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Endothelin-1 induces neutrophil-independent vascular injury in the rat gastric microcirculation

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Abstract

Local intra-arterial infusion of picomole quantities of endothelin-1 induced gastric vascular leakage of radiolabelled albumin. This leakage was partially inhibited by the platelet activating factor (PAF) receptor antagonist WEB 2086 (0.5–2 mg kg^{-1}), but was unaffected by the thromboxane synthase inhibitor OKY 1581 (5 mg kg^{-1}) or by pretreatment with anti-neutrophil serum. These results indicate a partial role of PAF, but demonstrate that neutrophils are not involved in the gastric vascular dysfunction induced by locally administered endothelin-1.

Keywords: Endothelin-1; Neutrophil; Inflammation

Neutrophils have been implicated in the gastric injury induced by a variety of agents including non-steroidal anti-inflammatory drugs (Wallace et al., 1990). Endothelin-1 has been shown to induce a significant and dose-dependent injury in the rat gastric mucosa following close-arterial administration (Lopez-Belmonte and Whittle, 1994). Moreover, endothelin-1 has been shown to aggregate polymorphonuclear leukocytes, an effect mediated, in part, via release of platelet activating factor (PAF; Gómez-Garre et al., 1992). In addition, both the macroscopic gastric mucosal damage and the increase in protein extravasation induced in the rat stomach by either local intra-arterial or systemic intravenous bolus administration of endothelin-1 can be inhibited by PAF receptor antagonists (Filep et al., 1991; Kurose et al., 1992; Sirois et al., 1992). These findings imply that neutrophils and PAF could be involved in the gastric microvascular injury induced by endothelin-1. In addition, thromboxane A_2 has also been shown to be involved in the vascular permeability changes provoked by endothelin-1 in several tissues (Sirois et al., 1992).

Local intra-arterial infusion of endothelin-1 has been previously shown to induce a time- and dose-dependent increase in gastric plasma extravasation which can be reduced by inhibitors of nitric oxide and prostaglandin synthesis (Lopez-Belmonte and Whittle, 1994). Thus, the present study has investigated the involvement of neutrophils, PAF and thromboxane A_2 in the increase in gastric plasma extravasation induced by local intra-arterial infusion of a low dose of endothelin-1.

Male Wistar rats (230–260 g body weight) were deprived of food but not water for 18–20 h prior to the experiment. The animals were anaesthetised with sodium pentobarbitone (60 mg kg^{-1}, i.p.), the stomach exposed by a midline incision and the left gastric artery cannulated with a 24 g Teflon cannula. Endothelin-1 (5 pmol kg^{-1} min^{-1}), PAF (2 ng kg^{-1} min^{-1}) or their respective vehicles (0.1 and 0.25% solution of bovine serum albumin in saline respectively) were infused close-arterially (13 μl min^{-1}) for a period of 10 min. Gastric vascular plasma leakage was determined as the extravasation of ^{125}I-labelled human serum albumin (2 μCi kg^{-1}, 1 ml kg^{-1}, i.v.) injected 10 min prior to endothelin-1 or PAF infusion (Lopez-Belmonte and Whittle, 1994).

Close-arterial infusion of PAF (2 ng kg^{-1} min^{-1}) induced a significant increase in plasma leakage after 10 min compared with infusion of its vehicle alone ($\Delta 47 \pm 6$ μl g^{-1} tissue, $n = 6$, $P < 0.001$). Pretreatment

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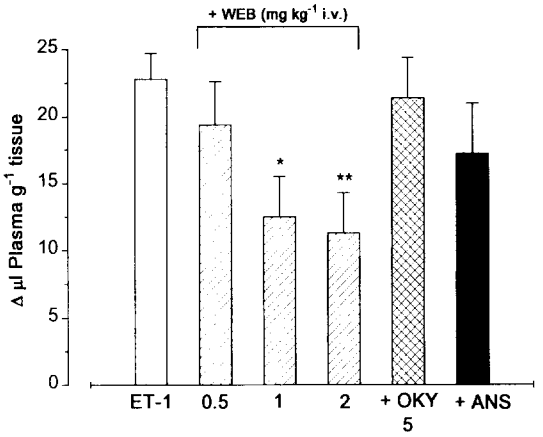

Fig. 1. Effect of WEB 2086 (0.5–2 mg kg^{-1} i.v.), OKY 1581 (5 mg kg^{-1} i.v.) and anti-neutrophil serum (ANS) on the increase in rat gastric vascular permeability induced by close-arterial infusion of endothelin-1 (ET-1; 5 pmol kg^{-1} min^{-1}, 10 min). Changes in vascular permeability are shown as albumin leakage $\Delta\mu l\ g^{-1}$ tissue. Results are the mean ± S.E.M. of 6 experiments in each group. Significant difference from ET-1 is given as $^{*}P < 0.05$; $^{**}P < 0.01$ (Student's *t*-test for unpaired data).

with the PAF receptor antagonist WEB 2086 (3-(4-(2-chlorophenyl)-9-methyl-6*H*-thieno(3,2-*f*)(1,2,4) triazolo-(4,3-*a*)(1,4)-diazepine-2-yl)-1-(4-morpholinyl)-1-propanone), at a dose of 1 mg kg^{-1} i.v., significantly inhibited the plasma leakage induced by this dose of PAF (90 ± 6% inhibition, $n = 6$, $P < 0.001$). Likewise, pretreatment of the rats with adsorbed anti-neutrophil serum (100 µl diluted to 500 µl with 0.9% saline, i.p., Accurate Chemical and Scientific Corp., USA) 4 h prior to the experiment reduced this plasma leakage induced by exogenous PAF (48 ± 12% inhibition, $n = 6$, $P < 0.05$). Neutropenia was confirmed by microscopic examination of the blood to determine the number of circulating neutrophils (modified Wright-Giesma stain; 94 ± 2% reduction, $n = 6$, $P < 0.001$).

Close-arterial infusion of endothelin-1 (5 pmol kg^{-1} min^{-1}) induced a significant increase in gastric plasma leakage when compared to infusion of its vehicle alone (Fig. 1). This increase in plasma leakage was inhibited by WEB 2086 (0.5–2 mg kg^{-1} i.v.), with 45 ± 13% and 52 ± 13% ($n = 6$; $P < 0.05$) inhibition at doses of 1 and 2 mg kg^{-1} respectively. However, pretreatment with anti-neutrophil serum had no significant effect on the increase in plasma leakage observed following close-arterial endothelin-1 administration (Fig. 1). Likewise the thromboxane synthase inhibitor OKY 1581 (sodium (*E*)-3[4-(3-pyridylmethyl) phenyl]-2-methyl-acrylate), in a dose (5 mg kg^{-1} i.v.) shown to near-maximally inhibit intestinal microvascular injury provoked by endotoxin and a nitric oxide synthase inhibitor (Laszlo et al., 1994), had no significant action on the plasma leakage induced by endothelin-1 (Fig. 1).

In contrast to several forms of experimental ulceration, circulating neutrophils were not found to play a significant role in the vascular injury induced by endothelin-1 in the stomach of the anaesthetised rat, similar to results obtained with endothelin-3 in the rat mesentery (Kurose et al., 1993). Moreover, these present results also show that endogenous PAF, but not thromboxane, mediates in part the increase in plasma extravasation in the rat stomach following local administration of a low dose of endothelin-1. However, complete abolition of this plasma leakage could not be accomplished with WEB 2086. These findings thus contrast with previous reports where PAF receptor antagonists, including WEB 2086, and thromboxane synthase inhibitors, including OKY 1581, were shown to inhibit substantially the protein extravasation and mucosal macroscopic injury induced endothelin-1 (Filep et al., 1991; Sirois et al., 1992; Kurose et al., 1992). Such an apparent discrepancy could result from the administration of higher bolus doses of endothelin-1 than those infused in the present study, which may trigger PAF and thromboxane release from other tissues such as the lungs or kidneys. This increase in circulating levels of these mediators could therefore contribute to the increased vascular permeability changes observed in the stomach in those studies.

Thus, the present study shows that local intra-arterial infusion of endothelin-1, at a dose that is known to cause macroscopic injury, results in a neutrophil-independent increase in gastric plasma extravasation which is mediated, in part, by PAF release. The source of this PAF is unknown, but may be secondary to direct injury of the vascular endothelium by endothelin-1.

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