammation that is followed by mucosal and submucosal edema, airway narrowing, and airway hyperresponsiveness. Inhibition of the leakage would therefore be essential for the treatment of bronchial asthma. Recently, several PAF-receptor antagonists have been reported to be effective in the airway microvascular leakage induced by PAF (2, 13). In addition, there are a few reports dealing with the effect of PAF-receptor antagonists on the leakage induced by antigens (13, 14).

As shown in our early papers, Y-24180 is a specific and long-acting PAF-receptor antagonist (15, 16), inhibits the vascular leakage induced by PAF in rat skin (17), and also prevents the late asthmatic response and eosinophil recruitment into the airway induced by antigen in guinea pigs (18). In a clinical study, Hozawa et al. (19) reported that Y-24180 can reduce bronchial hyperresponsiveness in patients with asthma. Therefore, the purposes of this study are to investigate the effect of Y-24180 on PAF- or antigen-induced airway microvascular leakage in guinea pigs and to determine the contribution of PAF and the other mediators to the antigen-induced leakage by using WEB 2086 (20), another PAF-receptor antagonist; OKY-046 (21) to inhibit the production of thromboxane (TX) A2; and ONO-1078 (22) to antagonize leukotriene (LT) D4 receptors.
MATERIALS AND METHODS

Animals

Male Hartley guinea pigs weighing 380–580 g were used. The male guinea pigs were purchased from Japan SLC, Inc. (Shizuoka), and were housed at a constant temperature (23 ± 2°C) and relative humidity (55 ± 5%). They were allowed free access to food and water.

Materials

Y-24180 ((±)-4-[2-(2-chlorophenyl)-2-[4-(2-isobutylphenyl)ethyl]-6,9-dimethyl-6H-thieno[3,2-f][1,2,4]triazolo-[4,3-a][1,4]diazepine), WEB 2086 (apafant), ONO-1078 (pronlukast hydrate) and ODY-046 (ozagrel hydrochloride) were synthesized in the chemical division of our research laboratories and were used as the test compounds. Other reagents were purchased from the following sources: Evans blue dye (Tokyo Chemical Industry Co., Ltd., Tokyo); ovalbumin, bradykinin and mepyramine (Sigma Chemical Co., St. Louis, MO, USA); hydroxypropylmethylcellulose (HPMC; 60 SH-4000; Shionetsu Chemical Industry Co., Ltd., Tokyo); heparin sodium (Takeda Chemical Industries Ltd., Osaka); paraformaldehyde (E. Merck, Darmstadt, Germany); urethane (ethyl carbamate; Kishida Chemical Co., Ltd., Osaka); LTD₄ and C₁₆-PAF (Funakoshi Co., Ltd., Tokyo); and histamine (Nacalai Tesque Inc., Kyoto). The test compounds were suspended or dissolved in a 0.5% solution of HPMC for oral administration. Urethane, Evans blue dye, ovalbumin, histamine and mepyramine were dissolved in saline. PAF was dissolved in ethanol, and LTD₄ and bradykinin were dissolved in saline. The solutions of PAF, LTD₄ and bradykinin were stored at −80°C until use, and they were diluted with saline just prior to use. Paraformaldehyde was dissolved in 0.1 M phosphate buffer (pH 7.4).

PAF-induced airway microvascular leakage

The airway microvascular leakage in guinea pigs was quantified by the extravasation of Evans blue dye according to a method based on the procedure reported by Evans et al. (13). On the basis of the data obtained in a previous experiment (15), the guinea pigs were anesthetized with 1.8 g/kg of intraperitoneal urethane at 3 hr after the oral administration of Y-24180 (15 mg/kg). After anesthesia, Evans blue dye was injected into an internal jugular vein. One minute later, LTD₄ (1 µg/kg), histamine (150 µg/kg) or bradykinin (100 µg/kg) were injected intravenously. Five minutes after the injection, the animals were perfused as described above.

Chemical mediators-induced airway microvascular leakage

Guinea pigs were anesthetized with urethane 3 hr after the oral administration of Y-24180 (10 mg/kg). After anesthesia, Evans blue dye was injected into an internal jugular vein. One minute later, LTD₄ (1 µg/kg), histamine (150 µg/kg) or bradykinin (100 µg/kg) were injected intravenously. Five minutes after the injection, the animals were perfused as described above.

Antigen-induced airway microvascular leakage

Sensitization procedure: Guinea pigs were sensitized by the method of Arimura et al. (23). In brief, the guinea pigs were sensitized to ovalbumin twice at interval of 1 week, by inhalation of an aerosolized solution of 1% ovalbumin dissolved in saline. The aerosol of ovalbumin solution was generated with an ultrasonic nebulizer (NE-U12; Omron Co., Ltd., Tokyo) and was administered to guinea pigs by placing them in an exposure chamber for 10 min. The guinea pigs were used for experiments 7–9 days after the second sensitization.

Antigen challenge: The guinea pigs were anesthetized with urethane 3 hr after the oral administration of Y-24180 or at 1 hr after that of the other test compounds. After anesthesia, Evans blue dye was injected into an internal jugular vein. After 1 min, mepyramine (0.1 mg/kg) was injected intravenously, followed 2 min later by ovalbumin (10 mg/kg) or saline. Five minutes after ovalbumin, the animals were perfused as described above.

Assay of tissue content of extravasated Evans blue dye: Evans blue dye was extracted from each of the tissues and measured according to the method of Harada et al. (24). In brief, the tissue was mixed with 2 ml of a medium composed of 7 ml acetone and 3 ml sodium sulphate (0.5% aqueous solution) in a test tube. The tube was left for 24 hr at room temperature with occasional mild shaking. Each preparation was then centrifuged at 1500 × g for 10 min. The amount of the dye in the supernatant was determined spectrophotometrically (MPS-2000; Shimadzu Co., Ltd., Kyoto) at 620 nm and calculated from a standard curve established with known amounts.
of Evans blue dye. The results are expressed as ng dye/mg of wet weight tissue.

Statistical analyses
The reported results represent the mean±S.E. The statistical significance was evaluated by the Dunnett method or Tukey method at P<0.05. The ED50 values and 95% confidence limits were calculated by linear regression analysis.

RESULTS

Effect of Y-24180 on PAF-induced airway microvascular leakage
The intravenously administration of PAF to guinea pigs caused a significant increase in the microvascular leakage in the trachea, main bronchi, CIA and PIA (Fig. 1). In the saline control, mean basal levels of Evans blue dye varied from 13.00 ng/mg in CIA to 22.97 ng/mg in PIA. After an intravenous injection with PAF (100 ng/kg), the amount of Evans blue dye in each tissue increased significantly (P<0.01) in comparison with the saline control; the contents of the dye were 113.32, 179.58, 103.02 and 85.45 ng/mg in the trachea, main bronchi, CIA and PIA, respectively. Thus, the responsiveness to PAF was strongest in the bronchi, and was weakest in the PIA. Y-24180 (0.1 mg/kg) inhibited PAF-induced microvascular leakage in all of the tissues. The ED50 value for each tissue was determined to be around 0.02 mg/kg for Y-24180, much lower than the value for WEB 2086, 0.1 mg/kg (Fig. 1).

![Graph showing effects of Y-24180 and WEB 2086 on microvascular leakage](image)

**Fig. 1.** Effect of Y-24180 and WEB 2086 on airway microvascular leakage induced by PAF in guinea pigs. Y-24180 and WEB 2086 were orally administered 3 or 1 hr before PAF (100 ng/kg, i.v.) injection, respectively. Results are expressed as ng dye/mg of wet weight tissue and are reported as the mean±S.E. (n=6). Significantly different from the PAF control (Dunnett method): *P<0.05, **P<0.01. ED50 values and 95% confidence limits (C.L.) were calculated by linear regression analysis.
Effect of Y-24180 on the airway microvascular leakage induced by chemical mediators other than PAF

The intravenous administration of each chemical mediator (LTD₄, 1 μg/kg; histamine, 150 μg/kg; or bradykinin, 100 μg/kg) caused a significant increase in the microvascular leakage in the trachea, main bronchi, CIA and PIA (Fig. 2). Even at a dose of 10 mg/kg, Y-24180 hardly inhibited the leakage induced by LTD₄, histamine or bradykinin in each tissue (Fig. 2).

Effect of Y-24180 on the antigen-induced airway microvascular leakage

As shown in Fig. 3, the content of Evans blue dye in the challenge control was increased by 702%, 956%, 173% and 974% in the trachea, main bronchi, CIA and PIA, respectively, compared to the corresponding value for each tissue in the saline control. At a dose of more than 0.1 mg/kg, Y-24180 significantly inhibited the antigen-induced microvascular leakage in the CIA and PIA. At a dose of 10 mg/kg, it inhibited the leakage in the main bronchi, while it did not inhibit the leakage in the trachea. In this reaction, WEB 2086 also showed a similar inhibitory effect, but the doses were much larger than those of Y-24180. On the other hand, OKY-046 (1–100 mg/kg) and ONO-1078 (1–100 mg/kg) produced no significant inhibition in any of the examined tissues.

DISCUSSION

First, the intravenously injected PAF was confirmed to induce airway microvascular leakage in guinea pigs, and Y-24180 was ascertained to inhibit significantly this
leakage at low doses. In this evaluation system, WEB 2086 exhibited an effect qualitatively similar to that of Y-24180. The inhibitory potency of WEB 2086 was weaker than that of Y-24180. Such a pharmacological superiority of Y-24180 over WEB 2086 should also be observed in experiments employing PAF-induced animal models, because a similar kind of difference between these compounds has been reported on models other than the guinea pig model (15–17). In subsequent experiments, Y-24180 was ascertained to inhibit neither the airway microvascular leakage induced by LTD₄ nor that by histamine. On the leakage induced by bradykinin, however, Y-24180 was partially effective, although this was not statistically significant. For example, Y-24180 exhibited 33% inhibition in the trachea. This finding is in agreement with that in a previous paper (25) in which the apparent bradykinin-induced leakage in guinea pigs was found to be also partially mediated by PAF, prostaglandins and other substances. All of these results demonstrate that Y-24180 acts as a selective PAF-receptor antagonist on airway microvascular leakage and that PAF is not involved in the airway inflammatory responses mediated by LTD₄ and those by histamine.

Secondly, Y-24180 significantly inhibited the antigen-
induced microvascular leakage in tissues of the guinea pig airway except for the trachea. As a consequence, our data demonstrate that the endogenous production of PAF appears to be an important step in the antigen-induced leakage. In this evaluation system, however, WEB 2086 was already judged to be ineffective at doses in which the compound completely antagonized the PAF-induced leakage in guinea pigs (13). Therefore, PAF has so far been believed to play no important role in the antigen-induced leakage; nevertheless, PAF is identified as one of the most potent mediators of airway inflammation. In the present study, WEB 2086 itself was also ascertained to be effective for the antigen-induced leakage in a similar manner as Y-24180, although the inhibitory potency of WEB 2086 was no more than one tenth that of Y-24180. We can still not sufficiently clarify the reason for such a discrepancy in the effect of WEB 2086. Between the two studies, however, there is a difference in the experimental protocol of sensitizing guinea pigs with antigen. The animals were sensitized with the antigen by intraperitoneal injection without a booster method in the previous study (13), but by inhalation with a booster method in the present study. Such a difference may account for the above-mentioned discrepancy. Actually, Amorim et al. (26) reported that the booster injection during the immunization process shifts the anaphylactic mouse edema from a PAF-independent to a PAF-dependent reaction.

When sensitized guinea pigs were challenged by antigen, the lung resistance was seen to correlate with extravasation of Evans blue dye into the intrapulmonary airways (27). Y-24180 and WEB 2086 markedly attenuated antigen-induced Evans blue dye extravasation in the intrapulmonary airways. The result demonstrates that the involvement of PAF in the antigen-induced airway microvascular leakage was greater in the intrapulmonary airways than in the trachea and main bronchi. We therefore think that the endogenous PAF should be produced mainly in the intrapulmonary airways when sensitized guinea pigs were challenged by antigen and that airway obstruction may be modulated by PAF.

On the other hand, the partial, although not statistically significant inhibition of the antigen-induced airway microvascular leakage produced by the pretreatment with high doses of either OKY-046 or ONO-1078 suggest that TX and LT should be partly involved in causing plasma exudation into the airway of sensitized guinea pigs. This view should be supported by the finding that the high doses of Y-24180 and WEB 2086 did not completely protect against antigen-induced airway microvascular leakage and that the inhibitory activity of the two compounds plateaued at their high doses. However, a previous study using ONO-1078 has shown that antigen-induced airway microvascular leakage was markedly mediated by leukotrienes (28). We therefore think that observations performed at 5 min after the antigen challenge is not long enough to examine the involvement of leukotrienes in the antigen-induced airway microvascular leakage because the actions of leukotrienes induce a late acceleration of leakage.

In conclusion, the results of the present study indicate that endogenous PAF plays a role in airway microvascular leakage following antigen challenge in actively sensitized guinea pigs. Because Y-24180, a PAF-receptor antagonist, was ascertained to exhibit an inhibitory effect on the leakage, this compound should be useful for clinical treatment of bronchial asthma.

Acknowledgments

The authors wish to thank H. Ishikawa, N. Tomomatsu and T. Masu for their technical assistance. Furthermore, we thank Drs. H. Ochi, T. Yokobe and M. Setoguchi for critical comments on the manuscript.

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