

BRIEF COMMUNICATION

Platelet-activating factor (PAF) induces wheal and flare skin reactions independent of mast cell degranulation

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Keywords

histamine; microdialysis; platelet-activating factor; prostaglandin D₂; wheal and flare.

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Abstract

Background: Platelet-activating factor (PAF) causes wheal and flare responses which are abrogated by H₁-antihistamines giving rise to the hypothesis that PAF-induced wheal development is secondary to histamine release from dermal mast cells. But is this hypothesis correct?

Methods: Wheal and flare responses were induced by intradermal injection of PAF, codeine and histamine in 14 healthy volunteers. Dermal histamine and PGD₂ contractions were measured using microdialysis.

Results: PAF, unlike histamine and codeine, did not cause a statistically significant rise in mean histamine levels with ten persons showing negligible histamine release. Codeine caused a significant but variable histamine release, ranging from 29 to 282 ng/ml. Codeine, but not PAF or histamine, caused a small but statistically significant release of PGD₂.

Conclusion: Wheal and flare reactions in human skin induced by PAF are not associated with histamine release and, therefore, appear to be independent of mast cell degranulation.

Platelet-activating factor (PAF) is a lipid-derived pro-inflammatory mediator, which has been implicated in the pathogenesis of allergic disease. Its possible involvement as a mediator of urticaria stems from observations in 1984 (1) that intradermal injection of PAF resulted in a wheal and flare response, a finding that has been replicated many times. Furthermore, cold water challenge of cold urticaria patients results in the release into the bloodstream of a PAF-like lipid (2).

The mechanism by which PAF causes wheal responses is unclear. That PAF-induced wheal development is secondary to histamine release from dermal mast cells has been suggested from the ability of a wide variety of histamine H₁-antihistamines, including azelastine, ketotifen (3), loratadine (4), cetirizine (5) and rupatadine (6), to abrogate the response.

But is this hypothesis correct? Although PAF releases histamine from human lung mast cells *in vitro* (7), it does not induce release from those dispersed from the dermis (7,8). In a microdialysis study (9), intradermal injection of 25 µl of 18 µM PAF induced a small and variable histamine release, approximately 10% of that released following injection of 750 µM codeine. Furthermore, release was reduced by local nerve blockade.

To address the mechanism of PAF-induced wheal and flare responses, we used microdialysis to recover histamine and

prostaglandin D₂ (PGD₂) from the skin following intradermal injection of PAF, codeine and histamine.

Methods

Participants

Fourteen healthy volunteers (11 men, 3 women; age, 20–36 years) participated in this exploratory study conducted at the Department of Dermatology and Allergy, Charité-Universitätsmedizin Berlin (*n* = 4) and Department of Dermatology, Hospital del Mar, Barcelona (*n* = 10). This study was conducted in accordance with the Declaration of Helsinki, International Conference on Harmonization Good Clinical Practice guidelines and FDA Institutional Review Board regulations and was approved by the Institutional Review Board/Independent Ethics Committee. All participants provided written informed consent.

Study design

Intradermal injections of 25 µl each of histamine (1 mg/ml, ALK Scherax, Germany), codeine (0.09%, Charité-Berlin

Table 1 Wheal and flare diameters and microdialysate levels of histamine and PGD₂ before and after intradermal injection of PAF, codeine and histamine

Provocant	Before provocation	After provocation	Significance of difference (<i>P</i>)
PAF			
Wheal diameter (mm)	–	11.0 ± 1.3	–
Flare diameter (mm)	–	20.7 ± 2.4	–
Histamine (ng/ml)	19.2 ± 4.4	23.9 ± 2.4	0.201
PGD ₂ (pg/ml)	2.6 ± 0.7	4.4 ± 0.7	0.150
Codeine			
Wheal diameter (mm)	–	13.6 ± 0.9	–
Flare diameter (mm)	–	37.7 ± 3.2	–
Histamine (ng/ml)	13.0 ± 4.2	128 ± 26	0.0006
PGD ₂ (pg/ml)	2.0 ± 0.6	19.8 ± 5.7	0.013
Histamine			
Wheal diameter (mm)	–	18.1 ± 1.1	–
Flare diameter (mm)	–	46.4 ± 2.6	–
Histamine (ng/ml)	12.3 ± 3.6	253 ± 11	<0.0001
PGD ₂ (pg/ml)	1.5 ± 0.4	4.7 ± 1.4	0.050

Intradermal injections of 25 µl each of PAF (18 µM), codeine (0.09%) and histamine (1 mg/ml) were made into the volar forearm of 9–10 individuals. Wheal and flare diameters were measured 10 min after provocation. Results are expressed as mean ± SEM. There were no statistically significant differences between the 'before provocation' levels of histamine or PGD₂.

pharmacy, Germany) and PAF (18 µM, Sigma Aldrich, UK) were performed in the volar forearm. At 5, 10, 15 and 30 min, 1, 2 and 3 h after provocation, wheal and flare diameters were measured with a transparent ruler as the mean of the largest diameter and the diameter at right angles to this.

Dermal microdialysis was used to determine release of histamine and PGD₂. Six microdialysis fibres (3000 kDa molecular mass cut-off) were inserted intradermally in parallel pairs (with a distance of 0.5 cm within a pair of fibres) into the volar surface of each forearm of volunteers under topical local anaesthesia (EMLA cream, Astra Pharmaceuticals, Kings Langley, UK) as previously described (10). Each membrane ran for a length of 20 mm, at a depth of approximately 0.7 mm. A 1-hour period was allowed for the recovery from local anaesthesia and trauma before the start of perfusion with Ringer's solution at a rate of 3 µl/min using a microinfusion pump. To assess mediator levels, dialysate was collected for 30 min before (baseline) and 20–30 min after intradermal injection of PAF, codeine and histamine, between each pair of fibres. Dialysate samples were collected in vials and stored at –80°C before analysis. Histamine was measured by a Hista-reader REFlab®, Copenhagen, Denmark and PGD₂ by ELISA (PGD₂ MOX EIA assay, Cayman).

Statistical analysis

Statistical significance was calculated using Student's *t*-test for paired data. Statistical significance was considered at *P* < 0.05 (two-sided).

Results

Intradermal injections of PAF, codeine and histamine resulted in typical wheal and flare type responses, which became maximal 5 to 15 min and then declined slowly over

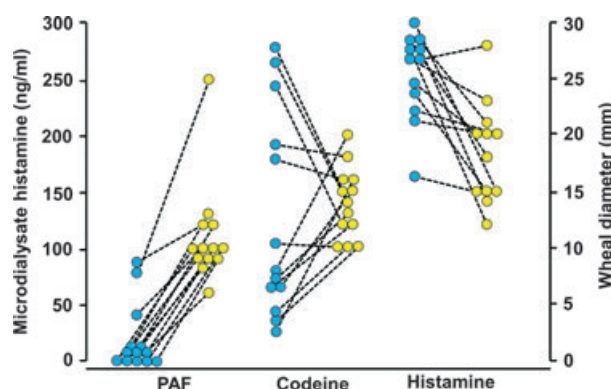


Figure 1 Individual concentrations of histamine recovered from the dermis by microdialysis (blue circles) and corresponding wheal responses (yellow circles) following intradermal injection of 25 µl of PAF (18 µM), codeine (0.09%) and histamine (1 mg/ml).

the next 2 to 3 h. At 10 min, the flare/wheal ratio for PAF of 2.0 ± 0.24 was significantly lower than those for histamine (2.7 ± 0.24 , *P* = 0.02) and codeine (2.8 ± 0.17 , *P* = 0.01).

Skin microdialysis demonstrated that PAF, unlike histamine and codeine, did not cause a statistically significant rise in mean histamine levels (Table 1). Examination of the individual results reveals that with PAF there was negligible histamine release in ten persons and only low levels in the others (Fig. 1). Codeine caused a significant but variable histamine release, ranging from 29 to 282 ng/ml. Dermal histamine levels after histamine injection were higher and more constant, ranging from 158 to 305 ng/ml. Codeine, but not PAF or histamine, caused a small but statistically significant release of PGD₂ (Table 1).

Following histamine injection, the mean histamine/wheel ratio (microdialysis histamine concentration divided by wheel diameter) is 14.1 ± 1.5 ng/ml/mm. The corresponding ratio for PAF is 2.5 ± 0.7 ng/ml/mm, suggesting that approximately 80% of the wheal response to PAF is mediated by PAF itself or an unidentified secondary mediator. The ratio for codeine is 9.2 ± 1.8 ng/ml/mm.

Discussion

The hypothesis that skin wheal and flare responses to PAF are secondary to mast cell activation, and histamine release appears to be incorrect. Our results clearly demonstrate that intradermal PAF injections induce robust wheals and flares, but do not induce significant release of histamine or PGD₂ from skin mast cells. Interestingly, histamine release by codeine, a nonimmunological histamine releaser (11), was surprisingly inconsistent in this study, indicating a wide intra-individual variability of mast cell sensitivity to this agent. The low level of PGD₂ generated with this agent is consistent with previous studies with similar histamine releasers (12).

The ability of PAF to cause an H₁-antihistamine-sensitive wheal and flare response in the absence of demonstrable histamine release is not without precedent. This has also been shown with bradykinin (13,14). The failure of cetirizine, which is particularly effective in blocking bradykinin responses, to interact with bradykinin B1 or B2 receptors (14) suggests cooperation between or heterodimerization of

the receptors for histamine and bradykinin, both of which are class A G-protein-coupled receptors (15). We suggest the same may be possible for histamine-H₁ and PAF receptors.

In conclusion, PAF-induced wheal and flare-induced skin reactions in human skin are not associated with histamine release and, therefore, appear to be independent of mast cell degranulation. These findings have implications for understanding the mechanisms of inflammatory skin diseases.

Author contributions

Karoline Krause, Ana Giménez-Arnau, Martin Church and Marcus Maurer were responsible for experimental design, statistics and preparation of the manuscript. Estela Martínez-Escala, Magi Farré-Albadalejo and Marina Abajian were responsible for the patient recruitment, practical aspects and assistance with preparation of the manuscript.

Conflict of interest

Karoline Krause has received honoraria from Dr. Pflieger GmbH and Novartis. Martin Church has been a speaker or consultant for Almirall, FAES Pharma, Menarini, MSD, UCB Pharma, Sanofi-Aventis and Uriach. Marcus Maurer has been a speaker or consultant for Almirall, FAES Pharma, Genentech, Menarini, MSD, Novartis, UCB Pharma, Sanofi-Aventis and Uriach. Ana Giménez-Arnau, Estela Martínez-Escala, Magi Farré-Albadalejo and Marina Abajian declare no conflict of interest.

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