Platelet-Activating Factor (PAF)-Induced Rhinitis and Involvement of PAF in Allergic Rhinitis in Guinea Pigs

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Abstract—The effects of inhaled PAF on the guinea pig nasal mucosa were investigated. Intranasal pressure (INP) was recorded as an index of intranasal resistance. To access the capillary permeability of nasal mucosa, exudation of Evans blue into the nasal lavage fluid was determined. Inhalations of histamine and PAF markedly and significantly increased INP and dye exudation into the nasal cavities. The two responses to PAF were about 20-fold and 70-fold stronger than those of histamine, respectively. A PAF antagonist, CV-3988, significantly antagonized both the PAF-induced increases in INP and dye exudation. Indomethacin and OKY-046 had no effect on the PAF-induced responses. FPL-55712 inhibited the PAF-induced increases in INP and dye exudation by 52% and 40%, respectively. Ovalbumin (OA) antigen challenge by inhalation to sensitized guinea pigs resulted in significant increases in both INP and dye exudation. These two responses to 30 mg/ml OA were inhibited by CV-3988 (10 mg/kg, i.v.) by 55% and 40%, respectively. From the above results, it is indicated that: 1) inhalation of PAF evokes rhinitis-like symptoms through activation of PAF receptors, 2) the PAF-induced rhinitis is, in a part, mediated by leukotrienes, and 3) PAF might be involved in allergic rhinitis.

Allergic rhinitis afflicts over 10% of the population and is prevalent in early spring for Japanese cedar pollen in Japan. Nowadays, allergic rhinitis is a world-wide social problem (1). Fundamental studies on allergic rhinitis, however, have been poorly performed, mainly because there are very few experimental rhinitis models using laboratory animals (2–5).

PAF was first described as a product of antigen-stimulated IgE-sensitized rabbit basophils, which is capable of aggregating rabbit platelets (6). In the lower airways, PAF is considered to be one of the important chemical mediators in bronchial asthma, because inhaled PAF can produce a bronchoconstriction (7, 8), prolonged airway hyperresponsiveness (9, 10) and airway inflammation (11), all characteristic of asthma. On the other hand, the relationship between PAF and rhinitis has been poorly understood.

In the present study, the effect of inhaled PAF on the guinea pig nasal mucosa was investigated in comparison with that of histamine. Also, the involvement of PAF in allergic rhinitis using a new experimental animal model was studied.

Materials and Methods

Procedures: Male Hartley guinea pigs (350–500 g) purchased from Tokyo Laboratory Animals, Inc. were anesthetized with pentobarbital sodium (30 mg/kg, i.p.) and fixed in a supine position. Animals were spontaneously respired through a tracheal cannula inserted into the lower trachea. A polyethylene cannula (Imamura, Size 9, o.d. of 3 mm, length of 5 cm) was inserted to a depth of 3–4 mm into the nasopharynx from the side of the larynx after making a 2–3-mm incision in the thyroid cartilage. The two duct pores, which are situated at the upper oral cavity wall and lead to the nasal cavities, were closed with Alon-alpha (Konoshi). Then, a
polyethylene cannula (Imamura, Size 6, o.d. of 2 mm, length of 2 cm) was inserted into each nostril to a depth of 2–3 mm. The two nostril cannulae were connected to a Y-shaped cannula with another polyethylene cannula (Imamura, Size 9).

The nasal cavities were ventilated between both cannulae inserted into the nasopharynx and bilateral nostrils, with an artificial respirator (Shinano, SN-480-7-10) (Fig. 1). A fixed volume of air was insufflated at a frequency of 70/min, from the nasopharynx to the nostrils. Intranasal resistance was recorded continuously as a change in intranasal pressure (INP). Intranasal pressure was measured with a pressure transducer (Nihon Kohden, T12-AD), carrier amplifier (Nihon Kohden, AP-621D) and DC amplifier (Nihon Kohden, AD-641G).

To induce rhinitis symptoms, histamine, PAF and ovalbumin (OA) were inhaled for 10 min into the nasal cavities by aerosolizing drug solutions in a specially devised plastic cylindrical chamber (diameter: 27 mm and height: 57 mm) (12) which was introduced in an ultrasonic nebulizer (Nihon Kohden, TUR-3200). The ultrasonic nebulizer was placed in the air-insufflating circuit so that the aerosolized mist was inhaled into the nasal cavities each time air was sent out from the respirator. The consumption volume of inhaled drug solutions in the chamber was about 0.3–0.6 ml for 10 min. In each animal, the effect of only one of the tested drugs was evaluated.

The experimental schedule was as follows: After intranasal pressure became stable following the operation, 0.9% saline was inhaled for 10 min into the nasal cavities to obtain a control change in intranasal pressure. Next, Evans blue (Merck; 30 mg/kg, i.v.) was injected i.v. into the brachial vein, 10 min prior to the 10-min inhalation of histamine, PAF or OA. Ten min after the end of inhalation of histamine, PAF or OA, the nasal cavities were lavaged with 3 ml saline using a 5 ml syringe, 5 times repeatedly for 2.5 min. The lavage fluid (yield: 2.8–3.0 ml) was centrifuged at 12,000 rpm for 5 min. The amount of Evans blue in the supernatant was determined colorimetrically at 620 nm. Thus, both the nasal congestion and nasal capillary permeability were determined in the respective animals.

For inducing allergic rhinitis, guinea pigs were sensitized with OA (10 µg) and Al(OH)₃ (1 mg), s.c., at the back in a volume of 0.5 ml, boosted with the same dosing two

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**Fig. 1.** Scheme of the present experimental method using guinea pigs. Intranasal resistance was recorded as a change in intranasal pressure. PAF, histamine or ovalbumin was inhaled into the nasal cavities with an ultrasonic nebulizer.
weeks later, and further challenged with inhalation of OA (10 mg/ml, 30 mg/ml) one week later. Antisera were collected, and reagin-like immunoglobulin titers were determined by 72 hr homologous PCA. The titer of antisera of immunized guinea pigs was 128 on the average.

As for processing, the date of nasal congestion, the nasal resistance change represented by intranasal pressure was obtained by deduction of the maximal change in intranasal pressure by inhalation of saline from that by inhalation of histamine, PAF or OA in each animal, during a 10-min inhalation period and a further succeeding 10-min period prior to the nasal washing.

**Drugs:** The drugs used were histamine dihydrochloride (Tokyo Kasei), platelet-activating factor (PAF, β-acetyl-γ-O-hexadecyl-L-α-phosphatidylcholine, Sigma), ovalbumin (OA, Sigma), CV-3988 (Takeda Chem. Ind.), indomethacin (Sigma), OKY-046 (Kissei) and FPL-55712 (presented by Nissan Kagaku). Histamine, OA, OKY-046 and CV-3988 were dissolved in saline. CV-3988 was given i.v. at doses of 3 and 10 mg/kg 5 min prior to the beginning of inhalation of PAF or OA. OKY-046 was given i.v. at a dose of 10 mg/kg 2 min prior to PAF inhalation. Indomethacin was dissolved in 0.02 N disodium carbonate (pH 8.1) and injected at a dose of 5 mg/kg, i.v. 30 min prior to PAF inhalation. PAF was dissolved in PBS/BSA buffer solution. The PBS/BSA buffer solution was prepared as follows: 0.15 M phosphate buffer solution (PBS, containing Na2HPO4 and NaH2PO4·2H2O) was adjusted to pH 7.4 with 1 N NaOH, and then bovine serum albumin (BSA; 2.5 mg/ml, Sigma) was added.

**Statistical analysis:** All values were expressed as the mean with S.E. Statistical significance of difference was determined by Student's t-test.

**Results**

**Effects on intranasal pressure:** A 10-min inhalation of saline into the nasal cavities sometimes resulted in a slight increase in intranasal pressure (as an index of intranasal resistance), which rapidly recovered to the baseline level after the end of inhalation. On the other hand, inhalation of histamine (0.03%, 0.1% and 0.3%) into the nasal cavities markedly increased intranasal pressure (Figs. 2 and 3). The increased pressure by 0.3% histamine was sustained even at 10 min after the end of inhalation.

**PAF (0.003%, 0.01% and 0.03%)** by a 10-min inhalation significantly increased intranasal pressure in a concentration-dependent manner (Figs. 2 and 3). The response to 0.03% PAF continued even at 10 min after the end of inhalation. PAF-inhalation groups showed significantly higher values of 1.31±0.36 cmH2O (N=6), for 0.003% PAF: 1.86±0.52 cmH2O (N=6), for 0.01% PAF and 3.38±0.93 cmH2O (N=8), for 0.03% PAF, as compared with the control value of 0.08±0.18 cmH2O (N=6). Such increased responses were not induced by inhalation of 0.03% lyso-PAF.

Estimated from the dose-response curves for the changes in intranasal pressure by PAF and histamine, the activity of PAF was about twenty times stronger than that of histamine in mole ratio.

**Effects on nasal capillary permeability:** The results of Evans blue dye exudation are shown in Fig. 4. The dye amount exudated in the saline group was 2.06±0.47 μg/ml (N=6). In the histamine (0.1%, 0.3%)-treated groups, the dye amounts exudated were significantly increased. In PAF (0.01%, 0.03%)-treated groups, the amounts were also significantly increased as compared with the PBS/BSA group (2.28±0.54 μg/ml, N=7): that is, 9.18±2.27 μg/ml (N=7) at 0.01% and 11.31±2.31 μg/ml (N=7) at 0.03%. The dye exudation was not increased by inhalation of 0.03% lyso-PAF (1.77±0.32 μg/ml, N=5).

Estimated from the dose-response curves for the dye exudations by PAF and histamine, the activity of PAF was about seventy times stronger than that of histamine in mole ratio.

**Effect of CV-3988 on the PAF-induced nasal responses:** The 0.01% PAF-induced increases in intranasal pressure and dye exudation were totally inhibited by pretreatment with the PAF antagonist CV-3988 (3 mg/kg,
Fig. 2. Typical recordings of the responses to aerosol administrations of a) 0.1% and 0.3% histamine (Hist) and b) 0.01% and 0.03% PAF into the nasal cavities of the guinea pig. Inhalation was performed for 10 min, as shown by underlines below the INP recordings. Saline or PBS/BSA was used as the control. BP: systemic blood pressure, HR: heart rate, and ΔINP: change in intranasal pressure as an index of intranasal resistance.

Fig. 3. Maximal changes in intranasal pressure by inhalations of histamine (0.03%, 0.1% and 0.3%) and PAF (0.003%, 0.01% and 0.03%) in guinea pigs. Each column represents the mean with S.E. of 5 to 8 animals. *: P<0.05, **: P<0.01 and ***: P<0.001 vs. saline or PBS/BSA.
Pretreatment with CV-3988 also caused a significant inhibition of the 0.03% PAF-induced responses.

**Effects of inhibitors of arachidonic acid metabolites on the PAF-induced nasal responses:** Neither the cyclooxygenase inhibitor indomethacin (5 mg/kg, i.v.) nor the thromboxane synthetase inhibitor OKY-046 (10 mg/kg, i.v.) influenced the 0.03% PAF-induced responses (Table 1). On the other hand, pretreatment with the leukotriene antagonist FPL-55712 (3 mg/kg, i.v.) significantly inhibited the 0.03% PAF-induced increases in intranasal pressure. The agent showed a tendency to inhibit the 0.03% PAF-induced dye exudation by 40%, which was not statistically significant. FPL-55712 (3 mg/kg, i.v.) had no effect on the 0.3% histamine-induced responses.

**Provoked allergic rhinitis:** Typical recordings of the change in intranasal pressure by OA antigen (10 mg/ml, 30 mg/ml) challenge by inhalation to sensitized guinea pigs are shown in Fig. 7. The 10-min inhalation of OA markedly increased intranasal pressure (Figs. 7 and 8). In sensitized guinea pigs, the maximal changes in intranasal pressure in the OA-inhalation groups (10 mg/ml, 30 mg/ml) were $1.07 \pm 0.25$ cmH$_2$O (N=10) and $2.48 \pm 0.49$ cmH$_2$O (N=8), respectively, which were
significantly higher than that in the saline inhalation group (0.02±0.20 cmH₂O, N=8). The results of Evans blue dye exudation are shown in Fig. 9. The OA-inhalation (10 mg/ml, 30 mg/ml) significantly increased the dye exudation. The above allergic rhinitis responses were not seen by inhalation of OA (30 mg/ml) to non-sensitized guinea pigs.

**Involvement of PAF in allergic rhinitis:** The OA-induced increases in intranasal pressure were significantly inhibited by CV-3988 (3 mg/kg, i.v.; 10 mg/kg, i.v.) (Fig. 8). CV-3988 (3 mg/kg, i.v.; 10 mg/kg, i.v.) inhibited the OA-induced dye exudation by 40% (Fig. 9). CV-3988 at a dose of 10 mg/kg, i.v. had no effect on the 0.1% histamine-induced responses.

Table 1. Effects of arachidonic acid cascade inhibitors on 0.03% PAF-induced increases in intranasal pressure (INP) and dye exudation in the nasal cavities

<table>
<thead>
<tr>
<th>Treatment</th>
<th>INP (% Change from control)</th>
<th>Dye concentration (% Change from control)</th>
</tr>
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<tbody>
<tr>
<td>Indomethacin (5 mg/kg, i.v.)</td>
<td>-11%</td>
<td>-13%</td>
</tr>
<tr>
<td>OKY-046 (10 mg/kg, i.v.)</td>
<td>+10%</td>
<td>+ 9%</td>
</tr>
<tr>
<td>FPL-55712 (3 mg/kg, i.v.)</td>
<td>-52%**</td>
<td>-40%</td>
</tr>
</tbody>
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Values are represented as the mean of 5 to 7 animals. Significant difference was assessed with Student’s t-test using original data. **P<0.01.

![Graph](image)

**Fig. 7.** Typical recordings of the responses to aerosol administrations of 10 mg/ml and 30 mg/ml ovalbumin (OA) into the nasal cavities of the sensitized guinea pig. Other explanations are as in Fig. 2.

Discussion

PAF is a naturally occurring lipid autacoid produced by a variety of inflammatory cells such as neutrophils, eosinophils, basophils, mast cells and macrophages (13). In the lower airways, the biological properties of PAF suggest its role as a mediator of bronchial asthma. PAF is a potent bronchoconstrictor in experimental animals and humans (14, 15). PAF is a particularly potent chemotactic agent for eosinophils (16), which are considered to be of primary importance in the development of late asthmatic responses and airway hyper-responsiveness. On the other hand, the relationship between PAF and rhinitis has not been adequately documented.

In the present study, PAF induced rhinitis-
like symptoms more strongly than histamine; the potency ratios between PAF and histamine were about 20-fold in nasal congestion and about 70-fold in dye exudation. Evans et al. (17) have demonstrated that i.v. injected PAF is extremely potent in increasing vascular permeability of the trachea and main bronchi of the guinea pig, being 10,000-fold more active than histamine. Konno et al. (18) have clinically found that PAF induces an increase in nasal airway resistance in patients with nasal allergy. The response to PAF was 4 times stronger than that to histamine. Mezawa (19) investigated the threshold concentrations of the chemical mediators for rhinitis symptoms in patients with allergic rhinitis, and found that PAF was approximately 1,000 times stronger than histamine in threshold concentration for rhinitis symptoms. These findings that PAF is stronger in potency than histamine for increasing nasal resistance are consistent with ours.

Histamine is present in mast cells of the submucosal layer and in free-floating mast cells of the mucous blanket of nasal cavities. Histamine is clinically considered to be one of the most important chemical mediators responsible for allergic rhinitis (20-23). Antihistamine drugs have been extensively used to treat allergic rhinitis. This category of drugs is reportedly effective on nasal hypersecretion and sneezing, but only has a slight effect on nasal congestion (1). This indicates that histamine is not the only mediator involved in allergic rhinitis. In the previous studies using animals, histamine evokes rhinitis-like symptoms. Histamine-inhalation can cause rhinitis-like nasal congestion and inflammation in rats (24) and evoke nasal hypersecretion (25).

CV-3988, a specific PAF-receptor antagonist, inhibited PAF-induced hypotension (26), platelet aggregation (27) and vascular permeability (28). In the present study, CV-3988 (3 mg/kg, i.v.; 10 mg/kg, i.v.) totally antagonized the PAF-induced increases in both intranasal pressure and dye exudation. In addition, inhalation of 0.03% lyso-PAF did not induce rhinitis-like symptoms. Therefore, the PAF-induced rhinitis-like symptoms are presumably evoked through activation of PAF receptors.

Fundamental studies on allergic rhinitis have been poorly performed. In order to define the mechanisms of allergic rhinitis and to develop effective therapeutic drugs for it, it is necessary to have good allergic rhinitis models. However, as yet, no adequate rhinitis models have been developed. In some conventional models, only the nasal capillary permeability is used as an index for rhinitis
symptoms or allergens dissolved in saline are artificially perfused in the nasal cavities (2, 4). In the present study, a new allergic rhinitis model was devised in which 1) the inhalation technique for allergen challenge was introduced and 2) reproducible determinations of nasal congestion and nasal inflammation were feasible. The sensitization procedure was that described by Pretolani et al. (29), which had been used for an allergic bronchial asthma model. For inducing the allergic asthma reaction, 10 mg/ml of OA is necessary as a challenge concentration (29). However, inhalation of 10 mg/ml of OA into the nasal cavities caused rather slight allergic rhinitis symptoms (Fig. 8). Therefore, inhalation of 30 mg/ml of OA was presently employed as well as 10 mg/ml. In the guinea pig allergic rhinitis model hitherto described (4), the sensitization period is more than one month, and the frequency of antigen administration for sensitization is more frequent.

In the present study, allergically induced rhinitis responses were also inhibited by CV-3988 (3 mg/kg, i.v.; 10 mg/kg, i.v.). CV-3988 in the doses used seems to specifically antagonize the PAF effects, since 10 mg/kg of CV-3988 had no effect on the histamine-induced rhinitis symptoms. Miadonna et al. (30) reported that PAF and lyso-PAF were detected in nasal secretions obtained from patients with hay fever that underwent local antigen challenge. PAF is also present in the nasal lavage fluid of OA-sensitized guinea pigs after topical challenge (31). The above findings suggest that PAF might be involved in allergic rhinitis.

Possible factors for causing nasal blockage in rhinitis may be the edema of nasal mucosa, vasodilation of sinusoidal venous spaces inside the mucosa and nasal hypersecretion. In increasing intranasal pressure, PAF was 20-fold stronger in potency than histamine; and in increasing capillary permeability, PAF was 70-fold stronger in potency than histamine. This difference of effectiveness ratio in these two responses indicates that the PAF-induced nasal congestion may more predominantly depend on an increase in capillary permeability than the histamine-induced nasal congestion.

In the present study, FPL-55712 significantly prevented the PAF-induced rhinitis-like symptoms, but not the histamine-induced ones. PAF has been shown to stimulate leukotriene production in cat pulmonary tissue (32) and rat lungs (33). PAF is also known to stimulate leukotriene B4 production in human neutrophils and eosinophils (34). Furthermore, the PAF-induced increase in airway responsiveness to histamine may be dependent on the release of lipoxygenase metabolites (35). It is thus suggested that PAF may partly cause rhinitis-like symptoms via the generation of leukotrienes.

In conclusion, the above findings suggest that: 1) inhalation of PAF evokes rhinitis-like symptoms through activation of PAF receptor, 2) the rhinitis-like symptoms evoked by PAF are, in a part, mediated by leukotrienes, and 3) PAF might be involved in allergic rhinitis.

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