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Colonic microvascular integrity in acute endotoxaemia: interactions between constitutive nitric oxide and 5-lipoxygenase products

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Abstract

Administration of the nitric oxide synthase inhibitor, N^G-nitro-L-arginine methyl ester (5 mg/kg s.c.) provoked acute microvascular injury (assessed by the leakage of radiolabelled human serum albumin) in the rat colon within 1 h, when administered concurrently with endotoxin (*Escherichia coli* lipopolysaccharide, 3 mg/kg i.v.). Pretreatment with the selective inhibitor of 5-lipoxygenase, BW A137C (*N*-[4-benzyloxybenzyl] acetohydroxamic acid; 1–20 mg/kg s.c., 15 min before endotoxin) attenuated such damage in a dose-dependent manner. These findings suggest a balance between protective constitutive nitric oxide and the detrimental actions of 5-lipoxygenase products in the maintenance of vascular integrity in the early stage of sepsis.

Keywords: 5-Lipoxygenase inhibitor; Nitric oxide (NO); Microvascular injury

Vascular endothelial dysfunction is known to have a key importance in the development of hypotension and increased vascular permeability during sepsis syndrome (Bone, 1991). In sepsis, pro-inflammatory mediators, such as the arachidonate 5-lipoxygenase products, the leukotrienes, are known to be released and are involved in the pathogenesis of tissue injury (Bone, 1991). However, the actions of these products may be modulated by endogenous protective factors. Nitric oxide, formed by the Ca^{2+}-dependent constitutive nitric oxide synthase plays an important vascular protective role in acute endotoxaemia, since its blockade leads to early microvascular injury following endotoxin administration (Hutcheson et al., 1990; László et al., 1994). In the present study, the interaction of constitutive nitric oxide with 5-lipoxygenase products on rat colonic microvascular integrity in the early phase endotoxaemia has been examined.

Male Wistar rats (225–275 g) were fasted overnight, but received water ad libitum. Under transient halothane anaesthesia, endotoxin (*Escherichia coli* lipopolysaccharide 0111:B4; Sigma Chemical Co., Poole, Dorset, UK; 3 mg/kg i.v.), [^{125}I]human serum albumin (Amersham, UK; 2 μg/kg i.v.) and nitric oxide synthase inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME; Sigma Chemical Co.; 5 mg/kg s.c.) were injected concurrently. This dose of L-NAME was selected as near-maximal from previous studies (László et al., 1994). The selective 5-lipoxygenase inhibitor, *N*-(4-benzyloxybenzyl)-acetohydroxamic acid (BW A137C; 1–20 mg/kg s.c.) in doses shown previously to inhibit the formation of 5-lipoxygenase products ex vivo (Tateson et al., 1988) was administered 15 min before endotoxin. For the evaluation of leakage of [^{125}I]human serum albumin, as a measure of vascular endothelial damage, under halothane anaesthesia, blood from the abdominal aorta was collected into syringes containing trisodium citrate and colonic tissue removed 1 h after endotoxin injection. Blood was centrifuged ($10\,000 \times g$, 10 min, 4° C) and the [^{125}I]human serum albumin content in the colon and plasma was determined in a gamma spectrometer (Nuclear Enterprises NE 1600). The albumin content in colonic tissue was calculated as described previously (László et al., 1994). Control values (from rats that received saline) were subtracted from test values and the data were expressed as Δ plasma leakage, μl plasma/g tissue. For statistical comparisons, analysis of variance with the Bonferroni test was utilised and differences were taken as significant when the probability was less than 5%.

A single bolus injection of endotoxin (3 mg/kg i.v.) or L-NAME (5 mg/kg s.c.) alone did not affect colonic plasma leakage over a 1 h period ($n = 8$–10). In con-

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trast, the concurrent administration of endotoxin (3 mg/kg i.v.) with L-NAME (5 mg/kg s.c.) elevated plasma leakage in the colon determined 1 h later. Pretreatment with BW A137C (1–20 mg/kg s.c., 15 min before endotoxin) dose-dependently attenuated acute colonic microvascular damage induced by the combination of endotoxin and L-NAME (Fig. 1). Administration of BW137C (20 mg/kg s.c.) alone or in combination with endotoxin alone, had no significant action on plasma leakage ($n = 4$).

Our present findings confirm that constitutively formed nitric oxide plays a beneficial role in the intestinal microcirculation during acute endotoxaemia, since its inhibition by L-NAME provoked early microvascular leakage in the colon following endotoxin administration, as demonstrated previously (László et al., 1994). Furthermore, in the current study, this acute increase in colonic vascular permeability was attenuated by the administration of BW A137C (Tateson et al., 1988), implicating the pathological involvement of 5-lipoxygenase products released in the early phase of sepsis. Previous studies have demonstrated that inhibition of leukotriene synthesis can ameliorate the hypotension, arterial hypoxaemia and the related tissue injury in sepsis (Fink et al., 1993). Furthermore, the release of leukotrienes during sepsis has been suggested to cause a decrease in mesenteric blood flow, increase in neutrophil adhesion, and increases of vascular permeability, either directly or through interactions of neutrophils with endothelial cells (see Bone, 1991 for review). Such neutrophil activation and adhesion may lead to the local release of platelet-activating factor and thromboxane A_2, also shown to be involved in the early phase of microvascular injury in this model (László et al., 1994). Constitutively synthesised nitric oxide may thus protect the microvasculature following acute endotoxin exposure by the prevention of adhesion of neutrophils and platelets to the endothelium and by promoting local vasodilatation (Kubes et al., 1991; Moncada, 1992; Mulder et al., 1994).

The present study thus suggests a protective role of constitutive nitric oxide against the pro-injurious actions of endogenously released leukotrienes or other 5-lipoxygenase products in the maintenance of microvascular integrity in the colon in the early phase of endotoxaemia.

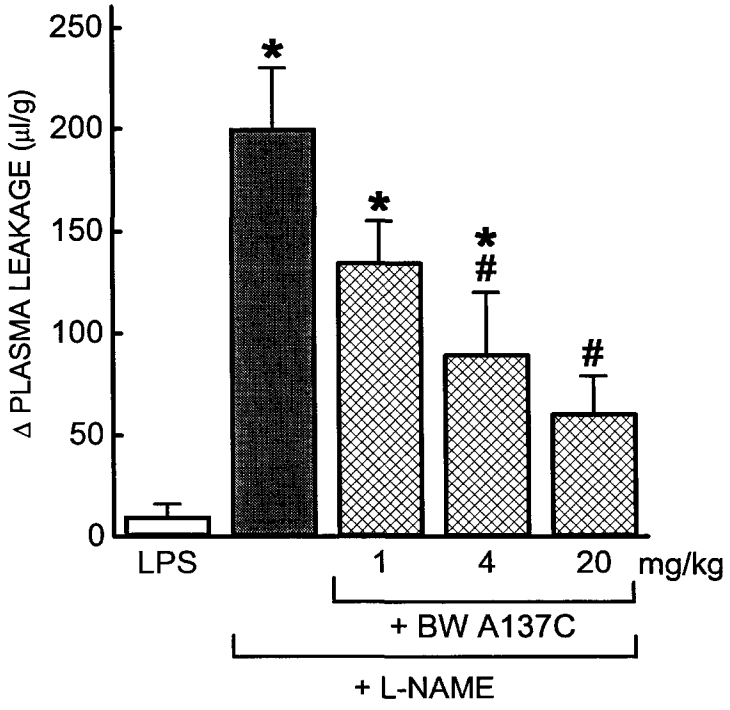

Fig. 1. Acute plasma leakage in the colon provoked by the concurrent administration of endotoxin (LPS; *E. coli* lipopolysaccharide 0111:B4; 3 mg/kg i.v.) and N^G-nitro-L-arginine methyl ester (L-NAME, 5 mg/kg s.c.), and its reduction by a selective 5-lipoxygenase inhibitor, BW A137C (1–20 mg/kg s.c., 15 min before endotoxin and L-NAME). Albumin leakage (expressed as plasma leakage, Δ μl/g tissue) was determined 1 h after challenge and shown as mean ± S.E.M., where $n = 7$–10 rats in a group. $^*P < 0.05$ compared to the endotoxin-alone group (open column), $^\#P < 0.05$ compared to the endotoxin + L-NAME group (grey column).

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