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Role of nitric oxide and platelet-activating factor in the initiation of indomethacin-provoked intestinal inflammation in rats

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Abstract

The effect of indomethacin following the concurrent administration of the nitric oxide (NO) synthase inhibitor N^G-nitro-L-arginine methyl ester (L-NAME) on acute intestinal microvascular permeability has been investigated in the rat. Administration of indomethacin (10 mg/kg, s.c.) or L-NAME (10 mg/kg, s.c.) alone did not affect jejunal and ileal vascular permeability over a 12 h period, as determined by the leakage of radiolabelled serum albumin. By contrast, when indomethacin (10 mg/kg, s.c.) was injected concurrently with L-NAME (2–10 mg/kg, s.c.) significant time-dependent plasma leakage occurred in intestinal tissues over 12 h, being apparent within 1 h. Pretreatment with L-arginine (300 mg/kg, s.c.) 15 min prior L-NAME prevented these changes in microvascular permeability. Likewise, pretreatment with the platelet-activating factor receptor antagonist, WEB 2086 ((3-[4-(2-chlorophenyl)-9-methyl-6H-thienol[3,2-f][1,2,4]triazolo-[4,3-a][1,4]diazepine-2-yl]-1-(4-morpholynil)-l-propanone), 0.1–1 mg/kg, s.c.) dose-dependently attenuated such damage. These findings suggest that following indomethacin administration, the early inhibition of NO synthase leads to acute microvascular injury involving platelet-activating factor in the rat jejunum and ileum, indicating a protective role of NO, formed by constitutive NO synthase. © 1998 Elsevier Science B.V.

Keywords: Indomethacin; Nitric oxide (NO); N^G-Nitro-L-arginine methyl ester (L-NAME); PAF (platelet-activating factor); WEB 2086; Inflammation, intestinal; Plasma leakage

1. Introduction

The non-steroidal anti-inflammatory drug indomethacin causes severe gastrointestinal complications including small bowel inflammation and ulceration leading to intestinal bleeding or perforation in experimental animals and man (Somogyi et al., 1969; Whittle, 1981; Bjarnason et al., 1987; Yamada et al., 1993).

It has been recently demonstrated that significant jejunal plasma leakage, as a measure of intestinal inflammation, occurred 18–48 h following the single subcutaneous injection of a 10 mg/kg dose of indomethacin in the conscious rat (Whittle et al., 1995). This microvascular injury was shown to be related to the induction of nitric oxide (NO) synthase, which was detected 18 h following the administration of indomethacin. It was proposed that excessive levels of NO, formed by the inducible NO synthase was involved in the pathogenesis of the small intestinal lesions (Whittle et al., 1995).

During the first 12 h period following indomethacin challenge there was no change in constitutive NO synthase activity nor any significant intestinal microvascular leakage (Whittle et al., 1995). Under physiological circumstances NO, formed from L-arginine by the constitutive NO synthase in the vascular endothelium, plays a key role in the maintenance of intestinal microvascular integrity and protects against damage (Whittle, 1994). Thus, inhibition of constitutive NO synthase causes extensive gastrointestinal microvascular injury following acute endotoxin challenge (László et al., 1994a), while the involvement of the pro-inflammatory mediator, platelet-activating factor in this acute damage was demonstrated by the use of platelet-activating factor receptor antagonists (László et al., 1994b). Therefore, the aim of our present study was to evaluate the role of constitutive NO synthase in the maintenance of intestinal microvascular integrity over the initial 12 h period following indomethacin challenge. In addition,

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we have studied the possible involvement of platelet-activating factor in the acute intestinal injury following NO synthase inhibition, using the platelet-activating factor antagonist WEB 2086 (Casals-Stanzel, 1987; László et al., 1994b).

2. Materials and methods

2.1. Experimental protocol

Male Wistar rats (225–275 g) were allowed free access to food and water during the experiments. The rats were injected (s.c.) with a 10 mg/kg dose of indomethacin (from a freshly prepared solution containing 10 mg/ml indomethacin) at the beginning of the study. This dose of indomethacin was demonstrated in previous experiments to induce the formation of intestinal lesions and microvascular injury (Whittle et al., 1995).

2.2. Plasma leakage

Under transient halothane anaesthesia, [^{125}I]human serum albumin (2 μCi/kg, containing approximately a 0.4 mg/kg dose of albumin) was injected into the tail vein, 2 h before autopsy and leakage of [^{125}I]human serum albumin was determined in segments of jejunal and ileal tissues 1, 3, 6 and 12 h after indomethacin administration. Blood was collected from the abdominal aorta into syringes containing trisodium citrate (final concentration 12.3 mM, i.e. 0.318%) and centrifuged (10 000 $\times$ g, 10 min, 4°C). The [^{125}I]human serum albumin content of plasma and whole segments of the jejunum and ileum (2–2.5 cm) including the luminal contents was determined in a gamma spectrometer (Nuclear Enterprises NE 1600) and the albumin content in the tissues was calculated as described previously (László et al., 1994a,b). Briefly, values of control tissue (pooled data from groups treated only with radioidinated albumin, i.e. the resting plasma extravasation) were subtracted from the values of treated tissue and the data were expressed as changes in vascular permeability (Δplasma leakage μl plasma/g wet tissue) following the correction for intravascular volume. Intravascular volumes were determined in additional groups of rats by administering radiolabelled albumin (2 μCi/kg) via the tail vein 2 min before tissue removal and expressed as μl/g.

2.3. Effects of L-NAME

In a time–response study, N^G-nitro-L-arginine methyl ester (L-NAME, 10 mg/kg, s.c.) was injected concurrently with indomethacin (10 mg/kg, s.c.). Plasma leakage in the jejunum and ileum was determined 1, 3, 6 and 12 h later. The dose of L-NAME was selected from previous studies on the basis of its potency in elevating systemic arterial blood pressure in the rat and acute microvascular leakage in the endotoxin-challenged rat, as described previously (Rees et al., 1990; László et al., 1994b).

In a dose–response study, L-NAME (2–10 mg/kg, s.c.) was administered concurrently with indomethacin (10 mg/kg, s.c.) and plasma leakage into jejunal and ileal tissues was determined after 6 h.

2.4. L-arginine inhibition of the effects of L-NAME

L-arginine (300 mg/kg, s.c.) was administered 15 min before L-NAME (10 mg/kg, s.c.) and indomethacin (10 mg/kg, s.c.). Plasma leakage in the jejunum and ileum was measured 6 h after L-NAME and indomethacin administration.

2.5. Actions of WEB 2086

The specific platelet-activating factor receptor antagonist, WEB 2086 ((3-[4-(2-chlorophenyl)-9-methyl-6H-thienol[3,2-f][1,2,4]triazolo-[4,3-a][1,4]diazepine-2-yl]-1-(4-morpholynil)-l-propanone) (Casals-Stanzel, 1987) 0.1–1 mg/kg, s.c.) in doses shown previously to be effective against exogenous platelet-activating factor (Casals-Stanzel, 1987) and in a model of endotoxin-induced intestinal leakage (László et al., 1994b) was administered 15 min before indomethacin (10 mg/kg, s.c.) and L-NAME (10 mg/kg, s.c.). Jejunal plasma leakage was determined 1 h later.

2.6. Materials

[^{125}I]-labelled human serum albumin was obtained from Amersham International (UK). WEB 2086 was from Boehringer Ingelheim K.G. All the other compounds were from Sigma Chemical Co. (Poole, Dorset, UK). Indomethacin was dissolved in isotonic (148.8 mM, i.e. 1.25%) $NaHCO_3$. All the other agents were solubilized in 154 mM (0.9%) NaCl.

2.7. Statistics

The data were expressed as mean $\pm$ S.E.M. from (n) rats per experimental group. For statistical comparisons, analysis of variance with the Bonferroni test were utilised. $P < 0.05$ was taken as significant.

3. Results

3.1. Resting plasma leakage and intravascular volumes

In control untreated animals, resting plasma leakage was 128 $\pm$ 6 and 110 $\pm$ 3 μl/g in the jejunum and ileum,

respectively ($n = 11$). The control values for intravascular plasma volume in the jejunum and ileum were of 64 ± 11 and 65 ± 3 μl/g, respectively ($n = 4$), which did not change throughout the 12 h experimental period in any of the groups (data not shown).

3.2. Effect of indomethacin or L-NAME alone

Indomethacin (10 mg/kg, s.c.) or L-NAME (10 mg/kg, s.c.) alone did not affect plasma leakage in the jejunum and ileum over 12 h (Fig. 1).

3.3. Effect of concurrent administration of L-NAME and indomethacin

In contrast, when L-NAME (10 mg/kg, s.c.) was administered concurrently with indomethacin (10 mg/kg, s.c.) significant time-dependent increase in plasma leakage occurred in the jejunum and ileum 1, 3, 6 and 12 h later, e.g. with an elevation of $\Delta 527 \pm 60$ and $\Delta 151 \pm 12$ μl/g tissue at 6 h, respectively ($n = 10$, $P < 0.001$) as shown in Fig. 1.

Increasing doses of L-NAME (2–10 mg/kg, s.c.) concurrently with indomethacin (10 mg/kg, s.c.) caused a dose-dependent enhancement of jejunal and ileal plasma leakage determined at 6 h as shown in Fig. 2.

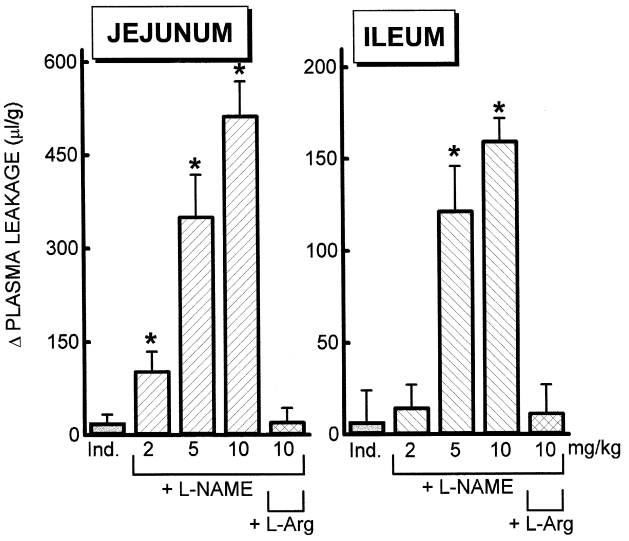

Fig. 2. Potentiation of indomethacin (Ind.; 10 mg/kg, s.c.)-induced plasma leakage (expressed as Δ μl/g tissue) by the concurrent administration of N^G-nitro-L-arginine methyl ester (L-NAME, 2–10 mg/kg, s.c.) and its reversal by the pretreatment with L-arginine (300 mg/kg, s.c., 15 min before challenge) in the rat jejunum (left panel) and ileum (right panel) 6 h later. Data are given as the mean ± S.E.M. of 6 rats per group; statistical significance is shown as $^{*}P < 0.05$ compared to the indomethacin alone groups.

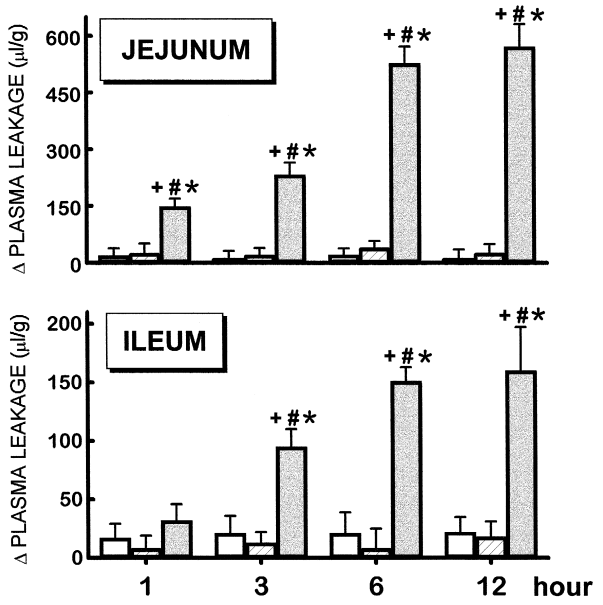

Fig. 1. Plasma leakage induced by indomethacin (10 mg/kg, s.c.) with or without the concurrent administration of N^G-nitro-L-arginine methyl ester (L-NAME, 10 mg/kg, s.c.) in the rat jejunum (upper panel) and ileum (lower panel) over 12 h. The columns show the leakage of plasma (Δ μl/g tissue) 1, 3, 6 and 12 h after the injection of indomethacin (hatched column), L-NAME (open column) and indomethacin with L-NAME (stippled column). Data are given as the mean ± S.E.M. of minimum 4 rats per group; statistical significance is shown as $^{*}P < 0.05$ compared to the control groups (untreated animals), $^{\#}P < 0.05$ compared to the indomethacin alone groups, $^{+}P < 0.05$ compared to the L-NAME alone groups.

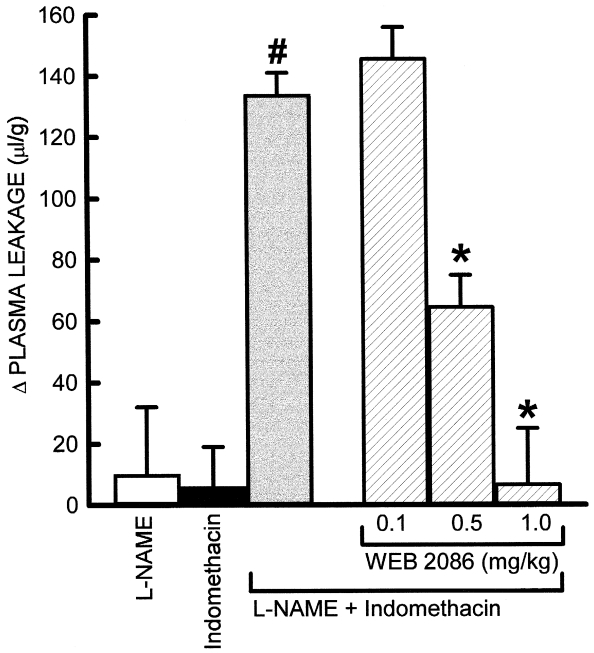

Fig. 3. Acute plasma leakage in the jejunum provoked by the concurrent administration of indomethacin (10 mg/kg, s.c.) and N^G-nitro-L-arginine methyl ester (L-NAME, 10 mg/kg, s.c.) and its reduction by a platelet-activating factor receptor antagonist, WEB 2086 (0.1–1 mg/kg, s.c., 15 min before indomethacin 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 $n = 4$ rats in a group. $^{\#}P < 0.05$ compared to the indomethacin alone group (black column), $^{*}P < 0.05$ compared to the indomethacin + L-NAME group (grey column).

3.4. L-arginine prevention of the effects of L-NAME

Administration of L-arginine (300 mg/kg, s.c.) 15 min before L-NAME (10 mg/kg, s.c.) and indomethacin (10 mg/kg, s.c.) abolished the increase in plasma leakage in the jejunum and ileum 6 h later (Fig. 2). L-arginine alone had no effect on jejunal and ileal plasma leakage ($\Delta 3 \pm 16$ and $\Delta 7 \pm 9$ μl/g tissue, respectively; $n = 4$).

3.5. Effect of WEB 2086

Pretreatment with WEB 2086 (0.1–1 mg/kg, s.c., 15 min before indomethacin) dose-dependently attenuated the acute jejunal microvascular damage induced by the combination of indomethacin (10 mg/kg, s.c.) and L-NAME (10 mg/kg, s.c.), determined after 1 h (Fig. 3).

4. Discussion

In previous studies, the development of microvascular injury in the rat jejunum, which commenced 18–48 h following subcutaneous administration of indomethacin was associated with the expression of inducible NO synthase at these later time-periods. This tissue injury could be inhibited by the delayed administration of L-NAME (Whittle et al., 1995). The present study confirms that the administration of a single parenteral dose of indomethacin (10 mg/kg, s.c.) did not provoke intestinal plasma leakage over the initial 12 h period. Administration of L-NAME alone also did not affect jejunal or ileal microvascular permeability over the 12 h period in the conscious rat, which extends our previous findings that neither L-NAME nor N^G-monomethyl-L-arginine (L-NMMA) alone had any action to provoke ileal or colonic plasma leakage over 5 h (László et al., 1994a). In contrast, when L-NAME was administered concurrently with indomethacin, a significant time- and dose-dependent plasma leakage occurred in the jejunum and ileum over the 12 h period, with potentiating actions being observed as early as 1 h after challenge and plateau responses after 6 h. These actions were abolished by pretreatment with the NO precursor, L-arginine, and suggest a beneficial role of NO formed by constitutive NO synthase in the maintenance of acute intestinal microvascular integrity following indomethacin challenge.

Protective actions of constitutively formed NO have been found in the gastrointestinal tract under various pathological circumstances. Thus, inhibition of NO synthase by L-NAME or L-NMMA following the acute administration of low doses of endotoxin was shown to provoke ileal and colonic microvascular injury (László et al., 1994a,b). Such enhanced plasma leakage would be unlikely to reflect a local reduction in intestinal blood flow by L-NAME, which would be expected to limit vascular leakage. Acute administration of L-NMMA likewise aggravated intestinal plasma leakage induced by high doses of endotoxin (Hutcheson et al., 1990). In addition, in studies in a model of colitis following trinitrobenzene-sulphonic acid administration, pretreatment with L-NAME augmented the colonic inflammation (Kiss et al., 1996).

Following acute challenge with endotoxin, NO, formed constitutively, has been demonstrated to effectively counteract the tissue damaging actions of locally released pro-inflammatory cytotoxic mediators, such as platelet-activating factor (Whittle, 1994). Our present results likewise suggest that during the initial phase following indomethacin administration, there is an early release of pro-inflammatory mediators, whose injurious effects on the intestinal microcirculation are attenuated by endogenous NO.

It has been proposed that the cytokine, tumour necrosis factor alpha is involved in the acute gastric injury caused by non-steroidal anti-inflammatory drugs (Santucci et al., 1994). Furthermore, the adhesion of neutrophils to intestinal vascular endothelium promoted by the inhibition of constitutive NO synthase (Kubes et al., 1991) involves platelet-activating factor (Arndt et al., 1993). In the present study the acute increase in jejunal vascular permeability provoked by the concurrent administration of indomethacin and L-NAME was attenuated by the administration of WEB 2086, implicates the early release of platelet-activating factor following indomethacin challenge. The potent vasodilator actions of NO and its ability to inhibit neutrophil adherence (Kubes et al., 1991) may protect the intestinal microcirculation against the occlusive actions of platelet-activating factor (Filep and Földes-Filep, 1993; Whittle, 1994). Indeed, *S*-nitroso-*N*-acetyl-penicillamine, a spontaneous generator of NO, inhibits gastrointestinal plasma leakage induced by platelet-activating factor (Boughton-Smith et al., 1992) and the increase of vascular permeability induced by platelet-activating factor in the gut can be augmented by L-NAME (Filep and Földes-Filep, 1993). However, a modulator role of NO in the regulation of the biosynthesis or release of platelet-activating factor cannot yet be excluded. Previous studies have also suggested a balance between the local actions of platelet-activating factor and NO, formed constitutively, in hypoxia-induced intestinal injury (Caplan et al., 1994).

The present study thus suggests a protective role of NO formed by the constitutive NO synthase against the acute injurious actions of endogenously released platelet-activating factor on microvascular integrity following administration of indomethacin. Such effects contrast with the subsequent injurious actions of excessive NO production by inducible NO synthase, involved in the delayed jejunal inflammation provoked by indomethacin (Whittle et al., 1995).

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