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Effect of human plasma-type platelet-activating factor acetylhydrolase in two anaphylactic shock models

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Abstract

The effect of human recombinant plasma-type platelet-activating factor (PAF) acetylhydrolase was examined in two murine models, PAF-induced death and active anaphylactic models. In the PAF-induced death model where mice were injected intravenously with 40 μg/kg of PAF, the administration of PAF acetylhydrolase reduced mortality in a dose-dependent manner, showing complete prevention of mortality at 1.0 mg/kg. Myeloperoxidase activity, the marker for neutrophils, was increased in the lung by PAF injection, and the PAF acetylhydrolase treatment significantly reversed the increase in myeloperoxidase activity. As in the PAF-induced model, PAF acetylhydrolase also decreased mortality in the active anaphylactic shock model where bovine serum albumin was injected intravenously to mice previously immunized with bovine serum albumin. The protective effect of PAF acetylhydrolase on mortality in this model was significant at 1.0 mg/kg. These results suggest that PAF is an important mediator in the lethality of systemic anaphylaxis, and that PAF acetylhydrolase may be beneficial for treatment of anaphylactic shock.

Keywords: PAF (platelet-activating factor); PAF acetylhydrolase; Anaphylactic shock

1. Introduction

Anaphylactic shock is an immediate and sometimes fatal allergic reaction to systemically administered antigens. The representative clinical features of the syndrome are suffocation, abrupt hypotension and cardiac dysfunction, resulting from binding of immunoglobulin E (IgE) on connective tissue mast cells and the subsequent release of various inflammatory mediators from the activated mast cells (Sheffer, 1985). Although it is well known that a number of mediators such as leukotrienes and platelet-activating factor (PAF) are involved in the reaction (Sheffer, 1985), the major mediator playing a lethal role in anaphylactic shock is not clearly identified.

1-*O*-hexadecyl-2-*O*-acetyl-sn-glycero-3-phosphocholine (PAF) is a phospholipid mediator with pleiotropic biological activities including platelet activation, airway constriction, hypotension, and vascular permeability (Imaizumi et al., 1995; Snyder, 1995). The action of PAF is abolished by the hydrolysis of the acetyl group at the sn-2 position of PAF to produce the biologically inactive lyso-PAF, a reaction which is catalyzed by a specific enzyme, PAF acetylhydrolase (Blank et al., 1981). There are at least three isozymes of PAF acetylhydrolase: an extracellular (plasma-type) and two intracellular (tissue-type) PAF acetylhydrolases. Plasma-type PAF acetylhydrolase is a 45-kDa monomeric enzyme which is usually associated with plasma lipoproteins (Tjoelker et al., 1995). An intracellular PAF acetylhydrolase, designated isoform Ib, is a heterotrimeric enzyme composed of three different subunits, α (45 kDa), β (30 kDa), and γ (29 kDa) (Hattori et al., 1994; 1995), whereas another subunit, isoform II, is a 40-kDa monomer with an amino acid sequence that has 41% identity with that of plasma-type PAF acetylhydrolase (Hattori et al., 1996).

In the present study, which is aimed to investigate the role of PAF in anaphylactic shock, we tried to block selectively the activity of PAF in two murine models, PAF-induced and active anaphylactic models, by adminis-

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tration of recombinant human plasma-type PAF acetylhydrolase. Our data clearly show that PAF is the crucial mediator inducing lethality of the mice in a systemic anaphylactic reaction.

2. Methods

2.1. Recombinant PAF acetylhydrolase

The recombinant PAF acetylhydrolase was produced and purified at ICOS (Bothell, WA). The specific activity of PAF acetylhydrolase used in this study was 13 mmol/h/mg.

2.2. PAF-induced sudden death model

PAF (Sigma, St. Louis, MO) was administered intravenously to male ICR mice (5 weeks old, Clea Japan, Japan), and mortality was examined 24 h after the PAF injection unless otherwise indicated. PAF acetylhydrolase (0.2 ml) in sodium phosphate buffer (40 mM, pH 7.5) (Hofbauer et al., 1998) was administered intravenously 15 min before the PAF injection. Control animals received the same volume of the buffer without PAF acetylhydrolase.

2.3. Active anaphylactic shock

Antigen-induced systemic anaphylaxis was evoked as described (Terashita et al., 1987). In brief, male ICR mice were sensitized by intraperitoneal injection of 1.0 mg of bovine serum albumin (Sigma) in conjunction with inactivated *Bordetella pertussis* (2×10^9 cells), and 2 weeks after the sensitization, the mice were challenged by intravenous injection of 2.0 mg of bovine serum albumin. Mortality was counted 24 h after the challenge. PAF acetylhydrolase (0.2 ml) or vehicle was administered intravenously 15 min before the challenge as described above.

2.4. Measurement of myeloperoxidase activity in the lung

The myeloperoxidase activity in the lung was measured basically as described previously (Bozeman et al., 1990). Briefly, myeloperoxidase was extracted from homogenized lung by sonication in 50 mM phosphate buffer (pH 6.0) containing 0.5% hexadecyltrimethylammonium bromide (HTAB) (Sigma) for 1 min. The specimens were then subjected to freezing and thawing three times and thereafter sonicated for 1 min once again. The suspensions were centrifuged at $10{,}000 \times g$ for 10 min and myeloperoxidase activity in the supernatant was determined by measuring the change in the absorbance at 620 nm in phosphate buffer (pH 5.4) containing 1.6 mM 3,3′,5,5′-tetramethylbenzidine (Sigma) and 0.3 mM hydrogen peroxide. An enzyme unit was defined as the amount of enzyme that produced an increase of 1 absorbance unit per min.

2.5. Statistical analysis

Mortality was evaluated according to the Fisher's exact method using StatView (Abacus Concepts, Berkeley, CA). The results for myeloperoxidase measurement represent the means ± S.E.M. and the differences between groups were analyzed using Student's *t*-test. Values of $P < 0.05$ were considered as significant.

2.6. Approval of animal experiments

The protocols of the animal experiments were approved by the local ethics committee.

3. Results

3.1. PAF-induced death in mice

Mice were injected intravenously with 5–40 μg/kg of PAF and the mortality rate was evaluated. As shown in Table 1, PAF injection caused the death of the mice in a dose-dependent manner, with an LD_{90} of ~40 μg/kg. This result is consistent with the data reported previously (Terashita et al., 1987; Myers et al., 1988). Mice treated with more than 10 μg/kg of PAF started to die approximately 15 min after the PAF injection, and no mice died on and after 1 h in any group.

3.2. Effect of PAF acetylhydrolase on mortality in PAF-induced death in mice

The effect of PAF acetylhydrolase was examined in the PAF-induced death model in which 40 μg/kg of PAF was injected. PAF acetylhydrolase was administered intravenously 15 min before the PAF injection and mortality was evaluated for 24 h after the PAF injection. As shown in Table 2, pretreatment with PAF acetylhydrolase resulted in a dose-dependent reduction in the mortality rate of the mice. PAF acetylhydrolase at 1.0 mg/kg completely pro-

Table 1
The effect of PAF on mortality in mice
PAF was injected intravenously to mice and mortality was determined 24 h after the PAF injection.

PAF (μg/kg)	Number of mice (dead/tested)	Mortality (%)
5	0/20	0
10	2/20	10
20	8/20	40
40	18/20	90

Table 2
The effect of PAF acetylhydrolase on mortality in PAF-induced death model in mice
40 μg/kg of PAF was injected intravenously to mice and mortality was determined 24 h after the PAF injection. PAF acetylhydrolase was administered intravenously 15 min before the PAF injection.

PAF acetylhydrolase (mg/kg)	Number of mice (dead/tested)	Mortality (%)
0[a]	6/12	50.0
0.01	2/12	16.7
0.1	1/12	8.3
1.0	0/12	0[b]

[a]Vehicle was administered instead of PAF acetylhydrolase.
[b]$P < 0.05$, as compared with the control (Fisher's exact method).

tected the mice from death induced by the PAF injection ($P < 0.05$).

3.3. Effect of PAF acetylhydrolase on myeloperoxidase activity in the lung

It has been shown that PAF injection induces neutrophil accumulation in the lung (Terashita et al., 1987). Next therefore, the effect of PAF acetylhydrolase on myeloperoxidase activity, a specific marker enzyme for neutrophils, was examined in the lung. As shown in Fig. 1, 40 μg/kg of PAF significantly increased myeloperoxidase activity in the lung 10 min after the PAF injection. Treatment with 1.0 mg/kg of PAF acetylhydrolase 15 min before the PAF injection almost completely reversed the PAF-induced myeloperoxidase activity to the normal level.

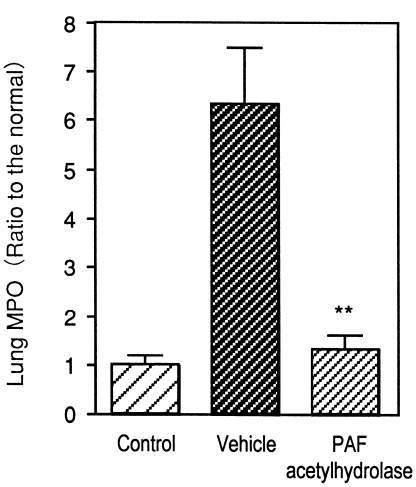

Fig. 1. The effect of PAF acetylhydrolase on myeloperoxidase activity in the lung in PAF-induced death model. A total of 40 μg/kg of PAF was injected intravenously to mice and myeloperoxidase activity in the lung was determined 10 min after the PAF injection. PAF acetylhydrolase was administered 15 min before the PAF injection. The data are presented as means ± S.E.M. for $n = 8$. $^{**}P < 0.01$, as compared with the vehicle group (Student's *t*-test).

Table 3
The effect of PAF acetylhydrolase on mortality in active anaphylactic shock model in mice
Mice were sensitized by injecting intraperitoneally 1.0 mg of bovine serum albumin in conjunction with inactivated *B. pertussis* (2×10^9 cells), and were challenged by intravenous injection of 2.0 mg of bovine serum albumin 2 weeks after sensitization. Mortality was determined 24 h after the challenge. PAF acetylhydrolase was administered intravenously 15 min before the challenge with bovine serum albumin.

PAF acetylhydrolase (mg/kg)	Number of mice (dead/tested)	Mortality (%)
0[a]	10/10	100
0.1	10/10	100
0.3	7/10	70
1.0	2/10	20[b]

[a]Vehicle was administered instead of PAF acetylhydrolase.
[b]$P < 0.01$, as compared with the control (Fisher's exact method).

3.4. Effect of PAF acetylhydrolase on mortality in active anaphylactic shock

The effect of PAF acetylhydrolase on mortality was further explored in an active anaphylactic model. As shown in Table 3, all the mice (10 out of 10 mice) died within 24 h when bovine serum albumin was injected intravenously to the mice which had been sensitized with bovine serum albumin 2 weeks before the intravenous injection of bovine serum albumin. Treatment with PAF acetylhydrolase 15 min before the intravenous injection of bovine serum albumin protected the mice from death caused by the challenge (Table 3). The effect of PAF acetylhydrolase was dose-dependent and protection was significant at 1.0 mg/kg ($P < 0.01$).

4. Discussion

Numerous studies have suggested the involvement of PAF in the pathology of systemic anaphylaxis. For example, most symptoms of the anaphylactic shock can be reproduced by the injection of PAF to animals (Imaizumi et al., 1995), and high level of PAF have been detected in the serum in rodent anaphylactic models (Pinckard et al., 1979). Also showing the importance of PAF in anaphylaxis is the fact that diverse PAF receptor antagonists significantly improve the lethality as well as bronchopulmatory response and hypotension (Arimura and Harada, 1991; Herbert et al., 1991; Hattori et al., 1996). However, the conclusion drawn from studies using such PAF receptor antagonists is not necessarily convincing as some PAF receptor antagonists exert various activities. For example, the PAF receptor receptor antagonist, CV-3988, rac-3-(*N*-*n*-octadecylcarbamoyloxy)-2-methoxypropyl 2-thiazolioethyl phosphate, which prevents death in anaphylactic models (Terashita et al., 1987), has been shown to markedly

inhibit PAF acetylhydrolase activity (Svetlov et al., 1996; Adachi et al., 1997). Such antagonists would prolong the half-life and biological actions of PAF, and therefore would not explain its beneficial effect in anaphylactic models. In the present study, administration of PAF acetylhydrolase significantly reduced the mortality of the mice in both PAF-induced (Table 2) and active anaphylactic models (Table 3). Since PAF acetylhydrolase is highly specific for degrading PAF (Tjoelker et al., 1995), our data are valuable for showing more clearly the key role of PAF in the death that occurs during the systemic anaphylactic reaction.

Recently, Ishii et al. (1998) presented evidence showing directly the dominant role of PAF in anaphylactic shock by using the PAF receptor knock-out mice. The resulting PAF receptor-deficient mice exhibited a lower mortality rate than the corresponding wild-type mice in systemic anaphylaxis elicited by antigen challenge, in agreement with our results. However, the mutant mice still show mild but significant symptoms of anaphylaxis and 14% of the mice (one out of seven) died within 50 min after the induction of anaphylaxis. It is therefore possible that other chemical mediators such as eicosanoids and histamine also participate in the lethality of anaphylaxis, while their contribution may be much less than that of PAF.

In the PAF-induced death model, there was a pronounced neutrophil accumulation in the lung 30 min following the PAF injection, judging from the increase in myeloperoxidase activity in the lung (Fig. 1). In contrast, no neutrophil accumulation was observed in the active anaphylactic model (data not shown), although mortality in this model was higher than that in the PAF-induced model where lung myeloperoxidase activity was significantly elevated (Tables 1 and 3). These findings suggest that neutrophil accumulation in the lung is not essential for the lethality seen in systemic anaphylaxis. The effect of PAF acetylhydrolase on mortality in the two models was almost the same, e.g., the minimum effective doses of PAF acetylhydrolase were both 1.0 mg/kg, suggesting that the amount of PAF produced in the active anaphylactic model is similar to that administered intravenously in the PAF-induced model. The difference in neutrophil accumulation in the lung between the two models might be explained by localization of PAF synthesized in the active anaphylaxis model and that of exogenously injected PAF.

In contrast to our results, the doses of some PAF receptor antagonists needed to block active anaphylactic shock in mice appear to be greater than those needed to protect in the PAF-induced death model (Terashita et al., 1987; Imanishi et al., 1994). This might indicate that the distribution of PAF acetylhydrolase and PAF receptor antagonists is different, i.e., PAF acetylhydrolase may be distributed to a location similar to the site where endogenous PAF is released, while the PAF receptor antagonists may not. Alternatively, the difference in effects between PAF acetylhydrolase and the PAF receptor antagonists might be explained by the difference in their half-lives. For instance, endogenous PAF is unlikely to be released rapidly after the antigen challenge (Terashita et al., 1987), and therefore, the PAF receptor antagonists with short half-lives, such as FPL-55712 (sodium 7-[3-(4-acetyl-3-hydroxy-2-propylphenoxy)-2-hydroxypropoxy]-4-oxo-8-propyl-4*H*-1-benzopyran-2-carboxylate) (Chand, 1979), would be metabolized before the endogenous PAF release. In this regard, it should be noted that PAF acetylhydrolase has a long half-life in vivo, forming a complex with lipoproteins (Stafforini et al., 1999).

In this study, mice were given human PAF acetylhydrolase but little is known about the cross-reactivity and cross-functionality between murine and human PAF acetylhydrolases. It has been shown that the composition of molecular species of PAF is not similar among species with respect to the length of the 1-*O*-alkyl chain. For example, the major 1-*O*-alkyl chain of PAF in the human is 16:0; whereas in the rat the rate is 18:0 (Mueller et al., 1984). The human PAF acetylhydrolase catalyzes hydrolysis of the sn-2 position of PAF and its analogues with short or oxidized residues at the sn-2, regardless of the length of the 1-*O*-alkyl chain, implying that human PAF acetylhydrolase degrades the major PAF of rats as well as that of humans. It is, therefore, conceivable that the human PAF acetylhydrolase would also act on the major species of PAF in mice, while there is no information regarding the structure of murine PAF.

In conclusion, PAF acetylhydrolase, an inactivating enzyme of PAF, significantly improved the mortality in PAF-induced and antigen-induced shock models. The data presented in this paper demonstrate that PAF plays an important role in the death caused in systemic anaphylaxis, and that PAF acetylhydrolase will be beneficial for treatment of patients with anaphylactic shock.

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