Platelet-activating factor, histamine, and tryptase levels in human anaphylaxis

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Background: Platelet-activating factor (PAF) is an important mediator and correlates with anaphylaxis severity. How well PAF correlates with severity relative to histamine or tryptase is not known.

Objective: To analyze the levels of PAF, histamine, and tryptase as a function of severity in patients with acute allergic reactions.

Methods: PAF, histamine, and tryptase levels were measured in blood samples collected from 23 healthy volunteers and from 41 patients during acute allergic reactions. Reactions were stratified by severity from grade 1 (least severe) to grade 3 (most severe).

Results: Among the 3 reaction grades, there were significant differences by ANOVA for PAF ($P < .0001$). The proportion of elevated PAF values increased across severity groups ($P = .0009$). Increased PAF levels were observed in 20%, 66.7%, and 100% of the patients with grades 1, 2, and 3 allergic reactions, respectively. While the proportion of elevated histamine values increased from 40% to 57% to 70% across grades 1, 2, and 3, respectively, these were not significantly different ($P = .40$). For tryptase, the proportion of elevated values increased monotonically from 0 in grade 1 to 4.8% in grade 2 to 60% in grade 3 ($P = .0002$).

Conclusions: The PAF level was significantly elevated in proportion to the severity of acute allergic reactions. Whereas the PAF level was elevated in all patients with severe anaphylaxis, this was not true for either histamine or tryptase. Neither histamine nor tryptase showed as good correlations with severity scores as did PAF. These data are consistent with a pivotal role for PAF as a mediator of anaphylaxis. (J Allergy Clin Immunol 2013;131:144-9.)

Key words: *Platelet-activating factor, histamine, tryptase, anaphylaxis, emergency department*

Abbreviations used
PAF: Platelet-activating factor
PAF-AH: PAF acetylhydrolase

Anaphylactic reactions are rapid, potentially fatal, immediate hypersensitivity reactions with multisystem involvement. The major manifestations include bronchoconstriction, pulmonary hypertension, systemic hypotension, and vascular leakage.[1,2] Biochemical mediators and chemotactic substances are released systemically during the degranulation of mast cells and basophils.[3] These include preformed chemicals, such as histamine, tryptase, chymase, and heparin, and newly synthesized lipid mediators, such as prostaglandin D2, leukotrienes, and platelet-activating factor (PAF).[4] PAF is a proinflammatory phospholipid synthesized and secreted by mast cells, monocytes, and fixed tissue macrophages.[5] The binding of PAF to its receptor on target cells—platelets, monocytes, macrophages, and neutrophils—results in many of the manifestations of acute allergic reactions and anaphylaxis.[2]

Exogenous PAF reproduces many of the manifestations of experimental anaphylaxis in animals.[6] *In vitro*, PAF is rapidly synthesized and released from antigen-stimulated mast cells and basophils.[7] *In vivo*, high levels of circulating PAF have been detected after antigen challenge in sensitized animals,[8] and in human anaphylaxis.[9,10] PAF receptor antagonists, which inhibit the binding of PAF to the receptor, reduce anaphylaxis-related mortality in animal models.[5,11] PAF receptor knockout mice exhibited a lower mortality rate to systemic anaphylaxis than did wild-type mice.[12] Moreover, enzymatic inactivation of PAF by PAF acetylhydrolase (PAF-AH) was shown to protect against anaphylaxis in mice pretreated with recombinant human PAF-AH.[13] Conversely, deficiency of PAF-AH predisposed patients to severe or fatal anaphylaxis.[10]

The purpose of this study was to analyze the levels of PAF, histamine, and tryptase as a function of severity in patients treated for acute allergic reactions.

METHODS

Study design

This was a cross-sectional study of 41 patients who presented to the emergency department of a university teaching hospital with acute allergic reactions. Twenty-three healthy volunteers served as controls. The study was approved by the Research Ethics Board at St Michael's Hospital. Informed consent was obtained from all volunteers, patients, or their parents or guardians.

Patients were recruited into the study if they met case-definition criteria for allergic reactions as described by Brown et al.[14] The severity of the allergic reactions was also evaluated according to the criteria described by Brown et al.[14] Grade 1 patients had acute allergic reactions with cutaneous involvement and no other organ system involvement. Those with grade 2 reactions had mild-to-moderate manifestations of anaphylaxis (systolic blood pressure > 90 mm Hg, respiratory rate < 25 breaths per minute). Patients with grade 3 reactions had severe manifestations, with cutaneous, gastrointestinal, and potentially life-threatening respiratory or cardiovascular signs and symptoms.

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Blood sample collection

Venous blood samples for the measurement of PAF level were collected in EDTA and immediately added to an equal volume of 20% acetic acid (to inactivate endogenous PAF-AH). For the measurement of histamine and tryptase levels, blood samples without anticoagulant were centrifuged for 10 minutes at 4°C to obtain cell-free serum. In all cases, blood was collected before the initiation of therapy.

Materials

PAF ^{3}H scintillation proximity assay system was supplied by Amersham Biosciences (Oakville, Ontario, Canada). All other chemicals were purchased from Sigma-Aldrich Canada Ltd (Oakville, Ontario, Canada).

Measurement of PAF level

Blood samples were assayed for PAF as described previously[10] by using the PAF-scintillation proximity assay system (Amersham Biosciences). PAF level was measured in the range of 0.2 to 12.8 ng/mL in the sample. Assay specificity and sensitivity were determined by the addition of known amounts of PAF and subjecting each concentration to the same extraction and purification procedures followed by scintillation proximity assay.

Histamine level was measured by ELISA according to manufacturer instructions (EIA Kit; Cayman Chemical, Ann Arbor, Mich). Samples (in triplicate) were centrifuged at 1600*g* for 20 minutes. The enzyme immune assay was based on the competition between unlabeled derivatized histamine and acetylcholinesterase linked to histamine (tracer) for limited specific mouse antihistamine antibody sites. Derivatization was needed to increase the affinity of histamine to the antibody and thereby increase the sensitivity of the assay.

The concentration of total tryptase was measured in triplicate serum samples by using UniCap-Tryptasefluoro-immunoassay (Pharmacia & Upjohn, Uppsala, Sweden).

Statistical analysis

The large SDs relative to the mean for the chemical mediators suggested that the distributions were skewed and log transformation should be considered. For subjects with 0 values for PAF (observed in controls only), 1 was added prior to log transformation. Values of the chemical mediators between groups were compared with the *t* test (for 2 groups) and ANOVA (for 3 or more groups) when considering the mediators on a continuous scale and the chi-square statistic for categorical variables (ie, considering the mediators as normal vs elevated), as appropriate. Analyses were conducted by using SAS, version 8.2 (SAS, Cary, NC).

RESULTS

Demographics

Forty-one patients and 23 healthy volunteers were enrolled in the study and included in the statistical analyses. The mean age of patients was 35.2 ± 13.9 years (range, 15-74 years), and 26 patients (63%) were female. The mean age of healthy volunteers was 30.8 ± 9.8 years (range, 20-51 years), and 7 volunteers (30%) were female. Prior to presentation in the emergency department, 36 of the 41 patients had known histories of allergy confirmed by appropriate diagnostic testing and were exposed to their respective allergen at the time of the acute reaction. The prevalence of asthma as a function of severity of allergic reactions was as follows: grade 1, 1 of 10 (10%); grade 2, 6 of 21 (28.5%); grade 3, 3 of 10 (30%). Allergic reactions were triggered by foods (n = 22), drugs (n = 12), and insect stings (n = 2), and the remainder were idiopathic (n = 5). Ten patients had grade 1 reactions, 21 patients had grade 2 reactions, and 10 patients had grade 3 reactions.

PAF, histamine, and tryptase levels in blood

Platelet activating factor. *Analysis on a continuous scale.* As a group, mean serum PAF levels among patients with acute allergic reactions (mean ± SD, 805 ± 595 pg/mL) were significantly greater than those among controls (127 ± 104 pg/mL) whether untransformed ($P < .0001$) or log-transformed ($P < .0001$). PAF levels were observed to be statistically significantly different among the patients with grades 1, 2, and 3 allergic reactions (ANOVA; $P < .0001$ untransformed or log-transformed). Both explained similar amounts of variation (R^2 65% untransformed; 57% transformed).

Comparisons of untransformed data demonstrated that PAF serum levels of patients with grade 3 allergic reaction were significantly different from those of patients with grades 1 and 2 allergic reactions (grade 3 vs 1, $P = .0006$, and grade 3 vs 2, $P = .0128$); however, PAF serum levels of patients with grades 1 and 2 allergic reactions were not significantly different (1 vs 2, $P = .0684$, NS). Comparisons between grades of log-transformed data showed that for the log-transformed values, the controls were significantly different than grade 1 ($P = .0002$), grade 2 ($P < .0001$), and grade 3 anaphylaxis ($P < .0001$), while for untransformed data, the controls were not different from grade 1 but were significantly different from grades 2 and 3 anaphylactic reactions.

Analysis as categorical data (normal vs elevated). Using the cutoff of 400 pg/mL as the upper limit of normal, PAF levels were elevated in 26 (63%) of allergic patients combined but in no controls ($P = .0006$ by χ^2 test with 1 degree of freedom). Across the 4 groups, the proportion that was elevated increased monotonically from 0 in controls to 2 (20%) of grade 1, 14 (66.7%) of grade 2, and all 10 (100%) of grade 3 reactions ($P < .0001$ with 3 degrees of freedom).

Histamine. *Analysis on a continuous scale.* Analysis of the untransformed data of serum histamine on a continuous scale demonstrated that serum histamine levels achieved only borderline significance when log-transformed (ANOVA; $P = .06$) among patients with grades 1, 2, and 3 anaphylaxis. Comparisons between grades showed significant differences between grade 1 versus grade 3 and grade 2 versus grade 3 (untransformed).

Tryptase. *Analysis on a continuous scale.* Analysis of tryptase serum levels showed these to be significantly different among patients in the 3 allergic reaction groups in both untransformed and log-transformed data (ANOVA; whether untransformed or log-transformed $P < .0001$). Comparisons between grades showed serum tryptase levels to be significantly different in patients with grade 3 allergic reaction than in patients with both grades 1 and 2 allergic reactions whether the data were untransformed or log-transformed (grade 1 vs 3, $P = .0006$; grade 2 vs 3, $P = .0086$). Serum tryptase levels were borderline significant between patients with grade 1 and grade 2 allergic reactions ($P = .0484$).

Comparison of 3 mediators among allergic patients

Analysis on a continuous scale. *PAF.* Among the 3 grades of allergic subjects themselves, there were significant differences by ANOVA for PAF ($P < .0001$ whether untransformed or log-transformed). The log-transformed data explained slightly more of the variation between groups. Comparisons between grades showed that for the untransformed data, grade 3 was significantly different from grades