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Increased intestinal formation of Paf in endotoxin-induced damage in the rat

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Platelet-activating factor (Paf) has been proposed as a mediator of the gastrointestinal damage in endotoxic shock. The formation of Paf in rat jejunal tissue, determined following extraction and bioassay on rabbit washed platelets has therefore been correlated with the induction of damage following endotoxin administration. Intravenous injection of *E. coli* endotoxin led to a time-dependent increase in the jejunal formation of Paf, which after 20 min was twenty fold greater than the control level. There was a significant correlation between elevated Paf release and intestinal hyperaemia and haemorrhage, thus supporting a role for Paf as a mediator of such damage.

Introduction Platelet-activating factor (Paf) has recently been proposed as a mediator of gastrointestinal damage associated with septic shock (Rosam *et al.*, 1986). Receptor antagonists of Paf prevent the gastrointestinal ulceration (Wallace & Whittle, 1986a) and the hypotension (Doebber *et al.*, 1985; Wallace & Whittle, 1986a) that follows endotoxin administration. In addition, Paf is present in the blood after infusion of endotoxin (Doebber *et al.*, 1985). If endogenous Paf does play a role in mediating endotoxin-induced gastrointestinal damage, increased levels should be detectable in the affected tissues. We have therefore investigated whether intravenous administration of endotoxin leads to changes in the levels of Paf formed by intestinal tissue and if these changes are related to macroscopic damage.

Methods Rats (male Wistar 250–225 g) which had been deprived of food, but not water for 18–20 h, were anaesthetized with sodium pentobarbitone (60 mg kg^{-1}, i.p.). Lipopolysaccharide from *E. coli* (0111:84, 50 mg kg^{-1}, Sigma) dissolved in isotonic saline was administered as a bolus injection via the tail vein, in a volume of 4 ml kg^{-1} and rats killed 5, 10 or 20 min later. Control animals received a similar volume of isotonic saline by the same route. Macroscopically visible small-intestinal damage, which was most apparent in the jejunum, was scored blind on a 0 (normal) to 3 (severe hyperaemia) scale.

Segments of jejunal tissue (300 mg) were added whole to 3 ml of 0.25% bovine serum albumen (BSA) in saline (0°C), and after vortexing for 30 s, the mixture was added to 6 ml of cold acetone (− 20°C) and after centrifugation (2000 g, 5 min) was extracted as described previously (Parente & Flower, 1985). After a further centrifugation, the Paf in the organic phase was characterized by thin layer chromatography (t.l.c.). The dried extract was redissolved in chloroform:methanol (1:1, v.v), and applied to t.l.c. plates which were developed in chloroform:methanol:water (65:35:6, v:v:v). Zones from the plate co-migrating with authentic [^{14}C]-Paf (1000 c.p.m., 58 mCi $mmol^{-1}$, Amersham) were transferred to 0.25% BSA/saline, vortexed for 15 s and re-extracted. Subsequently, the dried organic phase was resuspended in 20 mM Tris buffer (pH 8.0) containing 0.25% BSA, and the Paf activity bioassayed as the aggregation of rabbit washed platelets.

Rabbit blood, collected in tri-sodium citrate (0.32% final concentration), was centrifuged to prepare platelet-rich-plasma and the platelets were subsequently washed free of plasma with prostacyclin (Wellcome Foundation Ltd) and resuspended in a Tyrode solution, as previously described in detail (Radomski & Moncada, 1983). The activity of Paf in the extracts following t.l.c. separation was bioassayed against concentration-aggregation curves to authentic Paf (99% pure, Sigma) in 0.5 ml aliquots of the platelet suspension, by use of a Payton dual-channel aggregometer. The pro-aggregatory activity of the extracts was confirmed as Paf since it was completely inhibited by pre-incubating the platelet suspensions for 1 min with the Paf receptor antagonist kadsurenone (5 μM, a gift from T.Y. Shen, Merck, Sharp & Dohme).

Results The formation of Paf by jejunum, which in control tisue was 0.46 ± 0.20 ng g^{-1} of tissue (mean ± s.e.mean, $n = 12$) was increased substantially (5 × control, $P < 0.05$) 5 min after endotoxin injection. A further significant ($P < 0.01$) increase in Paf formation occurred at 10 min and 20 min after endotoxin (Figure 1).

As described previously, endotoxin produced

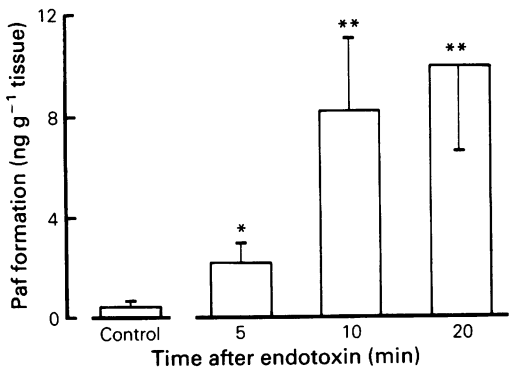

Figure 1 Formation of platelet activating factor (Paf) by jejunal tissue of rats receiving vehicle alone or at various times following lipopolysaccharide from *E. coli* (50 mg kg^{-1} i.v.). Results are expressed as mean ± s.e.mean of 9–12 rats in each group. $*P<0.05$, $**P<0.01$. (Students' t test for unpaired data).

macroscopic jejunal damage which was characterized by extensive hyperaemia and overt haemorrhage (Wallace & Whittle, 1986a) similar to that described following Paf infusion (Wallace & Whittle, 1986b). In the present study, endotoxin produced moderate damage at 5 min (damage score = 1.4 ± 0.5, $n = 9$, $P<0.05$, Mann Whitney U-test) and 10 min (1.5 ± 0.3, $n = 12$, $P<0.05$) which was more severe after 20 min (2.5 ± 0.3, $n = 12$, $P<0.01$). There was a significant correlation ($r = 0.61$, $P<0.05$, $n = 29$) between increases in Paf release and the jejunal damage score.

Discussion In the present study, rat jejunal tissue produced substantial amounts of Paf, even in the absence of any exogenous stimulant. Furthermore, intravenous administration of lipopolysaccharide from *E. coli* led to a time-dependent increase in the jejunal formation of Paf, which, after 20 min, was twenty fold greater than that of the control. A time-related appearance of Paf has previously been reported in the blood following endotoxin administration in the rat (Doebber *et al.*, 1985). Other studies have also demonstrated the elevated release of Paf from cells obtained from the peritoneal cavity after endotoxin administration in the rat (Inarrea *et al.*, 1985).

The increased formation of Paf by the jejunal tissue following endotoxin was accompanied by extensive hyperaemia and overt haemorrhage of the small intestine, particularly the jejunum, and there was a significant correlation between the elevated Paf release and damage. The characteristics of the gastrointestinal damage were similar to those previously observed following administration of exogenous Paf (Wallace & Whittle, 1986b). The mechanism underlying the jejunal damage induced by either Paf or endotoxin is not yet established, but may reflect changes in the microcirculation, coupled with substantial haemoconcentration, as suggested for the induction of gastric mucosal damage (Whittle *et al.*, 1986).

It is not yet known whether the increase in the jejunal formation of Paf following endotoxin administration is due to the infiltration of circulating blood cells such as neutrophils or platelets, or to the stimulation of resident cells including macrophages, mast cells or endothelial cells. The present results however, add further support to the postulated role for Paf as a mediator of the gastrointestinal ulceration that is associated with septic shock in the rat.

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