Platelet-activating factor (PAF) plays an important role in the immediate asthmatic response in guinea-pig by augmenting the response to histamine

¹Shigeaki Morooka, Masako Uchida & Noriaki Imanishi

Research Laboratories, Sumitomo Pharmaceuticals Co., Ltd, 1-98, Naka-3, Kasugade, Konohana-ku, Osaka, 554 Japan

1 To investigate the role of platelet activating factor (PAF) in the immediate asthmatic response, we examined the bronchial reactivity to histamine after administration of PAF to guinea-pigs or antigen challenge to passively sensitized guinea-pigs.

2 A bolus injection of PAF (20–40 ng kg⁻¹), which did not cause a significant increase in intrathoracic pressure (ITP), augmented the bronchial response to histamine almost 8 fold. This airway hyperreactivity was observed even 1 min after PAF treatment.

3 A subthreshold dose of antigen (0.01 mg kg⁻¹, i.v.) also provoked hyperreactivity to histamine, which became significant 6 and 11 min after the antigen treatment.

4 The specific PAF-antagonists, SM-10661 and CV-6209 (i.v.) dose-dependently inhibited both PAF- and antigen-induced airway hyperreactivities to histamine.

5 These results suggest that PAF plays an important role in antigen-induced acute airway responses by augmenting the activities of spasmogens.

Keywords: PAF; SM-10661; immediate asthmatic response in guinea-pig

Introduction

Platelet activating factor (PAF) is a phospholipid mediator released from various inflammatory cells and has been proposed as a mediator of asthma because of its capacity to mimic a range of asthmatic symptoms (Morley *et al.*, 1984). Sensitized guinea-pig lung releases PAF and its inactive metabolite lyso-PAF (Fitzgerald *et al.*, 1986). PAF can therefore be generated in sensitized guinea-pig lung in response to antigen challenge. In guinea-pigs, PAF induces acute bronchospasm (Vargaftig *et al.*, 1980) and causes intrathoracic accumulation of neutrophils and platelets (Dewar *et al.*, 1984).

Airway hyperreactivity is one of the characteristics of asthma which becomes more pronounced following inhalation of allergen by atopic subjects (Cockcroft *et al.*, 1977). Mazzoni *et al.* (1985) reported that airway responsiveness to bombesin increased after a 1 h infusion of PAF (600 ng kg⁻¹ h⁻¹) in guinea-pigs. It was revealed that inhalation of PAF caused sustained airway hyperreactivity to methacholine or histamine in man and guinea-pigs (Cuss *et al.*, 1986; Coyle *et al.*, 1988). Allergen-induced hyperresponsiveness 24 h after allergen inhalation in an allergic rabbit was modified by the PAF-antagonist, BN 52021 (Coyle *et al.*, 1989). These results indicate that PAF is involved in airway hyperreactivity. It has been presumed that airway hyperreactivity is a consequence of inflammatory events. It is thought that obstruction of the airway lumen by activation of various leucocytes, mucosal swelling and impaired mucus clearance play important roles in the occurrence of airway hyperreactivity (Cockcroft, 1983). However, physical obstruction due to inflammatory events in the airways needs several hours or days to become evident.

Recently, we observed that the selective PAF receptor antagonist, SM-10661 ((±)-*cis*-3,5-dimethyl-2-(3-pyridyl)-thiazolidin-4-one hydrochloride) inhibited not only PAF-induced increases in intrathoracic pressure (ITP) in anaesthetized guinea-pigs, but also rapid ITP increases by antigen challenge to passively sensitized guinea-pigs, although it had no effect on histamine-induced increase in ITP (Uchida *et al.*, 1989). These results led us to speculate the existence of interaction between PAF and histamine.

In this study, we investigate whether PAF-induced hyperreactivity to histamine can occur within several minutes and examine the role of this response in the immediate anaphylactic bronchospasm.

Methods

Animals

Male Dunkin-Hartley guinea-pigs (Japan SLC, Inc.) weighing 430–500 g were used throughout this study. The animals were sensitized by intravenous injection of 0.85 mg kg⁻¹ rabbit anti-egg albumin anti-serum (Seikagaku kogyo Co., Ltd., Japan) 18–24 h before antigen challenge.

Measurement of airway responses

Prior to the operation, the animals were anaesthetized with sodium pentobarbitone (30 mg kg⁻¹, i.p.) and sodium phenobarbitone (120 mg kg⁻¹, i.p.) and the trachea, carotid artery and jugular vein were cannulated for measurement of airway obstruction, systemic blood pressure and for the introduction of drugs respectively. Gallamine trietide (10 mg kg⁻¹, i.m.) was injected to prevent spontaneous breathing. The guinea-pigs were ventilated with a rodent ventilator (Harvard Inc.) with room air supplemented with oxygen at 60 strokes min⁻¹ with a stroke volume of 3–7 ml. The body temperature was maintained at 37°C with a heating pad throughout the experiment. Airway obstruction was measured according to the method of Konzett & Rössler (1940) using a differential pressure transducer (Ugo Basile) connected by the side-arm of the tracheal cannula to a bronchospasm transducer and expressed as ITP in cmH₂O. The intratracheal cannula was clipped before and after the experiment to obtain the maximal response.

Airway hyperreactivity induced by PAF

Histamine (3 µg kg⁻¹), PAF (20 ng kg⁻¹) or leukotriene D₄ (0.07 nmol kg⁻¹) was injected via the jugular vein and the ITP was measured as a prechallenge value. Then PAF (10–40 ng kg⁻¹, i.v.) was injected 15 min later. The histamine (3 µg kg⁻¹, i.v.), PAF (20 ng kg⁻¹) or leukotriene D₄

¹ Author for correspondence.

(0.07 nmol kg⁻¹) were injected 1, 6 and 11 min after the PAF administration. For the measurement of the duration of the PAF effect, histamine was administered at 31 and 61 min after PAF injection. For the control, saline containing 0.25% bovine serum albumin (BSA) was used instead of PAF. The phasic blood pressure and heart rate were continuously monitored via a heparinized arterial cannula connected to a pressure transducer and a recorder, respectively.

Airway hyperreactivity induced by antigen

Fifteen minutes after measurement of the prechallenge value by histamine as stated above in passively sensitized guinea-pigs, the animals were challenged intravenously with antigen (ovalbumin, 0.01 mg kg⁻¹). Histamine reactivity was measured 1, 6, 11, 31 and 61 min after antigen administration. For the control, saline was used instead of ovalbumin.

Effect of PAF-antagonists on the hyperreactivity induced by PAF or antigen

SM-10661 or CV-6209 was injected into the jugular vein 2 min before PAF or antigen administration. Histamine reactivity was estimated 6 min after PAF or antigen administration and % inhibition was calculated according to the equation shown below.

Increase in ITP induced by histamine, PAF, leukotriene D₄ or antigen

Normal or passively sensitized, anaesthetized guinea-pigs were injected intravenously with histamine, PAF, leukotriene D₄ or ovalbumin. The ITP peak value was measured as the % increase in ITP.

Effect of PAF or antigen on circulating cell count

Blood samples (20 µl) of normal or passively sensitized, anaesthetized guinea-pigs were drawn from cannula in the jugular vein before and after intravenous administration of PAF (40 ng kg⁻¹) or ovalbumin (0.01 mg kg⁻¹). After appropriate dilution, platelet and white blood cell counts were measured by Sysmex Microcell Counter F-800 (Toha Iyoh Denshi, Japan).

Materials

Histamine dihydrochloride (Nakalai Tesque Japan Inc.), leukotriene D₄ (Peptide Institute, Inc., Japan), sodium pentobarbitone (Pittmanmoor, U.S.A.), sodium phenobarbitone (Wako Junyaku, Japan), gallamine trietide and fatty acid-free bovine serum albumin (Sigma) were dissolved in physiological saline. Alkyl-PAF (mixture of C₁₆ and C₁₈, Funakoshi, Japan) was dissolved in 0.25% BSA saline buffer. SM-10661 ((±)-cis-3,5-dimethyl-2-(3-pyridyl)thiazolidin-4-one hydrochloride) was synthesized in the synthetic division of Sumitomo Pharmaceuticals Co., Ltd. CV-6209 (2-[N-acetyl-N-(2-methoxy-3-octadecylcarbamoyloxypropoxycarbonyl) ammomethyl]-1-ethylpyridinium chloride) was a gift from Takeda Chemical Industries. SM-10661 was dissolved in saline and the pH was adjusted to between 6 and 7 with 1N sodium hydroxide. CV-6209 was dissolved in 0.25% BSA-saline.

Statistical analysis

Data are presented as the mean ± s.e. of *n* observations. The % increase in ITP, % augmentation and % inhibition by the drugs were calculated according to the following formulae.

% increase in ITP

$$= \left[\frac{\text{airway response by histamine (cm)} - \text{baseline value (cm)}}{\text{occlusion value (cm)} - \text{baseline value (cm)}} \right] \times 100$$

% augmentation =

$$\left[\frac{\text{\% increase in ITP to histamine after PAF or antigen administration}}{\text{\% increase in ITP to histamine before PAF or antigen administration}} - 1 \right] \times 100$$

% inhibition =

$$1 - \left[\frac{\text{airway response by histamine under treatment with drug (cm)} - \text{baseline value (cm)}}{\text{airway response by histamine under treatment with vehicle (cm)} - \text{baseline value (cm)}} \right] \times 100$$

Significance was assessed by Dunnett's test and a *P* value of less than 0.05 was considered significant.

Results

Airway hyperreactivity to histamine induced by PAF

Administration of histamine caused a dose-dependent increase in ITP as shown in Figure 1. The ITP was elevated about 1.3 ± 0.3, 4.3 ± 1.1 or 80.4 ± 3.2% above the baseline at doses of 1, 3 or 10 µg kg⁻¹ respectively. Histamine, 3 µg kg⁻¹ caused a small but significant change in ITP and returned to the initial level within 1 min. This response was reproducible by repeated administration from -15 to 31 min, and the response was small but significantly increased at 61 min (Figure 2a). Intravenous administration of PAF also caused a dose-dependent increase in ITP and the ED₅₀ value was 86.5 ng kg⁻¹ (Figure 3a). At a dose of less than 50 ng kg⁻¹, the ITP returned to the baseline level within 1 min. However, at more than 50 ng kg⁻¹, the increase in ITP was observed for over 1 min. After the administration of 40 ng kg⁻¹ of PAF, which by itself caused a detectable but insignificant increase in

Figure 1 Dose-dependent increase in intrathoracic pressure (ITP) in response to histamine administration in anaesthetized guinea-pigs. ITP was measured by the Konzett-Rössler method. Each value represents the mean of *n* = 4; s.e.mean shown by vertical bars.

Figure 2 Time course of increase in intrathoracic pressure (ITP) in response to histamine before and after PAF treatment: (a) and (b) are representative traces of histamine (Hist) ($3 \mu\text{g kg}^{-1}$, i.v.) responsiveness before and after a bolus injection of 0.25% BSA-saline or 40 ng kg^{-1} of PAF to anaesthetized guinea-pigs, respectively. Control value of % increase in ITP in response to histamine was 3.8 ± 0.5 and % increase in ITP to PAF was 7.8 ± 1.5 ($n = 9$). (c) Represents the time course of the % increase in ITP in response to histamine ($3 \mu\text{g kg}^{-1}$, i.v.) after intravenous (●) PAF (40 ng kg^{-1} , $n = 9$) or (▲) 0.25% BSA-saline ($n = 5$) administration. Each value is expressed as % augmentation according to Methods and represents the mean with s.e. shown by vertical bars. Statistical significance; * $P < 0.05$; ** $P < 0.01$. Dunnett's test compared with the control value.

ITP, the response to histamine ($3 \mu\text{g kg}^{-1}$) was augmented significantly. The % augmentation at 1, 6, 11, 31 and 61 min was 620 ± 200 , 745 ± 125 , 880 ± 247 , 875 ± 125 and 810 ± 191 respectively (Figure 2b and 2c). Smaller doses of PAF (20 and 30 ng kg^{-1}) resulted in similar but smaller responses than that of 40 ng kg^{-1} of PAF. At a dose of 30 ng kg^{-1} , the responses at 6 and 11 min were significant and % augmentation was 565 ± 122 and 523 ± 103 respectively. At 20 ng kg^{-1} of PAF, the responses increased by 153 ± 101 and 246 ± 171 at 6 and 11 min, both of which were not significant. Thus these responses were induced dose-dependently at doses between 20 and 40 ng kg^{-1} of PAF. The dose-response curve at 6 min is shown in Figure 3b.

Smaller doses of histamine induced smaller increases in ITP after PAF treatment and when larger doses of histamine were used, it was impossible to obtain the % augmentation because the increase in ITP induced by histamine alone exceeds 100%. We therefore used a combination of 40 ng kg^{-1} of PAF and $3 \mu\text{g kg}^{-1}$ of histamine throughout the experiment.

Figure 3 Dose-response of (a) PAF-induced increase in intrathoracic pressure (ITP), ($n = 7-8$) and (b) increase in ITP in response to $3 \mu\text{g kg}^{-1}$ of histamine 6 min after PAF administration ($n = 4-6$) in guinea-pigs. Each value represents the mean with s.e. shown by vertical bars.

Effects of PAF-antagonists on PAF-induced airway hyperreactivity

The effects of two structurally unrelated PAF-antagonists were examined on PAF-induced hyperreactivity to histamine. Both PAF-antagonists did not affect the baseline level or influence the response of histamine (data not shown). When SM-10661 was administered intravenously 2 min before PAF, the hyperreactivity to histamine was inhibited dose-dependently. The ED_{50} value of SM-10661 at 6 min was 2.7 mg kg^{-1} (Figure 4a). Another PAF-antagonist, CV-6209, also dose-dependently inhibited the PAF-induced hyperreactivity to histamine with an ED_{50} value of 0.002 mg kg^{-1} (Figure 4b).

Airway hyperreactivity to leukotriene D_4 or PAF induced by PAF

Intravenous administration of leukotriene D_4 to anaesthetized normal guinea-pigs caused a dose-dependent increase in ITP (Figure 5a). Administration of leukotriene D_4 at a dose of $0.07 \text{ nmol kg}^{-1}$ caused a small but significant increase in ITP which returned to initial levels within 1 min. After treatment with 40 ng kg^{-1} of PAF, the % augmentation of ITP in response to the same dose of leukotriene D_4 was 193 ± 56 , 3987 ± 1246 and 4698 ± 701 at 1, 6 and 11 min respectively, and the values at 6 and 11 min were significant (Figure 5b). Similarly, the responses to 20 ng kg^{-1} of PAF itself, which did not cause significant increase in ITP, were also markedly increased and the % augmentation was 1244 ± 697 , 3316 ± 860 and 3711 ± 960 at 1, 6 and 11 min respectively

Figure 4 Inhibitory effects of PAF-antagonists on PAF (40 ng kg^{-1} , i.v.)-induced hyperreactivity to histamine in anaesthetized guinea-pigs at 6 min. PAF-antagonists were administered intravenously 2 min before PAF injection. The responses to histamine were dose-dependently inhibited by pretreatment with (a) SM-10661 ($n = 8-12$; control, $n = 14$) or (b) CV-6209 ($n = 5-7$; control, $n = 10$). Each value represents the mean with s.e. shown by vertical bars. Statistical significance; * $P < 0.05$; ** $P < 0.01$. Dunnett's test compared with the vehicle-treated group.

after PAF treatment as shown in Figure 5b. Larger doses of PAF provoked more than 100% increase in ITP as was seen with a large dose of histamine.

Airway hyperreactivity to histamine induced by ovalbumin in passively sensitized guinea-pigs

Figure 6a shows the dose-response curve of the increase in ITP by antigen administration. Ovalbumin-induced increases in ITP were apparent at doses over 0.02 mg kg^{-1} , i.v., in passively sensitized guinea-pigs. The degree of response to histamine ($3 \mu\text{g kg}^{-1}$) was the same as in nonsensitized guinea-pigs (Figure 7a). Administration of 0.01 mg kg^{-1} of antigen to passively sensitized guinea-pigs caused no marked increase in ITP ($1.6 \pm 0.3\%$, $n = 4$) and the baseline values did not change (Figure 7b). When histamine was administered 1 min after 0.01 mg kg^{-1} of antigen, the response was almost the same as the pre-challenge value. The increases in ITP caused by histamine returned to the baseline level within 1 min. However, the responses to histamine at 6, 11, 31 and 61 min after antigen treatment were far greater than the response before antigen administration. The % augmentation of ITP at 6, 11, 31 and 61 min was 1811 ± 418 , 1045 ± 226 , 1333 ± 478 and 1913 ± 554 , respectively (Figure 7b and c). The dose-response curve of % augmentation to antigen at 6 min is shown in Figure 6b.

Figure 5 (a) Dose-response to leukotriene D₄ (LTD₄)-induced increase in intrathoracic pressure (ITP) in anaesthetized guinea-pigs. (b) Time course of airway hyperreactivity to leukotriene D₄ (▲) or PAF (●), induced by PAF-treatment. Leukotriene D₄ ($0.07 \text{ nmol kg}^{-1}$) or PAF (20 ng kg^{-1}) were injected 15 min before and 1, 6 and 11 min after PAF (40 ng kg^{-1}) was administered to anaesthetized guinea-pigs. The prevalence of the % increase in ITP induced by leukotriene D₄ or PAF was 1.4 ± 0.3 or 1.0 ± 0.02 , respectively. Each value represents the mean (with s.e. shown by vertical bars) of $n = 5$. Statistical significance; * $P < 0.05$; ** $P < 0.01$. Dunnett's test compared with the prevalence.

Effects of PAF-antagonists on airway hyperreactivity to histamine induced by ovalbumin administration in passively sensitized guinea-pigs

When SM-10661 (i.v.) was administered 2 min before antigen treatment, the hyperreactivity to histamine was inhibited dose-dependently (Figure 8a). The ED₅₀ value at 6 min was 23.0 mg kg^{-1} . Another PAF-antagonist, CV-6209, also inhibited the response dose-dependently with an ED₅₀ of 1.4 mg kg^{-1} (Figure 8b).

Effect of PAF or antigen on circulating cell count

The circulating platelet and white cell counts in normal guinea-pigs 15 min before PAF administration were $(42.6 \pm 1.7) \times 10^4 \mu\text{l}^{-1}$ and $(33.6 \pm 5.9) \times 10^2 \mu\text{l}^{-1}$, respectively. One minute after PAF injection, platelet and white blood cell counts decreased to $(25.8 \pm 3.1) \times 10^4 \mu\text{l}^{-1}$ and $(23.2 \pm 3.5) \times 10^2 \mu\text{l}^{-1}$, respectively. The decrease of platelet count is statistically significant ($P < 0.01$, $n = 5$). Both platelet and white blood cell counts gradually recovered with time. In passively sensitized guinea-pigs, circulating platelet and white

Figure 6 Dose-response to (a) ovalbumin-induced increase in intra-thoracic pressure (ITP) 4 min after ovalbumin challenge ($n = 4-6$) and (b) increase in ITP in response to histamine ($3 \mu\text{g kg}^{-1}$, i.v.) 6 min after ovalbumin administration ($n = 4-6$) in passively sensitized anaesthetized guinea-pigs. Each value represents the mean with s.e. shown by vertical bars.

blood cell counts 15 min before antigen administration were $(45.2 \pm 2.8) \times 10^4 \mu\text{l}^{-1}$ and $(39.3 \pm 4.2) \times 10^2 \mu\text{l}^{-1}$, respectively. One minute after antigen administration, these cell counts did not change. However, at 6 min after antigen treatment, platelet and white blood cell counts decreased to $(12.0 \pm 1.2) \times 10^4 \mu\text{l}^{-1}$ ($P < 0.01$, $n = 4$) and $(23.0 \pm 2.6) \times 10^2 \mu\text{l}^{-1}$ ($P < 0.05$, $n = 4$), respectively. Both cells gradually recovered with time.

Discussion

We examined airway reactivity to histamine after PAF or antigen treatment to investigate the role of PAF in antigen-induced bronchial responses in passively sensitized guinea-pigs.

We showed that PAF, at doses of above 50 ng kg^{-1} , induced an increase in ITP in guinea-pigs. This result is almost comparable with the data reported by Vargaftig *et al.* (1980). Because of this PAF-induced increase in ITP, PAF has been considered a mediator of immediate asthmatic response.

We observed that a small dose of PAF, which provokes marginal increases in ITP, induced airway hyperreactivity to histamine, leukotriene D₄ and PAF within 1 min. These results suggest that PAF induces a nonspecific airway hyperreactivity in nonsensitized guinea-pigs. Anderson *et al.* (1988) reported that similar airway hyperreactivity to histamine was

Figure 7 Time course of the increase in intrathoracic pressure (ITP) in response to histamine before and after ovalbumin treatment: (a) and (b) are representative traces of histamine ($3 \mu\text{g kg}^{-1}$, i.v.) responsiveness before and after a bolus injection of saline or 0.01 mg kg^{-1} of ovalbumin to passively sensitized, anaesthetized guinea-pigs, respectively. The prevalue of the % increase in ITP in response to histamine was 3.3 ± 0.6 and the % increase in ITP to antigen was 0.4 ± 0.2 ($n = 9$). (c) Represents the time course of the % augmentation of ITP in response to histamine ($3 \mu\text{g kg}^{-1}$, i.v.) after intravenous (●) ovalbumin (0.01 mg kg^{-1} , $n = 9$) or (▲) saline ($n = 8$) administration. Each value is expressed as % augmentation according to Methods and represents the mean with s.e. shown by vertical bars. Statistical significance; * $P < 0.05$, ** $P < 0.01$. Dunnett's test compared with the prevalue.

induced by 20 ng kg^{-1} of PAF in guinea-pigs. Our data were correlated with the potentiation rate at this dose, but we showed that larger doses of PAF provoked more marked increases in ITP in response to spasmogens. In addition, we observed that PAF-induced hyperreactivity to histamine lasted for as long as 61 min. In man, such a lasting airway hyperreactivity is likely to augment the responses to subsequent antigen stimulation in asthmatic patients.

Mazzoni *et al.* (1985) found that continuous administration of PAF ($600 \text{ ng kg}^{-1} \text{ h}^{-1}$) induced airway hyperreactivity to bombesin. We have not yet examined the potentiation of the response to bombesin, but we noted that a bolus injection of PAF was sufficient to induce hyperreactivity to histamine. Furthermore, we showed that a lower dose of PAF was sufficient to induce hyperreactivity.

The specific PAF antagonist, SM-10661 (Komuro *et al.*, 1990), administered 2 min before PAF injection dose-

Figure 8 Inhibitory effects of PAF-antagonists on ovalbumin (0.01 mg kg^{-1} , i.v.)-induced hyperreactivity to histamine in passively sensitized, anaesthetized guinea-pigs at 6 min. PAF antagonists were administered intravenously 2 min before ovalbumin injection. The responses to histamine were dose-dependently inhibited by pretreatment with (a) SM-10661 ($n = 5-9$; control, $n = 8$) or (b) CV-6209 ($n = 5-8$; control, $n = 7$). Each value represents the mean with s.e. shown by vertical bars. Statistical significance; * $P < 0.05$, ** $P < 0.01$. Dunnett's test compared with the vehicle treated group.

dependently inhibited the hyperreactivity to histamine. In previous studies, we showed that SM-10661 inhibited PAF-induced platelet aggregation but did not influence ADP-, collagen- or calcium ionophore A-23187-induced platelet aggregation at concentrations up to $400 \mu\text{M}$. SM-10661 at doses of up to 100 mg kg^{-1} also did not inhibit airway responses induced by histamine ($8 \mu\text{g kg}^{-1}$, i.v.) or leukotriene D_4 (2.0 nmol kg^{-1} , i.v.). Furthermore it influenced neither the antigen-induced release of histamine nor leukotriene D_4 from passively sensitized guinea-pig chopped lung at concentrations up to 10^{-4} M (Uchida *et al.*, 1989). Another specific PAF-antagonist, CV-6209 (Terashita *et al.*, 1987), also inhibited PAF-induced hyperreactivity to histamine. Therefore, this PAF-induced airway hyperreactivity to spasmogens seems to be mediated via PAF receptors.

We found that the administration of antigen, at levels which did not cause an increase in ITP, to passively sensitized guinea-pigs, induced hyperreactivity to histamine 6 min after antigen treatment. The dose of antigen required to induce hyperreactivity to histamine was about one tenth the dose of antigen which alone induced the increase in ITP. The hyperreactivity was also observed for 61 min after the antigen administration. Popa *et al.* (1973) also reported that 30 min to 3 h after antigen-induced apparent bronchospasm, hyperreactivity to histamine occurred in sensitized guinea-pigs. Non-specific airway hyperreactivity to i.v. histamine or acetylcholine which was elicited by aerosol allergen challenge in

sensitized guinea-pigs 24 h after challenge, persisted for at least 48 h and was inhibited by PAF-antagonist, ketotifen or methylprednisolone (Havill *et al.*, 1990). In the present experiments, we found that the hyperreactivity was induced within 6 min after the administration of ovalbumin, at a dose which was insufficient to induce an increase in ITP. Our results suggest that the hyperreactivity is induced earlier after antigen administration than has been known so far.

The PAF-antagonists, SM-10661 and CV-6209, inhibited the antigen-induced hyperreactivity to histamine. These results may suggest that PAF is involved in this response. The antigen may induce the synthesis of PAF, which induces hyperreactivity, but the amount of histamine and leukotrienes released by antigen administration are not enough to induce increases in ITP. In contrast to the PAF-induced hyperreactivity to histamine, augmentation of histamine response at 1 min after antigen administration was not observed. It was presumed that mast cell or basophil activation accounted for the symptoms of allergic asthma. It is known that the time-courses of extracellular appearance of lipid derived mediators such as PAF, leukotriene C_4 , leukotriene B_4 and thromboxane A_2 are superimposable and there is a short lag period until these mediators are released after antigen challenge (Mencia-Huerta *et al.*, 1983). This may explain why the hyperreactivity to histamine could not be observed 1 min after the antigen treatment. It should also be noted that there is a difference between the doses of PAF-antagonists required to inhibit PAF- and antigen-induced hyperreactivity. In addition, the ratio of inhibitory potency of the two PAF-antagonists on PAF and antigen were not the same. Bussolino *et al.* (1986) reported that intracellularly synthesized PAF does not tend to be released from the cell. Stewart *et al.* (1988) and Stewart & Dusting (1990) found that PAF receptor antagonists inhibited bradykinin-induced prostacyclin (PGI_2) generation in bovine arteriole endothelial cells whereas exogenous PAF failed to stimulate PGI_2 synthesis. They suggested the existence of external and internal PAF receptors or receptor subtypes. Hwang (1988) also described the existence of PAF receptor subtypes. Therefore, the differences in amounts of SM-10661 and CV-6209 required for PAF- and antigen-induced hyperreactivity may be due to these mechanisms. These points require further investigation.

There have been several reports stating that PAF can induce airway hyperreactivity to spasmogens in man (Cuss *et al.*, 1986; Rubin *et al.*, 1987), monkeys (Patterson *et al.*, 1987), dogs (Chung *et al.*, 1986) or sheep (Christman *et al.*, 1987). Although the exact mechanism of hyperreactivity has not yet been elucidated, PAF-induced inflammatory events, such as leucocyte invasion of the thorax, oedema formation and impaired mucus clearance are thought to have important roles (Lellouch-Tubiana *et al.*, 1988). Despite a lack of correlation between airway hyperreactivity and the presence of eosinophils in bronchial alveolar fluids (Havill *et al.*, 1990), the role of eosinophils has generally been a topic of much attention (Durham & Kay, 1985; Sanjar *et al.*, 1989). Cockcroft *et al.* (1983) and Sanjar *et al.* (1989) found that the accumulation of sufficient eosinophils in the airway lumen requires more than 1 h or days. However, we observed that PAF or antigen administration induced the decrement of circulating white blood cells at 1 min or 6 min respectively, which implies the accumulation of white blood cells in the airway. So, the role of white blood cells such as eosinophils cannot be neglected in the rapid PAF- or antigen-induced hyperreactivity which we observed. PAF can activate guinea-pig platelets and Coyle *et al.* (1988) reported that the platelet-depletion of the ragweed-sensitized rabbit, inhibited the development of bronchial hyperresponsiveness 24 h after antigen exposure which was thought to be the consequence of the eosinophil accumulation. Platelet aggregates and migrated polymorphonuclear neutrophils were seen in the constricted arterioles 1 min after the intravenous administration of 100 ng kg^{-1} of PAF (Lellouch-Tubiana *et al.*, 1985). In the present study, we observed that the circulating platelet number decreases either

1 min after PAF injection or 6 min after antigen administration which correlates well with the development of airway hyperreactivity. These results may indicate the importance of platelets in the development of airway hyperresponsiveness. The precise role of platelets or white blood cells in PAF- or antigen-induced immediate airway response should be investigated. It is also necessary to examine whether antigen-induced thrombocytopaenia and leucopaenia are PAF-dependent.

PAF-antagonists reportedly inhibit late anaphylactic bronchospasm in guinea-pigs (Hutson *et al.*, 1988; Uchida *et al.*,

1989) and sheep (Tomioka *et al.*, 1989). These results indicated that PAF is involved in late anaphylactic response. In the present experiments, we revealed that PAF also may play an important role in the immediate anaphylactic response by elevating reactivity to spasmogens.

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