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Effects of serum albumin, indomethacin and histamine H_1-antagonists on Paf-acether-induced inflammatory responses in the skin of experimental animals and man

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1 Cutaneous responses to synthetic platelet activating factor (Paf-acether) have been studied in guinea-pig and human skin.

2 Intradermal injection of Paf-acether elicited an acute inflammatory response in guinea-pig skin (assessed by means of radioisotopic techniques) and acute oedema formation in human skin (assessed by means of weal volume and flare area).

3 Acute inflammatory responses in guinea-pig and human skin are potentiated by the presence of serum albumin, a phospholipid carrier.

4 Acute inflammatory responses induced by Paf-acether in guinea-pig and human skin are not significantly affected by concomitant administration of the cyclo-oxygenase inhibitor, indomethacin.

5 Acute inflammatory responses induced by Paf-acether in guinea-pig and human skin are slightly modified by the H_1-receptor antagonists, mepyramine and chlorpheniramine.

6 These results indicate that the acute inflammatory response induced by Paf-acether is independent of cyclo-oxygenase products of arachidonic acid and that histamine release has a minor contribution to the inflammatory response induced by Paf-acether.

Introduction

Originally described as platelet activating factor, Paf-acether is now recognized as the ether-linked phospholipid, 1-O-alkyl-2-acetyl-sn-glyceryl-3-phosphorylcholine, a potent biologically active material that can be released from a variety of inflammatory cells (Page *et al.*, 1983). On intradermal injection, Paf-acether has been shown to elicit acute extravasation of plasma protein, accompanied by platelet and neutrophil accumulation in the skin of experimental animals (Morley *et al.*, 1983; Pirotzky *et al.*, 1984). In man, intradermal injection of Paf-acether elicits a biphasic inflammatory response reminiscent of that induced by antigen in suitably sensitized individuals (Archer *et al.*, 1984; Basran *et al.*, 1984). The mechanism of this inflammatory response has not been previously characterized. In the present study, the effects of histamine H_1-antagonists, a cyclo-oxygenase inhibitor and a phospholipid carrier on Paf-acether-induced inflammatory responses in the skin of guinea-pigs and of normal human volunteers have been evaluated.

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Methods

Experimental animals

Male Dunkin-Hartley guinea-pigs (450–500 g body weight) were used throughout.

Assessment of plasma protein extravasation

The flank skin of animals was shaved at least 2 h before experiments and intradermal injections (0.1 ml) were allocated to marked sites according to balanced Latin square designs.

Plasma protein extravasation was measured using isotopically labelled albumin (Morley *et al.*, 1983).

Each animal was injected intravenously with 0.5 ml Evans blue dye (2.5% w/v in phosphate buffered saline (PBS); Sigma) containing 1.5 μCi [^{125}I]-human serum albumin [^{125}I]-HSA; Amersham International, PLC). Forty minutes after intradermal injection, the animals were killed and bled (1 ml blood was collected). Discs of skin containing whole lesions were removed with a metal wad punch and counted in an automatic gamma spectrometer (Nuclear Enterprises). Extravasation of plasma protein into each lesion was calculated by expressing responses as equivalent μl of whole blood (^{125}I counts in lesions/^{125}I counts in 1 μl of whole blood) and subtracting the values obtained at sites injected with diluent alone.

Subjects

Twenty four healthy, non-atopic volunteers, aged between 21 and 39 years, gave informed consent to participate in the study. Permission for these studies was granted by the Hospital Ethical Committee.

Assessment of cutaneous inflammatory responses in human skin

Six volunteers received intradermal injections of coded solutions of Paf-acether at doses of 10, 30 and 100 ng, both in the presence and absence of 0.25% human serum albumin. Solutions were prepared in PBS and a constant volume (50 μl) injected at marked sites on the flexor aspect of the forearms.

Twelve subjects were given intradermal injections of coded solutions containing Paf-acether (200 ng), histamine acid phosphate (0.5 μg base) or the histamine liberator, codeine phosphate (10 μg) alone, or admixed with chlorpheniramine (1.5 μg). Skin-fold thickness was measured using a low-tension spring-loaded thickness gauge (Mitutoyo, Japan) immediately before injection and 15 min after injection, when weal responses were maximal. Two orthogonal weal diameters were measured and weal volume calculated as weal area × half the increase in skin-fold thickness. Flare area was determined by measurement of two orthogonal diameters 5 min after injection, the time of maximal response. The presence or absence of a late-onset area of erythema at the site of the resolved weal was recorded.

In a separate series of experiments, 12 subjects received intradermal injections of Paf-acether (200 ng) and weal volume and flare area were assessed 15 and 5 min after administration, respectively. Weal volume measurement was followed by ingestion of indomethacin (25 mg) and, 1 h later, Paf-acether was administered at an equivalent injection site on the opposite

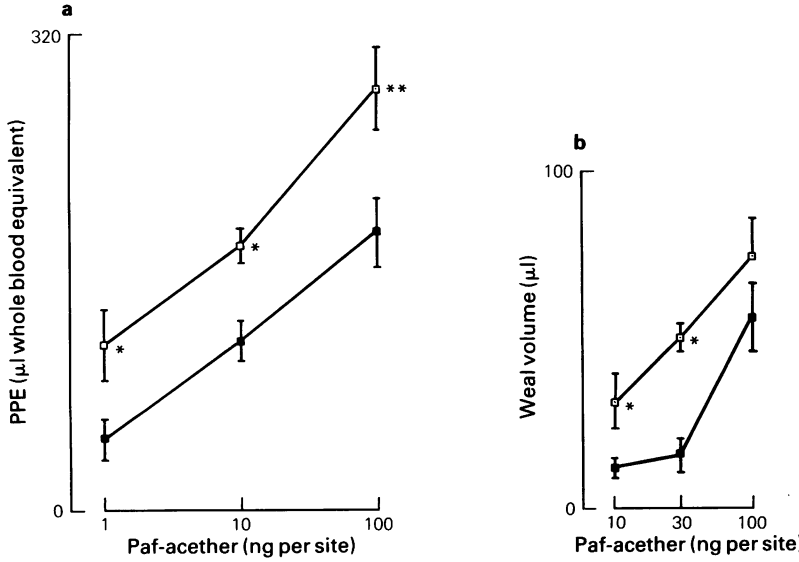

Figure 1 (a) Plasma protein extravasation (PPE) in guinea-pig skin in response to Paf-acether in the absence (■) or presence (□) of bovine serum albumin (BSA; 2.5 mg ml^{-1}). PPE has been expressed as equivalent μl of whole blood ([^{125}I]-HSA counts in skin sites/[^{125}I]-HSA counts in 1 μl blood). (b) Weal volume responses in human forearm to intradermal injections of Paf-acether in 50 μl saline alone (■) or containing human serum albumin (HSA; 2.5 mg ml^{-1}) (□). Weal volumes have been calculated from weal area × half the increase in skin-fold thickness. Each point represents the mean value and vertical lines indicate s.e.mean; $n = 3$ (a), $n = 6$ (b)., Significant differences from corresponding responses in the absence of BSA (a) or HSA (b) are indicated by * $P < 0.05$, ** $P < 0.005$, *** $P < 0.001$ (Student's paired t test).

forearm. Weal volumes and flare areas were measured and the presence or absence of a late-onset response was recorded.

Drugs

Drugs used were: batches of Paf-acether (gifts from Professor J.J. Godfroid and Dr J. Benveniste, Paris, France); lyso-Paf (Bachem); indomethacin (Merck Sharp & Dohme); chlorpheniramine maleate (Allen & Hanburys); codeine phosphate (McCarthy's) and mepyramine maleate (May & Baker). For studies in guinea-pig skin, histamine acid phosphate was obtained from B.D.H. and, for studies in human skin, from McCarthy's. All solutions were prepared in sterile Dulbecco's phosphate buffered saline (PBS; Gibco Europe) alone or containing 0.25% human serum albumin (Blood transfusion department, Guy's Hospital) for studies in human skin, or 0.25% bovine serum albumin (Sigma) for studies in the guinea-pig.

Results

(a) *Guinea-pig skin*

Effect of 0.25% bovine serum albumin When Paf-acether was administered in PBS containing bovine serum albumin, plasma protein extravasation was significantly greater than responses to equal doses of Paf-acether administered in PBS alone (Figure 1a).

Effect of indomethacin Inclusion of indomethacin (2 μg) in the injection solution caused no significant reduction of plasma protein extravasation induced by Paf-acether (3 ng). (Paf-acether alone: 77 ± 9 μl; Paf-acether + indomethacin: 68.2 ± 7 μl; $n = 6$).

Effect of mepyramine Inclusion of mepyramine (1.5 μg) in the injection solution caused a modest (<25%) reduction of plasma protein extravasation induced by Paf-acether (1 and 10 ng per site) (Figure

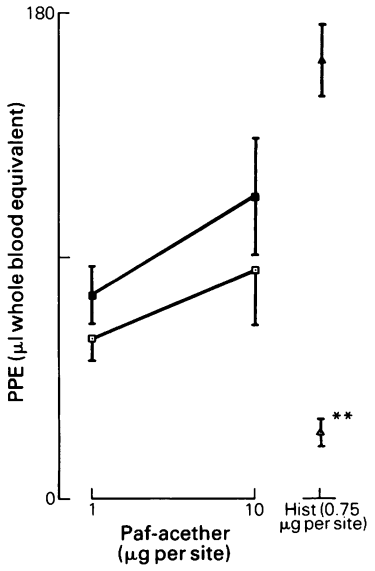

Figure 2 Plasma protein extravasation (PPE) responses in guinea-pig skin in response to Paf-acether (■,□) or histamine (Hist; ▲,△), in the absence (closed symbols) or presence (open symbols) of mepyramine (1.5 μg per site). PPE has been expressed as equivalent μl of whole blood (^{125}I]-HSA counts in skin sites/[^{125}I]-HSA counts in 1 μl blood). Each point represents the mean value and vertical lines indicate s.e.mean ($n = 3$). Significant differences from corresponding responses in the absence of mepyramine are indicated by ** $P < 0.01$ (paired t test).

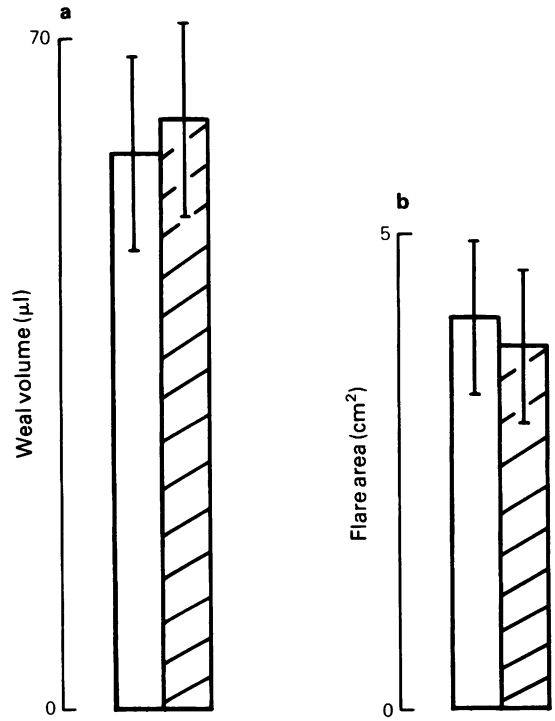

Figure 3 (a) Weal volume and (b) flare area responses in human forearm to intradermal injections of Paf-acether (200 ng) before (open columns) and after (hatched columns) ingestion of indomethacin (25 mg). Weal volumes were calculated from weal area × half the increase in skin-fold thickness measured at 15 min; flare area was measured at 5 min. Each column represents the mean response and vertical bars indicate s.e.mean ($n = 12$).

2). In these animals, the response to histamine in the presence of mepyramine was reduced by >85% (Figure 2).

(b) *Human skin*

Effect of human serum albumin The presence of human serum albumin (0.25%) caused an enhancement of the weal response induced by Paf-acether, reaching statistical significance for the doses, 10 and 30 ng per site (Figure 1b). Human serum albumin (0.25%) also significantly increased the flare response induced by Paf-acether (10 and 30 ng per site) (Table 1). The presence of 0.25% human serum albumin did not influence the incidence of late-onset responses.

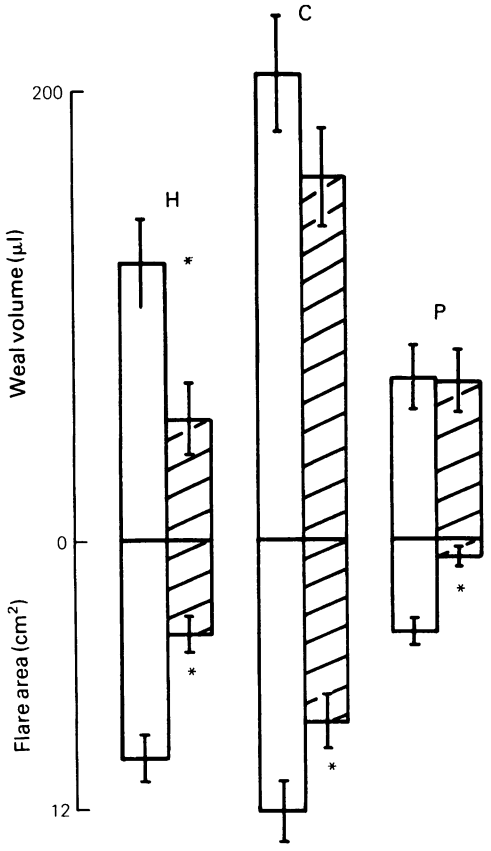

Figure 4 Weal volume and flare area responses in human forearm to intradermal injections of Paf-acether (P; 100 ng) codeine phosphate (C; 10 μg) or histamine (H; 0.5 μg) alone (open columns); or admixed with 1.5 μg chlorpheniramine (hatched columns). Weal volume was calculated from weal area × half the increase in skin-fold measured at 12 min. Flare area was calculated from 2 orthogonal diameters measured 5 min after injection. Each column represents the mean value and vertical bars indicate s.e.mean ($n = 10$). *Significantly different from corresponding response in the absence of chlorpheniramine.

Table 1 Flare responses to Paf-acether in human forearm in the absence and presence of human serum albumin (HSA)

Paf-acether (ng per site)	*Flare responses** (cm^2) *HSA absent*	*HSA present*	P
10	1.5 ± 0.4	3.5 ± 0.8	<0.025
30	2.1 ± 0.5	4.4 ± 1.2	<0.05
100	5.6 ± 0.9	6.1 ± 0.6	NS

* Flare areas were calculated from measurements of two orthogonal diameters made 5 min after intradermal injections of Paf-acether. Values are means ± s.e.mean ($n = 6$).

Effect of indomethacin Ingestion of indomethacin (25 mg) had no significant effect on either the early weal and flare responses induced by intradermal Paf-acether (Figure 3) or the incidence of late-onset responses, which occurred in 4 out of 12 subjects.

Effect of chlorpheniramine The weal volume responses to histamine and codeine were significantly reduced by concurrent administration of chlorpheniramine. However, local administration of chlorpheniramine (1.5 μg) did not significantly affect the weal response to Paf-acether (Figure 4) but there was a significant reduction in the flare responses induced by all three agents in the presence of chlorpheniramine (Figure 4).

Discussion

The ability of Paf-acether to elicit an acute inflammatory response in the skin of experimental animals and man is well documented. The present observations indicate that Paf-acether elicits acute oedema in both guinea-pig and human skin, by a mechanism that is independent of the release of endogenous histamine. However, the finding that chlorpheniramine reduces the acute flare response induced by Paf-acether in man suggests that this aspect of the response may involve histamine release. The inability of indomethacin to modify inflammatory responses induced by Paf-acether suggests that the cyclo-oxygenase metabolites of arachidonic acid do not play a part in the response to Paf-acether in skin, even though inflammatory responses induced by Paf-acether can be potentiated by inclusion of exogenous prostaglandins in the injection vehicle (Wedmore & Williams, 1981; Morley *et al.*, 1983; Archer *et al.*, 1984).

The addition of human serum albumin to Paf-acether has been shown to modify its biological properties (Hoppens *et al.*, 1983) and this effect may be attributed to the capacity of albumin to bind Paf-acether (Benveniste, 1974). The present results accord with previous observations obtained utilizing Paf-acether in the absence of albumin, but indicate that the presence of albumin has augmentative effects on the acute inflammatory responses induced by Paf-acether.

In experimental animals, Paf-acether has an effect on vascular endothelium that is independent of its ability to activate platelets or polymorphonuclear leucocytes (Humphrey *et al.*, 1982; Pirotzky *et al.*, 1984). The results of the present study are consistent with a direct effect of Paf-acether on vascular endothelium in man, since oedema formation in human skin is not a direct or indirect consequence of liberation of either the vasoactive amine, histamine, or the cyclo-oxygenase metabolites of arachidonic acid. However, some involvement of histamine in responses to Paf-acether is evident from the capacity of chlorpheniramine to inhibit the acute flare response. Presumably, this is due to histamine release in an amount insufficient to induce oedema. We would suggest that Paf-acether should be considered as a potential inflammatory mediator in both guinea-pig and human skin.

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