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Short Communication

Actions of isoform-selective and non-selective nitric oxide synthase inhibitors on endotoxin-induced vascular leakage in rat colon

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Abstract

The effects of the nitric oxide (NO) synthase inhibitor, *N*-(3-(aminomethyl)benzyl)-acetamidine (1400W) which is selective for the inducible isoform of NO synthase, on rat colonic microvascular injury provoked by *Escherichia coli* endotoxin (3 mg/kg i.v.) has been compared to those of aminoguanidine (25–50 mg/kg, s.c.), N^G-iminoethyl-L-ornithine (L-NIO, 15–30 mg/kg, s.c.) and N^G-nitro-L-arginine methyl ester (L-NAME, 2–5 mg/kg, s.c.). Administration of aminoguanidine, L-NIO or L-NAME concurrently with endotoxin provoked microvascular albumin leakage 1 h later, presumably by inhibiting constitutive NO synthase, whereas 1400W (0.1–10 mg/kg, s.c.) had no such effect. Administration of all these agents during the expression of inducible NO synthase (i.e. 3 h after endotoxin challenge) attenuated the subsequent endotoxin-provoked albumin leakage 1 h later. Moreover, concurrent administration of 1400W (0.2–5 mg/kg, s.c.; doses that did not affect systemic arterial blood pressure) with endotoxin suppressed the subsequent rise in albumin leakage after 5 h. These findings indicate that 1400W is a potent inhibitor of colonic microvascular injury associated with induction of NO synthase in vivo. 1400W will thus be useful to investigate in vivo the therapeutic potential of a selective inducible NO synthase inhibitor in inflammation. © 1997 Elsevier Science B.V.

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1. Introduction

Nitric oxide (NO) formed by calcium-dependent constitutive NO synthases plays an important role in the maintenance of vascular integrity under physiological and pathophysiological circumstances (Kubes et al., 1991; Kubes and Granger, 1992; László et al., 1994). Inhibition of the constitutive NO synthase elevates microvascular permeability under physiological (Kubes and Granger, 1992) and pathological (László et al., 1994) conditions, and promotes the acute adhesion of neutrophils to the vascular endothelium of mesenteric venules leading to further microvascular dysfunction (Kubes et al., 1992; Lopez-Belmonte and Whittle, 1995). NO can also be formed by an inducible calcium-independent isoform of NO synthase following challenge with cytokines or endotoxin. Expression of inducible NO synthase and the consequent overproduction of NO is associated with cytotoxic and proinflammatory actions in the gastrointestinal tract, that includes epithelial cell injury and microvascular damage (Boughton-Smith et al., 1993; Tepperman et al., 1994).

In previous studies, concurrent administration of NO synthase inhibitors that are not isoform-selective, such as N^G-monomethyl-L-arginine (L-NMMA) with endotoxin challenge, substantially augmented the early phase of microvascular injury in the gastrointestinal tract, as determined by the leakage of albumin. This was attributed to the inhibition of constitutive NO synthase (Boughton-Smith et al., 1993; László et al., 1994). By contrast, delay of administration of L-NMMA until 3 h following endotoxin challenge, at a time of detectable expression of inducible NO synthase, reduced the subsequent vascular leakage in the gut (Boughton-Smith et al., 1993; László et al., 1994). Moreover, concurrent administration of the proposed inducible isoform selective inhibitor, aminoguanidine (Corbett et al., 1992), likewise enhanced the early phase of

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vascular leakage provoked by endotoxin, while inhibiting the subsequent plasma leakage, indicating poor selectivity for the inducible NO synthase enzyme in vivo in this model (László et al., 1995).

More recently, the pharmacological profile of a highly potent and selective inhibitor of inducible NO synthase, 1400W (*N*-(3-(aminomethyl)benzyl)acetamidine) has been reported (Garvey et al., 1997). Preliminary studies with this agent indicated that it had selective actions on endotoxin-provoked vascular leakage in the rat intestine. In the current study, the actions of 1400W on the early and late phases of vascular leakage in the rat colon has been investigated, and its actions compared to other NO synthase inhibitors, aminoguanidine, N^G-iminoethyl-L-ornithine (L-NIO; Rees et al., 1990) and N^G-nitro-L-arginine methyl ester (L-NAME).

2. Materials and methods

2.1. Experimental protocol

Male Wistar rats (225–275 g) were fasted overnight, but received water ad libitum. Under halothane anaesthesia endotoxin (*Escherichia coli* lipopolysaccharide 0111:B4; Sigma Chemical, Poole, Dorset, 3 mg/kg i.v.), ^{125}I-human serum albumin (Amersham, UK; 2 μCi/kg i.v.) were injected. For evaluation of vascular permeability changes tissues were removed 1, 4 or 5 h after endotoxin.

2.2. Albumin leakage

As a measure of vascular damage, leakage of ^{125}I-human serum albumin was determined in the colon. Under halothane anaesthesia, blood was collected from the abdominal aorta into syringes containing trisodium citrate (final concentration 0.318%) and centrifuged (10 000 × *g*, 10 min, 4°C). The ^{125}I-human serum albumin content of the colon and plasma was determined in gamma spectrometer (Nuclear Enterprises NE 1600) and the albumin content in colonic tissue was calculated, taking into account any changes in colonic blood volume as described previously (Boughton-Smith et al., 1993). Control values (from rats that had received saline) were subtracted from the treated values and the data were expressed as Δ albumin leakage, μl albumin/g wet tissue.

2.3. Actions of early and delayed administration of 1400W and other NO synthase inhibitors

1400W (*N*-(3-iminoethyl)benzyl)acetamidine (a kind gift from Dr. Richard Knowles, GlaxoWellcome Research, Stevenage; 0.1–10 mg/kg s.c.), aminoguanidine hemisulphate (Sigma Chemical, Dorset; 25–50 mg/kg s.c.), L-NIO (N^G-iminoethyl-L-ornithine, GlaxoWellcome Research; 15–30 mg/kg, s.c.) and L-NAME (N^G-nitro-L-arginine methyl ester, Sigma; 2–5 mg/kg, s.c.) were injected concurrently with endotoxin. The doses of NO synthase inhibitors have been selected to be sub- and near-maximal on the basis of previous studies (Rees et al., 1990; László et al., 1995; Garvey et al., 1997). Albumin leakage in the colon was determined 1 h later.

In further studies, 1400W (0.1–1 mg/kg, s.c.), aminoguanidine (25–50 mg/kg, s.c.), L-NIO (15–30 mg/kg, s.c.) or L-NAME (2–5 mg/kg, s.c.) were administered 3 h after endotoxin challenge, and colonic albumin leakage was investigated 1 h later (i.e. 4 h after endotoxin).

2.4. Effects of concurrent administration of 1400W or L-NAME with endotoxin on late phase albumin leakage

1400W (0.2–5 mg/kg, s.c.) or L-NAME (1–5 mg/kg, s.c.) was administered concurrently with endotoxin. Albumin leakage in the colon was determined 5 h after endotoxin challenge.

2.5. Blood pressure

Under pentobarbitone anaesthesia (60 mg/kg, i.p.) systemic arterial blood pressure was measured from the right carotid artery of rats using a pressure transducer (Elcomatic) connected with a Grass Polygraph. The blood pressure was monitored over a 1 h period following the administration of 1400W (10 mg/kg, s.c.) or L-NAME (5 mg/kg, s.c.)

2.6. Statistical analysis

For statistical comparisons, analysis of variance with the Bonferroni test was utilised. Differences were taken as significant when probability was less than 5%.

3. Results

3.1. Actions of early and delayed administration of 1400W and other NO synthase inhibitors

A single bolus injection of endotoxin (3 mg/kg, i.v.) did not affect colonic albumin leakage over a 1 h period. Concurrent administration of 1400W (0.1–1 mg/kg, s.c.) with endotoxin did not affect plasma leakage in the colon after 1 h (Fig. 1). Furthermore, administration of 1400W (10 mg/kg, s.c.) failed to augment albumin leakage (Δ 2 ± 5 μl/g, $n = 4$) determined 1 h after endotoxin. By contrast, concurrent administration of aminoguanidine (25–50 mg/kg, s.c.), L-NIO (15–30 mg/kg, s.c.) or L-NAME (2–5 mg/kg, s.c.) caused a significant dose-dependent increase in colonic albumin leakage 1 h after endotoxin challenge (Fig. 1). When the NO synthase inhibitors, 1400W (10 mg/kg, s.c.), aminoguanidine (50 mg/kg, s.c.), L-NIO (30 mg/kg, s.c.) or L-NAME (5

mg/kg, s.c.) were administered alone in the absence of endotoxin challenge, no significant albumin leakage could be observed 1 h later ($n = 4$–5, data not shown).

When 1400W (0.1–1 mg/kg), aminoguanidine (25–50 mg/kg), L-NIO (15–30 mg/kg) or L-NAME (2–5 mg/kg) were administered (s.c.) 3 h after endotoxin, a significant inhibition (by a maximum of 96 ± 4%, 88 ± 5%, 82 ± 6% or 80 ± 8%, respectively; n = min 4, $P < 0.001$) of endotoxin-provoked colonic albumin leakage (Δ 102 ± 9 μl/g, $n = 8$, $P < 0.001$) occurred 1 h later, i.e. 4 h after endotoxin challenge (Fig. 1).

The intravascular volumes did not change significantly by any of the treatments (endotoxin alone, NO synthase inhibitors alone or in combination with endotoxin) nor at any time-points, being 68 ± 12 μl/g in the colon under basal conditions ($n = 3$–4 for each, data are not shown).

3.2. Effects of 1400W and L-NAME administered concurrently with endotoxin on albumin leakage

A significant albumin leakage in the colon was observed 5 h after endotoxin injection (Δ 132 ± 13 μl/g, $n = 12$, $P < 0.001$) as shown in Fig. 2. This late phase albumin leakage was dose-dependently attenuated by the concurrent administration of 1400W (0.2–5 mg/kg, s.c.) at the time of endotoxin challenge (Fig. 2). However,

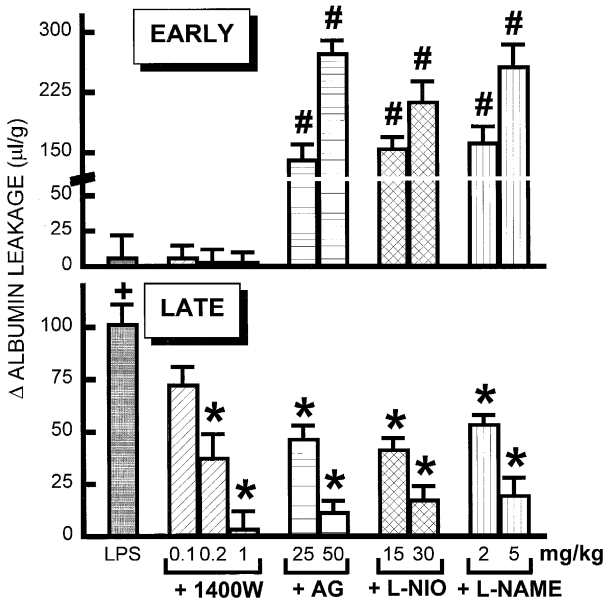

Fig. 1. Actions of concurrent or delayed administration of 1400W (0.1–1 mg/kg, s.c.), aminoguanidine (AG; 25–50 mg/kg, s.c.), L-NIO (15–30 mg/kg, s.c.) and L-NAME (2–5 mg/kg, s.c.) on the early phase (upper panel; after 1 h) and late phase (lower panel; after 4 h) of colonic albumin leakage (expressed as Δ μl/g tissue) following lipopolysaccharide (LPS; 3 mg/kg, i.v.) administration in the rat. Nitric oxide synthase inhibitors were administered concurrently with (early), or 3 h after (late), LPS challenge. Data are expressed as S.E.M., where n is 3–8 rats per group; statistical significances are shown as increased albumin leakage induced by LPS ($^{+}$ $P < 0.05$), potentiation ($^{\#}$ $P < 0.05$) or inhibition (* $P < 0.05$) of LPS-induced vascular permeability.

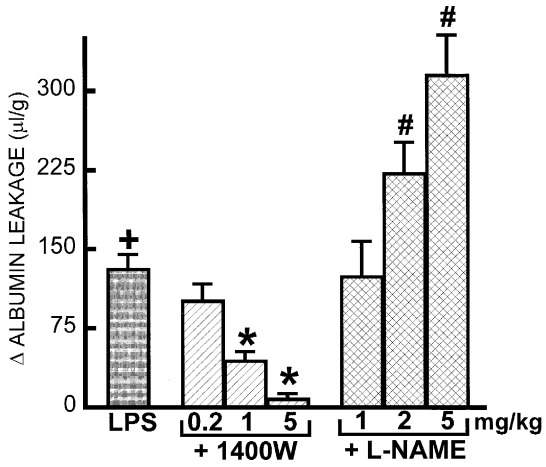

Fig. 2. Effects of concurrent administration of 1400W (0.2–5 mg/kg, s.c.) or L-NAME (1–5 mg/kg, s.c.) with lipopolysaccharide (LPS; 3 mg/kg, i.v.) on albumin leakage (expressed as Δ μl/g tissue) five hours after LPS in the rat colon. Data are expressed as S.E.M., where n is 5–7 rats per group; statistical significances are shown as increased albumin leakage induced by LPS ($^{+}$ $P < 0.05$), potentiation ($^{\#}$ $P < 0.05$) or inhibition (* $P < 0.05$) of LPS-induced vascular permeability.

administration of L-NAME (1–5 mg/kg, s.c.) concurrently with endotoxin significantly aggravated this colonic albumin leakage (Fig. 2).

The intravascular volumes did not change significantly by any of these treatments (endotoxin alone, NO synthase inhibitors alone or in combination with endotoxin) 5 h later, being 72 ± 9 μl/g in the colon under control conditions at 5 h ($n = 3$–4 for each, data are not shown).

3.3. Blood pressure

Over a 1 h investigation period, administration of L-NAME (5 mg/kg, s.c.) caused a significant increase in systemic arterial blood pressure, reaching a maximum value of Δ 57 ± 5 mm Hg after 30 min ($P < 0.001$, $n = 5$). In contrast, following the administration of 1400W (10 mg/kg, s.c.) the blood pressure remained unchanged throughout the 1 h experimental period (being Δ 1 ± 4 mm Hg after 30 min, $n = 4$).

4. Discussion

In the present comparative study, concurrent administration of inhibitors of NO synthases, such as aminoguanidine, L-NIO and L-NAME, with endotoxin provoked albumin leakage in the colon within 1 h, an indicator of acute vascular injury. This acute augmentation of vascular leakage by these agents, including aminoguanidine, is likely to be the response to the inhibition of the constitutive NO synthase (László et al., 1994). By contrast, the selective inhibitor of inducible NO synthase, 1400W had no effect in this early phase of endotoxaemia. Moreover, whereas L-NAME caused an elevation of blood pressure, consid-

ered to reflect the inhibition of constitutively formed NO (Moncada and Higgs, 1995), 1400W had no such effect at the doses used.

The expression of inducible NO synthase 3 h after endotoxin administration in the rat colon is associated with an increase in vascular permeability (Boughton-Smith et al., 1993). Administration of non-selective NO synthase inhibitors, such as L-NMMA (Boughton-Smith et al., 1993; László et al., 1994) at the time of NO synthase induction (3 h after challenge) ameliorated this vascular leakage. The present study confirms these previous findings, since this late phase of endotoxin-induced vascular leakage was inhibited by the delayed administration of aminoguanidine, L-NIO, L-NAME or 1400W. The doses of L-NIO, L-NAME and aminoguanidine that inhibited this late phase of vascular injury were comparable to those that augmented the early phase, indicating little or no selectivity of action of these NO synthase inhibitors in vivo. Vascular leakage provoked by endotoxin challenge, determined 5 h later, was also inhibited by 1400W when it was administered concurrently with endotoxin. Such findings contrasted with the actions of concurrent administration of L-NAME, which produced a substantiated enhancement of vascular leakage after 5 h.

The current model of early and late phase vascular leakage in the gut provoked by endotoxin appears to be useful in defining the selectivity in vivo of proposed isoform-selective NO synthase inhibitors. Using this model, our findings suggest that 1400W is a potent and selective inhibitor of the late phase intestinal microvascular injury associated with induction of NO synthase in vivo. In contrast to the isoform non-selective NO synthase inhibitors, that includes aminoguanidine in this in vivo model, 1400W, even at doses some 50 times those inhibiting the late phase of endotoxin-induced vascular damage, had no detrimental effect on the early phase following challenge. Therefore, 1400W will be useful to explore the therapeutic potential of selective inhibition of inducible NO synthase in vivo in experimental inflammation and injury of the gut and other tissues.

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