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Platelet-activating factor-induced contraction of human isolated bronchus

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ABSTRACT: In recent years, platelet-activating factor (PAF) has been strongly implicated as a mediator involved in asthma. In non-asthmatic subjects, aerosolized PAF has been shown to cause bronchoconstriction. The mechanism of this *in vivo* effect is unknown. We have previously shown that PAF causes a contraction of human isolated bronchus that varies in magnitude between patients, and within tissues from the same patient. To examine the possibility that this variability in contraction was secondary to PAF-induced release of mediators from inflammatory or epithelial cells within the tissue, we examined the relationship between contractile responses to PAF and the presence of inflammatory or epithelial cells.

We studied eight tissues from five patients. Of the eight tissues, four contracted, whilst four failed to contract, to PAF (7×10^{-7} M). After the contractile response to PAF had been assessed by observing changes in isometric tone *in vitro*, bronchial rings were examined histologically to enable the quantification of inflammatory cell numbers and intact epithelium. No significant correlation was observed between the magnitude of contractions and numbers of eosinophils, neutrophils, lymphocytes, plasma cells, total cells or percentage intact epithelium.

We conclude that it is unlikely that the variability in response to PAF in human isolated airways is related to the variability in inflammatory cell numbers or to the presence of epithelium. Thus, the contraction induced by PAF is probably not mediated *via* the release of a secondary mediator from the particular cells examined in this study.

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Platelet-activating factor (PAF) is a naturally occurring phospholipid, which causes bronchoconstriction and a contraction of isolated airway tissue from animals [1, 2] and man [3, 4]. The mechanism by which PAF produces these effects both *in vivo* and *in vitro* is unknown, however, a number of inflammatory mediators have been implicated.

In animals, the PAF-induced bronchoconstriction is thought to involve thromboxane A_2 [5], prostaglandins [6] and leukotrienes [1]. In man, histamine [7] and lipoxygenase [8] products have been implicated.

Little is known of the mechanism of the direct contractile effect of PAF on human isolated airway tissue, as few studies have shown that PAF induces a contraction. However, in animal isolated airway tissue, PAF has been shown to induce a contraction mediated by endogenous histamine [9], leukotrienes [10] and thromboxane A_2 [11].

The source of these inflammatory mediators which may be implicated in the PAF-induced effects is unknown. However, they may be released from inflammatory or epithelial cells within the tissue after exposure to PAF. Specific PAF receptors have been identified on inflammatory cells. Thus, eosinophils [12] and neutrophils [13] have specific PAF receptors. After exposure to PAF, eosinophils release leukotriene C_4 (LTC_4) [14], neutrophils release leukotriene B_4 (LTB_4) [15], whilst epithelial cells release 15-hydroxyeicosatetraenoic acid (15-HETE) [16].

We have previously shown that PAF causes a variable contraction of human isolated airways [4]. Because of the variable nature of this response, we propose that the *in vitro* PAF-induced effects are secondary to the release of mediators from inflammatory or epithelial cells. This study was undertaken to investigate the mechanism of the variable contraction induced by PAF. Specifically, we looked for a relationship between extent of inflammatory cell infiltrate within the airway tissue and presence of a contractile response to PAF.

Material and methods

Human lung was obtained from specimens resected at thoracotomy as described previously [17]. Macroscopically normal tissue was supplied by the hospital

pathologist and transported to the laboratory in Krebs-Henseleit solution (composition mM: NaCl, 118.4; KCl 4.7; $CaCl_2 \cdot 2H_2O$ 2.5; $MgSO_4 \cdot 7H_2O$, 1.2; KH_2PO_4 1.2; $NaHCO_3$ 25.0; and (D)-glucose 11.1) at 4°C, that had been saturated with 5% CO_2 in O_2 Bronchi, 2–3 mm in internal diameter, were dissected free from surrounding parenchymal tissue and cut into rings 4–5 mm long. The tissue was then stored overnight in Krebs-Henseleit solution at 4°C. The following day, bronchial rings were secured to tissue hooks and suspended in 5 ml double-jacketed organ baths under a load of 1 g. The load was continually adjusted to 1 g throughout the 1–3 h period of equilibration and then remained unaltered for the duration of the experiment. The bathing solution was maintained at 37°C, bubbled with 5% CO_2 in O_2 and exchanged every 20 min, until the tissue baseline became stable. Changes in force of contraction were measure isometrically by means of Grass FTO3 transducers and recorded on a Grass 7P polygraph chart recorder.

To assess the direct effects of PAF, a bolus dose of 7×10^{-7}M PAF was added to the organ bath and the effect on tone observed. After the contraction to PAF had plateaued, the tissues were washed every 20 min until baseline tone had returned. In the absence of a contraction to PAF, the tissues were washed 10 min after PAF addition, and then twice at 20 min intervals. Once baseline tone had re-established, a maximal contractile response to carbachol (10^{-3} M) was then obtained in all tissues. When the contraction to carbachol had plateaued, the tissue was removed from the organ bath, dehydrated and blocked in paraffin using standard histological procedures. The paraffin blocked tissues were then cut into 10 μm sections and stained with haemotoxylin and eosin.

The sections were viewed using a ×25 objective on an Olympus BH-2 microscope. Fifteen 10 μm sections in total were sampled at 40 μm intervals from each bronchial ring. The cross-sectional area examined in each 10 μm section was a radial area 1.26 mm (two fields) wide from the lumen. Every 10 μm section was assessed for eosinophil, neutrophil, lymphocyte and plasma cell numbers. The epithelium was assessed in these same sections using the JAVA™ computerized image analysis system (Jandel Video Analysis Software, Jandel Scientific, Corte Madera, CA, USA). The length of the basement membrane in each section was measured and the total length of intact epithelium covering this basement membrane was expressed as percentage coverage of the basement membrane.

Drugs and solutions

The following drugs and solutions were used: bovine serum albumin (BSA) fraction V; carbamylcholine chloride (carbachol); 1-0-alkyl-2-acetyl-sn-glyceryl-3-phosphorylcholine (platelet-activating factor, PAF); (all from Sigma).

Stock solutions of carbachol were prepared in distilled water, stored in 1 ml aliquots at -20°C and thawed as required. BSA 0.1% (w/v) in Krebs-Henseleit solution was made on the day of the experiment from BSA powder, which was stored desiccated at -20°C. PAF stock solution, 2 mg PAF·ml^{-1} chloroform, was stored at -20°C. Dilute solutions of PAF were made on the day of the experiment by removing an aliquot of PAF from the stock solution, evaporating the chloroform under nitrogen and dissolving the remaining solid in 0.1% (w/v) BSA in Krebs-Henseleit solution. This solution was sonicated to ensure that the PAF was completely dissolved.

Table 1. – PAF-induced contraction, inflammatory cell numbers and percentage epithelium from individual bronchial tissues

Patient Tissue no.	A t1	A t2	B t3	B t4	C t5	C t6	D t7	E t8
PAF contraction mg tension	250	380	345	415	0	0	0	0
PAF contraction % carb. max	26	25	22	24	0	0	0	0
Eosinophils	40	8	12	22	22	42	8	7
Neutrophils	55	17	50	91	47	176	11	8
Lymphocytes	1442	485	401	346	298	430	1625	366
Plasma cells	922	428	155	162	209	340	1119	349
Combined cells	2459	938	618	621	576	988	2763	730
% epithelium	53	71	74	58	53	51	74	85

PAF-induced contraction from the eight selected tissues expressed both as mg tension and as a percentage of carbachol maximum (carb. max.). Individual cell numbers and combined cell numbers from separate tissues are expressed as total cells counted over the fifteen 10 μm sections. Percentage epithelium is expressed as the mean percentage epithelium coverage of basement membrane over the fifteen 10 μm sections. PAF: platelet-activating factor.

Table 2. – Values of r^2 obtained from regression analysis of the individual cell types, combined cell numbers and percentage epithelium *versus* PAF contractility expressed both as mg tension and percentage carbachol maximum

	PAF contraction mg tension	PAF contraction % carbachol max.	p
Eosinohils	0.008	0.004	>0.05
Neutrophils	0.003	0.006	>0.05
Lymphocytes	0.024	0.0008	>0.05
Plasma cells	0.067	0.007	>0.05
Combined cells	0.042	0.0004	>0.05
% epithelium	0.012	0.0005	>0.05

PAF: platelet-activating factor.

Analysis of results

The contractions to the bolus dose of PAF (7×10^{-7} M) were expressed both as mg tension and as percentage of the maximal carbachol response (10^{-3} M).

Inflammatory cell numbers were counted in each individual 10 μm section and were expressed as individual cell types and as combined cells over the 15×10 μm sections. The epithelium, expressed as a percentage coverage of basement membrane, was assessed in each individual 10 μm section and expressed as an average for the fifteen, 10 μm sections.

Regression analysis was used to compare individual cell numbers, combined cell numbers and percentage epithelium, with contractility to PAF expressed as both mg tension and percentage of carbachol maximum. A value for r^2 was obtained from the regression analysis. The significance of the r value was determined using the number of tissues (n) and a significance value of 5%.

Results

The contraction induced by PAF 7×10^{-7} M ranged from 250–415 mg or from 22–26% of carbachol maximum in the eight tissues from five patients studied (table 1). Combined cell numbers ranged from 618–2,763 over the area studied, whilst the percentage epithelium ranged from 51–85% (table 1). No significant correlation was found between PAF contractility (expressed as mg or as % carbachol maximum) and cell numbers (whether expressed as individual cell type or as combined cell numbers) or percentage epithelium. Table 2 shows r^2 values for the regression analysis.

Discussion

The results of the present experiments show that in human isolated bronchus there is no correlation between the contractility to PAF 7×10^{-7} M and the quantity of eosinophils, neutrophils, lymphocytes, plasma cells, combined cell numbers or presence of epithelium found in a particular tissue. These results suggest that the variability in PAF responses is not related to the variability in inflammatory cell numbers or presence of epithelium and that the contraction in response to PAF is probably not mediated *via* the release of a secondary mediator from the particular cells examined in this study.

The variability in the PAF responses observed *in vivo* [7, 18] and *in vitro* [4] suggests that PAF may be having an indirect mode of action. SMITH *et al.* [7] observed intersubject variability in the PAF response *in vivo*, which they suggested was due to an indirect mechanism of action of PAF. One possible explanation for the observed variability in PAF responses is that the PAF-induced effects are caused by secondary mediators that have been released from inflammatory cells within the tissue. Prostaglandins, leukotrienes, histamine and thromboxane A_2 have been implicated in the PAF-induced effects in animals [6, 9–11], whilst in man, evidence suggests that histamine [7] and leukotrienes [8] are involved. Although there is good evidence that the PAF-induced bronchoconstriction *in vivo* and contraction of isolated animal airway tissue *in vitro* is in part mediated by secondary mediators, the present experiments have shown that the source of these secondary mediators in human airway tissue is unlikely to be an inflammatory or epithelial cell. Since the results indicate that the contraction induced by PAF is probably not mediated *via* the release of a mediator from the inflammatory cells examined, it is possible that PAF could be acting on a cell type not examined in this study. Macrophages and mast cells were not examined in this study as there is little evidence that PAF causes the release of contractile mediators from either of these cell types in human lung.

To investigate the possibility that the responses to PAF were related not merely to the presence of cells but to the activation status of the cell, it would have been ideal to study a marker of activation of specific cell types. However, the protocol of warming the tissues to 37°C from 4°C, incubating the tissues in the organ bath for hours and subjecting them to high doses of carbachol would make this approach impractical. Furthermore, the state of the tissues after sectioning did not allow such fine examination to differentiate the degree of cellular degranulation between tissue samples. The possibility therefore remains that differences in cell activation status or degree of degranulation may have accounted for differences in response to PAF.

In vivo studies indicate that leukotrienes are the most likely mediator to be involved in PAF-induced bronchoconstriction [8]. However, the present study and a separate study in which various antagonists/inhibitors of histamine, leukotrienes, prostaglandins and thromboxane A_2 did not alter the PAF-induced contraction (unpublished results), would suggest this is not the case in human isolated bronchus. Moreover, the nature of the contractile response to PAF [4], an easily reversible contraction which plateaus between 5–15 min, would also indicate that leukotrienes are not mediating the response *in vitro*.

Thus, the possibility exists that PAF is acting directly on receptors on the smooth muscle. PAF receptors have been located on guinea-pig smooth muscle [19], as well as in human lung membrane preparations [20, 21]. The location of the PAF receptors within lung membrane preparations is unknown, as they contain not only airway smooth muscle but also vascular smooth muscle and parenchymal tissue. The hypothesis that PAF could act directly on the smooth muscle is supported by the fact that PAF stimulation of human isolated smooth muscle cells causes a rise in cytosolic calcium [22]. This rise in cytosolic calcium was large enough to induce smooth muscle contraction.

If PAF is acting directly on the smooth muscle it could be expected that all viable tissues would respond to PAF. However, this was not the case in either our previous work [4] or the present study. One explanation for the variable response to PAF would be that the PAF receptors may have been desensitized through a prior exposure to PAF itself. This exposure may have occurred *in vivo* prior to resection or *in vitro* as a result of tissue trauma during dissection of the bronchial rings. The variability in PAF responses within tissues from the same patient could reflect a variability in exposure and thus desensitization to PAF. This phenomenon has been reported in guinea-pig trachea [23], human parenchyma [24], rabbit platelets [25], and has also been observed in human isolated airways [4]. It is possible that tissues which failed to respond to PAF may have had prior exposure to PAF *in vivo* or *in vitro* resulting in desentization of the receptor. The mechanism of this desensitization may be similar to that reported by SHUKLA *et al.* [25] in rabbit platelets, where phosphorylation of receptor coupled proteins resulted in desensitization of the PAF receptor.

This study has shown that the variability in contractile responses to PAF in human isolated airways is not related to the variability in inflammatory cell numbers and that the contraction induced by PAF is probably not secondary to the release of mediators from inflammatory or epithelial cells within the tissue. How PAF is inducing a contraction and what is responsible for the variability in the contractile responses in human airway tissue awaits determination.

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