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PAF formation by human gastrointestinal mucosa/submucosa in-vitro: release by ricinoleic acid, and inhibition by 5-aminosalicylic acid

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Abstract—Human isolated gastrointestinal mucosa/submucosa incubated with ricinoleic acid (12·5–100 μg mL^{-1}) or the calcium ionophore A23187 (10 μg mL^{-1}) released platelet-activating factor (PAF) as determined by a scintillation proximity assay after extraction and purification. 5-Aminosalicylic acid (25–100 μg mL^{-1}) inhibited PAF release by ricinoleic acid in a concentration-dependent manner, and 50 μg mL^{-1} reduced the effect of A23187. We suggest that PAF may play a role in the laxation and mucosal damage by ricinoleic acid released from castor oil.

Platelet-activating factor (PAF), a phospholipid with several biological activities, is produced by different cell types (Parente & Flower 1985) and tissues (Rachmilewitz et al 1990) in response to various stimuli. A substantial release of PAF occurs from incubated intestine of rats with induced experimental colitis (Mascolo et al 1990) and from inflamed colon of patients with ulcerative colitis (Rachmilewitz et al 1990). PAF possesses prodiarrhoeagenic secretory effects in rat isolated intestine (Buckley & Hoult 1989). Rats treated with castor oil also have intestinal release of PAF (Pinto et al 1989), together with intestinal hyperaemia and intraluminal release of acid phosphatase (a marker of cellular damage). These findings suggest a role for PAF as a mediator of the laxation and the intestinal damage induced by castor oil. However, experiments so far have mainly been performed in animals, and their relevance to man has not been determined. The present study has examined the effect of ricinoleic acid, the active constituent in castor oil, on PAF release from human isolated gastrointestinal mucosa.

Materials and methods

Human gastrointestinal tissues (colon, ileum and stomach) were taken at least 5 cm from any macroscopically detected lesions in surgical specimens removed for benign or malignant disease. As far as we are aware, the patients had not within the previous 4–6 days consumed any drug known to affect eicosanoid synthesis. Samples were transported to the laboratory at ambient temperature within 30 min of removal and then transferred to ice-cold 154 mM NaCl (saline). The layer of mucosa/submucosa was carefully cut off from the underlying muscle while the tissue was bathed in saline, cut finely with scissors and washed with saline. Accurately weighed aliquots (200 ± 10 mg) were suspended in 2 mL of 0·25% bovine serum albumin/saline with drugs or vehicle. The drugs used were: ricinoleic acid (6·25, 12·5, 25, 50 and 100 μg mL^{-1}), calcium inonophore A23187 (10 μg mL^{-1}), and 5-aminosalicylic acid (5-ASA; 25, 50 and 100 μg mL^{-1}). Solvents were: for ricinoleic acid, 10 mg mL^{-1} ethanol; for A23187, 0·2 mg mL^{-1} DMSO; and for 5-ASA, 10 mg mL^{-1} saline.

Samples were incubated in a shaking water bath (37°C, 30 min), and PAF was then extracted and purified by thin-layer chromatography (Pinto et al 1989). Briefly, cold acetone (2 mL, −20°C) was added to the incubate, centrifuged (2000 *g*, 5 min), and the acetone/water phase was extracted with 2 mL chloroform. After evaporation to dryness, the residue was redissolved in 75 μL of chloroform/methanol (1:1), applied to thin layer chromatography plates (silica gel, Kodak) and developed in chloroform/methanol/water (65:35:6) together with authentic standard PAF (Sigma, Poole). Zones co-migrating with authentic PAF, visualized by exposure to iodine, were re-extracted, dried, and assayed for PAF using a scintillation proximity radioimmunoassay (Amersham, SPRIA, TRK 990; sensitivity 20 pg, intra- and inter-assay coefficients of variations 4·7–6·7% and 2·9–8·3%). The amount of radiolabelled PAF bound to the fluomicrospheres was determined by direct counting in the vials, using a Packard scintillation counter 2200C (window 0 to 999, 4 min).

The above procedure gave a mean recovery of 85·3 ± 5·2% for standard PAF. Percent cross-reactions of the antiserum used were PAF 100; lyso-PAF < 0·01; octadecyl-2-acetyl GPC PAF 40; 1-hexadecanoyl-2-acetyl GPC 0·06; 1-octadecanoyl-2-acetyl GPC 0·05; 1-hexadecanoyl-2-lyso GPC 0·01; phosphatidylcho-

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line <0·04; lyso-phosphatidylcholine <0·02; arachidonic acid <0·01.

Results are shown as means ± s.e.m., and analysed statistically by Student's *t*-test or, because the variance was zero in some groups, by the Wilcoxon test (both 2-tailed).

Results

Human stomach, ileum or colon tissue incubated in 0·25% bovine serum albumin/saline did not release detected amounts of PAF (<0·4 ng g^{-1}/30 min). In the presence of ricinoleic acid 12·5–100 $\mu g\ mL^{-1}$ the colonic incubates usually showed concentration-related increases in PAF accumulation. The lowest drug concentration (6·25 $\mu g\ mL^{-1}$) had no effect, and the response to 100 $\mu g\ mL^{-1}$ showed no further increase over that obtained with 50 $\mu g\ mL^{-1}$ (Table 1). With ileum and stomach tissue, ricinoleic acid (12·5–100 $\mu g\ mL^{-1}$) added to the incubation medium also caused a concentration-related increase of PAF accumulation, but usually to a lesser extent than with colonic tissue. The calcium ionophore A23187 10 $\mu g\ mL^{-1}$ also stimulated PAF release (Table 1).

Release of PAF by ricinoleic acid was inhibited 5–100% by 5-ASA 25–100 $\mu g\ mL^{-1}$ in a concentration-related manner (Table 2), and 5-ASA 50 $\mu g\ mL^{-1}$ also reduced the release of PAF (by 36·8%) in response to the calcium ionophore A23187 10 $\mu g\ mL^{-1}$.

Discussion

The laxative effect of castor oil results from the ricinoleic acid which is released by hydrolysis. In addition, ricinoleic acid causes damage throughout the intestine (erosions, desquamation of surface cells, and infiltration of several cell types) (Gaginella et al 1977; Bretagne et al 1981). There is no macroscopic or microscopic damage to the stomach, because the castor oil is hydrolysed in the intestine.

The present study was undertaken as a result of evidence that PAF could induce mucosal damage (Wallace & Whittle 1986) and that castor oil increased intestinal PAF production (Pinto et al 1989). Rachmilewitz et al (1990) found, using a bioassay, that the calcium ionophore A23187 released PAF from the colon mucosa, and that this release was inhibited by 5-ASA in amounts therapeutically relevant to the treatment of ulcerative colitis (the compound is poorly absorbed from the colon, and high local concentrations may occur near the mucosal surface). In addition to confirming these results, but using the more definitive scintillation proximity assay, we similarly obtained tissue release of PAF into the incubation fluid with other regions of the alimentary tract using either A23187 or ricinoleic acid. Furthermore, we found that 5-ASA inhibited the release of PAF by ricinoleic acid. Buckley & Hoult (1989) demonstrated that PAF has pro-diarrhoeagenic secretory effects, so that both the laxation and the mucosal damage caused by castor oil might involve PAF. The mechanism is not understood, however, and the source of PAF is not clear since it can be produced by mast cells, platelets, monocytes, macrophages, eosinophils, neutrophils and basophils.

Table 1. Effects of ricinoleic acid and calcium ionophore A23187 on the accumulation of PAF in incubates of human colon, ileum and stomach mucosa/submucosa.

Drugs ($\mu g\ mL^{-1}$)	Colon (n)	Ileum (n)	Stomach (n)
Vehicle control	<0·4 (12)	<0·4 (4)	<0·4 (4)
Ricinoleic acid			
6·25	<0·4 (10)	<0·4 (4)	<0·4 (4)
12·5	2·7 ± 0·3 (10)	4·1 ± 1·4 (4)	2·6 ± 0·4 (4)
25·0	8·5 ± 1·6 (10)	7·8 ± 1·7 (4)	3·7 ± 0·4 (4)
50·0	18·5 ± 2·1 (12)	10·0 ± 2·7 (3)	9·5 ± 3·0 (4)
100·0	16·4 ± 4·2 (4)	ND	8·5 ± 1·9 (4)
Calcium ionophore			
10·0	8·4 ± 1·4 (6)	12·6 ± 2·1 (4)	6·8 ± 1·1 (4)

Each specimen was assayed in duplicate, and therefore the numbers in parentheses (n) represent double the number of separate specimens taken. One ileal sample was lost. Results are expressed as means ± s.e.m. ng g^{-1}/30 min. In all the experiments where n = 10–12, the *P* values compared with controls were <0·01–<0·001 (Wilcoxon test). With the ileum and stomach there were not enough results for nonparametric analysis, but nevertheless all tissues with at least 12·5 $\mu g\ mL^{-1}$ ricinoleic acid showed PAF release. ND = not determined.

Table 2. Effects of ricinoleic acid and 5-aminosalicylic acid (5-ASA) on PAF accumulation in incubates of human colon mucosa/submucosa.

Drugs ($\mu g\ mL^{-1}$)	PAF	n
Vehicle control	<0·4	12
Ricinoleic acid 50	18·5 ± 2·1	12
5-ASA 100	<0·4	10
Ricinoleic acid 50 + 5-ASA 25	17·6 ± 0·9	10
Ricinoleic acid 50 + 5-ASA 50	9·0 ± 0·9*	10
Ricinoleic acid 50 + 5-ASA 100	<0·4**	10

Results are expressed as means ± s.e.m. ng g^{-1}/30 min. *$P < 0·001$ (Student's *t*-test), **$P < 0·001$ (Wilcoxon test). The tissues were assayed in duplicate, and the numbers of samples (n) therefore represent double the number of separate specimens taken.

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