Characterization of platelet-activating factor–induced cutaneous edema and erythema in dogs

Miwa Watanabe
Hironori Osada
Sunao Shimizu
Shun Goto DVM
Makoto Nagai DVM, PhD
Junsuke Shirai DVM, PhD
Kazuo Sasaki DVM, PhD
Minoru Shimoda DVM, PhD
Hiroshi Itoh DVM, PhD
Keitaro Ohmori DVM, PhD

Received August 21, 2015. Accepted October 1, 2015.

From the Cooperative Department of Veterinary Medicine, Faculty of Agriculture, Tokyo University of Agriculture and Technology, 3-5-8 Saiwai-cho, Fuchu, Tokyo 183-8509, Japan.

Address correspondence to Dr. Ohmori (k-ohmori@cc.tuat.ac.jp).

OBJECTIVE
To characterize platelet-activating factor (PAF)–induced edema and erythema in the skin of dogs and compare those reactions with histamine-induced cutaneous reactions.

ANIMALS
6 healthy Beagles.

PROCEDURES
Experiments were performed at ≥ 2-week intervals. Each dog received ID injections (5 µg/site) of PAF C16, PAF C18, lyso-PAF, and histamine. Edema (mean diameter) and erythema scores (none, mild, moderate, or severe) were assessed 30 minutes after the injections. Dogs received ID injections of PAF and histamine each with various concentrations of WEB 2086 (PAF receptor antagonist) or underwent ID testing with PAF and histamine before and 3 hours after oral administration of cetirizine hydrochloride or prednisolone (at 2 doses each).

RESULTS
ID injections of PAF C16 and PAF C18, but not lyso-PAF, induced comparable levels of edema and erythema. The PAF-induced edema and erythema peaked at 30 minutes and lasted for 6 hours after the injection; histamine-induced edema and erythema peaked at 30 minutes and lasted for 3 hours after the injection. Edema sizes and erythema scores were significantly smaller and lower, respectively, for PAF than for histamine. The WEB 2086 inhibited PAF-induced but not histamine-induced edema and erythema. Cetirizine slightly, but significantly, repressed PAF-induced edema and erythema as well as histamine-induced cutaneous reactions. Prednisolone suppressed both PAF-induced and histamine-induced edema and erythema.

CONCLUSIONS AND CLINICAL RELEVANCE
In canine skin, the duration of PAF-induced inflammation was longer than that of histamine-induced inflammation. The PAF- and histamine-induced cutaneous reactions were effectively suppressed by oral administration of prednisolone. The importance of PAF in dogs with anaphylaxis and allergic disorders warrants further investigation. (Am J Vet Res 2016;77:969–975)

Platelet-activating factor (1-O-hexadecyl-2-acetyl-sn-glycero-3-phosphocholine) is a phospholipid produced by a variety of cells, including mast cells, basophils, eosinophils, neutrophils, and endothelial cells. Platelet-activating factor was originally identified as a lipid mediator capable of inducing platelet aggregation and anaphylaxis. Previous studies have also revealed that PAF secreted by basophils and neutrophils has a critical role in mouse models of IgG-mediated anaphylaxis. Therefore, in addition to histamine, it is now evident that PAF is also an essential mediator for development of anaphylaxis. Platelet-activating factor is not stored in cells but is rapidly synthesized through a remodeling pathway and a de novo pathway. Most PAFs are produced through a remodeling pathway as a result of inflammatory stimuli. In this pathway, phospholipase A₂ cleaves membrane phospholipids at the sn-2 position (second carbon molecule as determined by the stereospecific numbering system), resulting in the production of an inactive intermediate, lyso-PAF. Platelet-activating factor acetyltransferase adds an acetyl group to the sn-2 position of lyso-PAF to produce bioactive PAF. Through the removal of the acetyl group by PAF acetylhydrolase, PAF can be degraded and converted into inactive lyso-PAF. Serum PAF acetylhydrolase activity appears to be inversely correlated

ABBREVIATIONS
H1R Histamine H1 receptor
PAF Platelet-activating factor
PAFRR Platelet-activating factor receptor

AJVR • Vol 77 • No. 9 • September 2016 969

Unauthenticated | Downloaded 01/10/24 12:16 AM UTC
with the severity of anaphylaxis in humans. The molecular forms of PAF differ depending on the carbon chain length and the degree of unsaturation at the sn-1 position. The major molecular forms of PAF in vivo are PAF C16 and PAF C18 or mixtures of these homologs.

Platelet-activating factor binds to its specific receptor PAFR, which is expressed on platelets, monocytes, macrophages, neutrophils, vascular endothelial cells, and vascular smooth muscle cells. Binding of PAF to PAFRs on these cells leads to mobilization of intracellular calcium and activates several kinases, including protein kinase C and mitogen-activated protein kinase. The intracellular cascades activated by PAFRs trigger increased vascular permeability, circulatory collapse, decreased cardiac output, and acute inflammation, which are associated with development of anaphylaxis and allergic diseases. In addition, although precise mechanisms have yet to be determined, PAF is also involved in nonallergic inflammatory diseases, including sepsis, atherosclerosis, and tumorigenesis.

Platelet-activating factor–induced inflammation has not been fully characterized in dogs. In humans, PAF-mediated inflammation has been analyzed in the skin12-16 because PAF is a potent inducer of anaphylaxis. In previous studies,17,18 PAF has been injected ID into dogs; however, the studies were designed to investigate the effect of rupatadine (a dual antagonist of histamine and PAF), and PAF-induced cutaneous reactions were not evaluated in detail. Thus, the purpose of the study reported here was to characterize PAF-induced edema and erythema in the skin of dogs and compare those reactions with histamine-induced cutaneous reactions.

Materials and Methods

Animals

Six healthy sexually intact male Beagles were used in the study. The mean age ± SD of the dogs was 3.3 ± 2.4 years (range, 0.8 to 5.7 years), and mean body weight was 10.4 ± 0.9 kg (range, 9.4 to 12.0 kg). The dogs were housed in individual cages and fed a commercial diet once daily. Water was provided ad libitum. The ambient temperature was maintained at 24°C (range, 23°C to 25°C). Lights were automatically turned on at 07:00 hours and off at 19:00 hours. All procedures were approved by the Institutional Animal Care and Use Committee of Tokyo University of Agriculture and Technology.

ID testing

The hair on the left lateral aspect of the thorax of each dog was clipped 1 day before each experiment. For the test injections, PAF C16, PAF C18, lyso-PAF, and histamine were diluted with saline solution and used at a concentration of 0.1 mg/mL, acid. In previous studies, PAF has been injected ID into dogs; however, the studies were designed to investigate the effect of rupatadine (a dual antagonist of histamine and PAF), and PAF-induced cutaneous reactions were not evaluated in detail. Thus, the purpose of the study reported here was to characterize PAF-induced edema and erythema in the skin of dogs and compare those reactions with histamine-induced cutaneous reactions.

Administration of WEB 2086, cetirizine, and prednisolone

To elucidate possible mechanisms of PAF-induced cutaneous reactions, experiments were performed with WEB 2086, cetirizine, and prednisolone at ≥ 2-week intervals. The PAFR antagonist WEB 2086 was diluted with saline solution, and increasing concentrations of WEB 2086 (0, 10, 25,
and 50 µg) were each mixed with 5 µg of PAF C16 or histamine. Each mixture (total volume, 0.05 mL) was ID injected at a different skin site in each of the 6 dogs. Erythema and wheal formation were assessed 30 minutes after the injections. In 4 separate experiments, 2 doses of cetirizine (2 mg/kg and 10 mg/kg), which is an H1R antagonist, and 2 doses of prednisolone (1 and 2 mg/kg), which is a synthetic glucocorticoid, were orally administered to each of the 6 dogs. In each of these experiments, ID testing was performed before and 3 hours after the administration of cetirizine or prednisolone in each dog. For the pretreatment ID testing, PAF C16 and histamine were injected as described and erythema and wheal formation were assessed 30 minutes after the injections. For ID testing at the 3-hour time point, PAF C16 and histamine were injected as described at sites different from those used for the pretreatment ID testing, and erythema and wheal formation were assessed 30 minutes after the injections. Adverse events were not observed in the dogs that received WEB 2086, cetirizine, or prednisolone.

**Statistical analysis**

The normality of all data was analyzed by the Shapiro-Wilk test. Edema sizes were not normally distributed; therefore, edema sizes and erythema scores were analyzed by the Kruskal-Wallis test, followed by the Steel test as a post hoc analysis. In the dose-dependent experiment with PAF C16 and the experiments with WEB 2086, cetirizine, and prednisolone, edema sizes were tested by 1-way repeated-measures ANOVA, followed by the Bonferroni test. Erythema scores were analyzed by the Friedman test, followed by the Scheffe test. Erythema scores were compared between 2 groups by the Mann-Whitney U test or the Wilcoxon signed rank test. Statistical analyses were performed with commercial software. A value of $P < 0.05$ was considered significant.

![Figure 2](image1.png)

**Figure 2**—Mean ± SE cutaneous reactivity (edema sizes [A] and erythema scores [B]) following ID injection of PAF C16, PAF C18, lyso-PAF, and histamine (5 µg each/site) in 6 dogs. An equivalent volume of saline (0.9% NaCl) solution was injected ID as a negative control. Each dog received each treatment. Edema sizes and erythema scores were determined 30 minutes after ID injections were performed. Edema sizes and erythema scores were analyzed by the Kruskal-Wallis test, followed by the Steel test. Because edema sizes and erythema scores induced by the ID injections of PAF C16 and PAF C18 were not significantly different, PAF C16 was used in the subsequent experiments. *Value is significantly ($P < 0.05$) different from that for the saline solution injection. A bar over 2 columns indicates no significant difference (NS) between the 2 values. SS = Saline solution. See Figure 1 for key.

![Figure 3](image2.png)

**Figure 3**—Mean ± SE cutaneous reactivity (edema sizes [A] and erythema scores [B]) following ID injections of PAF C16 in increasing concentrations in 6 dogs. Concentrations of PAF C16 injected ID ranged from 0.3125 to 10 µg/site; an equivalent volume of saline solution was injected ID to represent a dose of 0 µg of PAF. Each dog received each treatment. Edema sizes and erythema scores were determined 30 minutes after ID injections were performed. Edema sizes were tested by 1-way repeated-measures ANOVA, followed by the Bonferroni test. Erythema scores were analyzed by the Friedman test, followed by the Scheffe test. See Figures 1 and 2 for key.
Results

Characterization of cutaneous reactivity to PAF

Among the 6 dogs, ID injections of PAF C16, PAF C18, lyso-PAF, and histamine were associated with significantly (P < 0.05) larger edema sizes, compared with the effect of the ID injection of saline solution (Figure 2). Erythema scores for ID injections of PAF C16, PAF C18, and histamine were significantly (P < 0.05) higher than that for the ID injection of saline solution. Intradermal injection of lyso-PAF did not elicit erythema.

Because edema sizes and erythema scores induced by the ID injections of PAF C16 and PAF C18 were not significantly different (P > 0.05; Figure 2), PAF C16 was used in the subsequent experiments. Intradermal injection of increasing concentrations of PAF C16 resulted in increasingly severe edema and erythema in a dose-dependent manner at 30 minutes after the injection (Figure 3). Edema and erythema caused by PAF C16 peaked at 30 minutes and lasted for 6 hours after the ID injection, whereas those changes induced by histamine peaked at 30 minutes and lasted for 3 hours after the ID injection (Figure 4). Edema and erythema were not observed at 12, 24, and 48 hours after the ID injections of PAF C16 and histamine (data not shown). Edema sizes were significantly (P < 0.05) larger for histamine than for PAF C16 at 15 minutes, 30 minutes, and 1 hour after the injection. Erythema scores were significantly (P < 0.05) higher for histamine than for PAF C16 at 15 minutes after the ID injection.

Effects of a PAFR antagonist on cutaneous reactivity to PAF

Among the 6 dogs, ID injections of increasing concentrations of WEB 2086 significantly (P < 0.05) inhibited PAF-induced edema and erythema in a dose-dependent manner. However, WEB 2086 did not suppress histamine-induced edema and erythema (P > 0.05; Figure 5).

Effects of an H1R antagonist on ID reactivity to PAF

Among the 6 dogs, ID injections of increasing

Figure 4—Mean ± SE cutaneous reactivity (edema sizes [A] and erythema scores [B]) at intervals following ID injection (5 µg each/site) of PAF C16 (black bars) and histamine (white bars) in 6 dogs. Each dog received each treatment. Edema sizes and erythema scores were determined at 5, 15, 30 minutes and 1, 3, 6, 12, 24, and 48 hours after ID injections were performed. Edema sizes and erythema scores of PAF C16 and histamine were compared by use of an unpaired t test and the Mann-Whitney U test, respectively. *Values bracketed by the bar are significantly (P < 0.05) different. See Figure 1 for remainder of key.

Figure 5—Mean ± SE cutaneous reactivity (edema sizes [A] and erythema scores [B]) following ID injection (5 µg each/site) of PAF C16 and histamine each with increasing concentrations of WEB 2086 in 6 dogs. Concentrations of WEB 2086 injected ID ranged from 10 to 50 µg/site; an equivalent volume of SS was injected ID to represent a dose of 0 µg of WEB 2086. Each dog received each treatment. Edema sizes and erythema scores were determined 30 minutes after ID injections were performed. Edema sizes for ID injections containing PAF C16 were analyzed by 1-way repeated-measures ANOVA, followed by the Bonferroni test. Erythema scores for ID injections containing PAF C16 were analyzed by the Friedman test, followed by the Scheffe test. Edema sizes and erythema scores for ID injections containing histamine were analyzed by a paired t test and the Wilcoxon signed rank test, respectively. See Figures 1 and 2 for key.
concentrations of cetirizine slightly, but significantly \((P < 0.05)\), inhibited PAF-induced edema and erythema. Also, cetirizine inhibited histamine-induced reactions in a dose-dependent manner \((P < 0.05; \text{Figure 6})\).

**Effects of prednisolone on cutaneous reactivity to PAF**

Among the 6 dogs, ID injections of increasing concentrations of prednisolone significantly \((P < 0.05)\) inhibited PAF-induced edema and erythema in a dose-dependent manner. Prednisolone inhibited histamine-induced edema and inhibited erythema in a dose-dependent manner \((P < 0.05; \text{Figure 7})\).

**Discussion**

In the present study, ID injections of PAF C16 and PAF C18 induced wheals characterized by edema and erythema in the skin of dogs. The biological activity of PAF is determined by the type (or mixture) of its homologs, the major ones of which are PAF C16 and PAF C18.\(^{11}\) In a previous study,\(^{11}\) PAF C18 was shown to be more active as a chemoattractant of human neutrophils than PAF C16. In contrast, in the same study,\(^{11}\) cutaneous inflammatory reactions induced by PAF C16 and PAF C18 in humans were comparable. The present study revealed that the effects in canine skin of PAF C16 were not significantly different from those of PAF C18. Therefore, it can be concluded that PAF-induced cutaneous edema and erythema in the skin of dogs are not affected by the type of PAF homologs.

Although lyso-PAF, an intermediate of PAF, has been considered inactive in most biological processes,\(^{1}\) the biochemical nature of lyso-PAF remains unclear. A recent study\(^{20}\) revealed inhibitory effects of lyso-PAF on human neutrophil and platelet activation. In addition, murine and human eosinophils were shown to degranulate in response to PAF and lyso-PAF simulation.\(^{21}\) In the present study, an ID injection of lyso-PAF induced a slight amount of edema, compared with the effect of an ID injection of saline solution; however, the slight lyso-PAF-induced edema was not accompanied by erythema, indicating that the edema was not a result of an inflammatory response. Although the mechanism by which lyso-PAF caused the slight amount of edema remains unclear, the results of the present study have indicated that...
the administered ID dose of lyso-PAF was not sufficient to induce inflammatory responses in the dogs' skin.

In anaphylaxis and allergic disorders, histamine is rapidly secreted after degranulation of mast cells and basophils as a preformed mediator in response to allergen stimulation.22 On the other hand, PAF is produced and secreted within minutes as a newly generated lipid-derived mediator in response to various stimuli by several types of cells, including mast cells, basophils, neutrophils, eosinophils, and macrophages.1 In the present study, edema sizes and erythema scores for PAF C16 were significantly smaller and lower than those induced by histamine at early time points from 15 minutes to 1 hour after the ID injections. However, edema and erythema caused by PAF C16 lasted for 6 hours after the ID injection, which was longer than the edema and erythema induced by ID injection of histamine. These results suggested that histamine is involved in the initiation of anaphylaxis and allergic reactions, whereas PAF contributes to the initiation and prolongation of these reactions. Histamine and PAF may have both redundant and nonredundant roles in anaphylaxis and allergic diseases in dogs, depending on the phase of the reactions.

To elucidate possible mechanisms that regulate PAF-induced edema and erythema, the dogs in the present study received separate ID injections of PAF or histamine, each mixed with a PAFR antagonist, WEB 2086. The results clearly indicated that WEB 2086 potently inhibited PAF-induced but not histamine-induced cutaneous reactions in a dose-dependent manner, indicating that PAFR signaling is crucial for development of PAF-associated edema and erythema in the skin of dogs.

Results of the present study also indicated that an H1R antagonist, cetirizine, slightly but significantly reduced PAF-induced cutaneous reactions in dogs, suggesting that PAF-induced edema and erythema are at least in part mediated through H1Rs. Similar findings were reported for humans, in which several H1R antagonists, including cetirizine, suppressed PAF-induced wheal development in the skin.14,15,23 An ID injection of PAF was shown to induce histamine release in the human skin, which was reduced by local nerve blockade.15 Platelet-activating factor cannot induce histamine release from human skin-devoid mast cells because of the absence of PAFRs on these cells, although PAF can directly elicit degranulation of human lung-derived and peripheral blood-derived mast cells through PAFR expression on those cells.24 Direct activation of H1Rs by PAF is unlikely on the basis of their structures. It is therefore possible that PAF activates PAFR-expressing neurons in the skin, thereby releasing neuropeptides that stimulate skin mast cells to release histamine. However, a recent study16 revealed that cutaneous reactions induced by PAF were not associated with histamine release and mast cell activation in the skin of humans.

Further studies are required to clarify the role of H1Rs in PAF-induced edema and erythema.

In the present study, administration of prednisolone attenuated PAF-induced and histamine-induced cutaneous edema and erythema. The oral doses of prednisolone in this study were 1 and 2 mg/kg, which are commonly used as an anti-inflammatory dose and an immunosuppressive dose, respectively, in veterinary medicine. Therefore, it is conceivable that prednisolone, when administered as an anti-inflammatory dose or an immunosuppressive dose, can be an effective treatment option for PAF-mediated inflammation in dogs.

In humans with anaphylaxis, mean serum PAF concentrations have been reported to be 805 pg/mL.6 However, actual PAF concentrations in serum and local tissues during anaphylaxis are considered to be much higher because PAF is rapidly degraded, having a half-life of approximately 5 minutes in plasma and whole blood.25 Actual PAF concentrations associated with various diseases have never been determined owing to the technical difficulty of doing so. In the present study in dogs, PAF was injected ID at a dose of 5 µg/site (except for doses used in the dose-dependent experiment). The PAF dose was determined according to the histamine dose (5 µg/site) that has been recommended for ID testing in dogs.19 By use of the same dose, we were able to compare the ability of PAF with that of histamine to induce cutaneous reactions.

Accumulated evidence indicates that PAF has pivotal roles in various diseases in humans, including anaphylaxis and allergic and nonallergic diseases.3 In addition, PAF and PAFRs have been investigated as possible therapeutic targets for PAF-mediated inflammatory diseases in humans.1 In the present study, PAF-induced edema and erythema in the skin of dogs have been characterized. These findings provide a rationale to further examine the role of PAF in the pathogenesis of anaphylaxis and allergic and nonallergic diseases in dogs.

Footnotes

a. Science Diet Adult, Hill’s-Colgate (Japan) Ltd, Tokyo, Japan.
b. PAF C16 (β-acetyl-α-hexadecyl-L-α-phosphatidylcholine hydrate), Sigma-Aldrich Corp, St Louis, Mo.
c. PAF C18 (β-acetyl-α-octadecyl-L-α-phosphatidylcholine), Sigma-Aldrich Corp, St Louis, Mo.
d. Lyso-PAF (1-O-palmitoyl-sn-glycero-3-phosphocholine), Sigma-Aldrich Corp, St Louis, Mo.
e. Histamine, Sigma-Aldrich Corp, St Louis, Mo.
f. WEB 2086, Sigma-Aldrich Corp, St Louis, Mo.
g. Cetirizine hydrochloride tablets, NIPRO, Osaka, Japan.
h. Prednisolone, Pfizer, Tokyo, Japan.
i. Ekuseru-Toukei 2015, Social Survey Research Information Co Ltd, Tokyo, Japan.

References


2. Benveniste J. Platelet-activating factor, a new mediator of


