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Induction of nitric oxide synthase and microvascular injury in the rat jejunum provoked by indomethacin

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1 The role of nitric oxide (NO) formed by the inducible isoform of NO synthase (NOS) in the generation of indomethacin-induced intestinal microvascular leakage was investigated in the rat.

2 Indomethacin (10 mg kg^{-1}, s.c.) provoked an elevation of vascular leakage of radiolabelled human serum albumin in the jejunum over 48 h, commencing 18 h after its administration. This was associated with the induction of a calcium-independent NOS, as assessed by the conversion of radiolabelled L-arginine to citrulline.

3 Pretreatment with the glucocorticoid, dexamethasone (1 mg kg^{-1} day^{-1}, s.c.) inhibited the induction of NOS and reduced jejunal microvascular leakage, determined 24 and 48 h after indomethacin.

4 Administration of the broad-spectrum antibiotic, ampicillin (800 mg kg^{-1} day^{-1}, p.o.) likewise inhibited both the induction of NOS and the plasma leakage observed 24 and 48 h after indomethacin.

5 Ampicillin pretreatment did not, however, inhibit the induction of NOS, determined 5 h following endotoxin (3 mg kg^{-1} i.v.) challenge. Furthermore, incubation with ampicillin (1 mM, 10 min) did not inhibit the activity of the calcium-independent isoform *in vitro*.

6 Administration of the NOS inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME, 2-10 mg kg^{-1}, s.c.), at the time of the detectable expression of the inducible NOS (18 h after indomethacin), dose-dependently attenuated the plasma leakage, determined 6 later. This effect was reversed by pretreatment with L-arginine (300 mg kg^{-1}, s.c.) 15 min before L-NAME.

7 These findings suggest that induction of a calcium-independent NOS following indomethacin administration involves gut bacteria and leads to microvascular injury in the rat jejunum.

Keywords: Nitric oxide; antibiotics; bacterial invasion; intestinal inflammation; endothelial dysfunction; dexamethasone; NO synthase inhibitors; N^G-nitro-L-arginine methyl ester; inducible NO synthase

Introduction

Gastrointestinal complications commonly occur in animals and man following administration of non-steroid anti-inflammatory drugs. These effects provoked by such agents as indomethacin include intestinal bleeding or perforation arising from severe mucosal inflammation (Hucker *et al.*, 1966; Somogyi *et al.*, 1969; Whittle, 1981; 1992; Bjarnason *et al.*, 1987). However, the biochemical mechanisms leading to the pathophysiological events underlying such intestinal injury are not fully clear (Whittle, 1992). Although the involvement of the gut bacterial flora in the generation of indomethacin-induced intestinal inflammation has been implicated, the mechanistic role of these bacteria or their products in the pathogenic process has not been defined (Fang *et al.*, 1977; Robert & Asano, 1977; Weissenborn *et al.*, 1985; Yamada *et al.*, 1993a).

The parenteral administration of bacterial endotoxins causes acute and the more chronic microvascular damage and inflammation in the small and large bowel (Wallace *et al.*, 1987; Hutcheson *et al.*, 1990; Boughton-Smith *et al.*, 1993b; Laszlo *et al.*, 1994). The microvascular changes that occur several hours following endotoxin challenge correlate with the expression of a Ca^{2+}-independent inducible nitric oxide (NO) synthase (Boughton-Smith *et al.*, 1993b; Laszlo *et al.*, 1995). The inducible isoform of NO synthase (NOS) can be detected in many tissues including the vascular smooth muscle and endothelium, in intestinal epithelium and in inflammatory cells (Radomski *et al.*, 1990; Moncada *et al.*, 1991; Tepperman *et al.*, 1993; Bandaletova *et al.*, 1993; Whittle, 1994). The sustained production of NO under these conditions is considered to bring about the observed cellular injury in endothelial and epithelial cells (Palmer *et al.*, 1992; Tepperman *et al.*, 1993), a process that may involve its interaction with the superoxide anion to produce the reactive peroxynitrite moiety or the formation of other radicals (Beckman *et al.*, 1990; Hogg *et al.*, 1992).

In the present study, the role of inducible NOS in the development of indomethacin-induced intestinal injury has been investigated. As an index of microvascular permeability changes that accompany inflammation, the leakage of radiolabelled albumin in the jejunum was measured at various times following indomethacin administration over 48 h, and correlated with the induction of a calcium-independent NOS. To evaluate the role of bacteria, the effects of the broad-spectrum antibiotic, ampicillin on microvascular leakage and induction of NOS produced by indomethacin have been determined. Moreover, the actions of the NOS inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME), as well as the corticosteroid, dexamethasone, which prevents expression of the inducible NOS (Radomski *et al.*, 1990) on this extravasation induced by indomethacin have also been evaluated.

Methods

Male Wistar rats (225-275 g) were allowed free access to water and food during the experiments. The rats were injected with indomethacin (10 mg kg^{-1}, s.c.) at the beginning of the study, a dose shown from previous studies to provoke the formation of intestinal lesions, particularly in the jejunal region (Whittle, 1981).

Plasma leakage

Under transient halothane anaesthesia, ^{125}I-labelled human serum albumin ([^{125}I]-HSA, 2 μCi kg^{-1}) was injected into a tail vein, 2 h before autopsy. The leakage of [^{125}I]-HSA was subsequently determined in segments of jejunal tissues (3 cm; re-

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moved 10–15 cm from the pyloric sphincter) taken from rats terminally anaesthetized with halothane, 3, 6, 12, 18, 24 and 48 h after indomethacin administration. 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]-HSA content of the plasma and segments of the jejunum was determined in a gamma-spectrometer (Nuclear Enterprises NE 1600) and the albumin content in the tissues was calculated. Values from control tissue were subtracted from the values of treated tissue and the data were expressed as plasma leakage, Δ plasma μl g^{-1} tissue, corrected for intravascular volume as previously described (Boughton-Smith *et al.*, 1993b). Intravascular volume was determined in a separate group of rats by the administration of [^{125}I]-HSA via the tail vein at each time point, 2 min prior to tissue removal.

Nitric oxide synthase activity

NOS activity was determined as the conversion of radiolabelled L-arginine to citrulline by the methods described previously (Salter *et al.*, 1991; Boughton-Smith *et al.*, 1993b). Segments of the jejunum (3 cm; taken 10–15 cm from the pyloric sphincter) were removed from rats terminally anaesthetized with halothane 3, 6, 12, 18, 24 or 48 h after indomethacin administration. Tissues were homogenized (15 s) in ice-cold buffer (tissue 250 mg ml^{-1}, HEPES 10 mM, sucrose 32 mM, dithiothreitol 1 mM, EDTA 0.1 mM, soybean trypsin inhibitor 10 mg ml^{-1}, leupeptin 10 μM ml^{-1}, and aprotonin 2 μl ml^{-1}) adjusted to pH 7.4 (8 M NaOH) followed by centrifugation for 20 min on 10,000 *g* at 4°C. A 40 μl sample of supernatant was incubated for 10 min at 37°C in 110 μl of reaction buffer comprising (final concentrations); KH_2PO_4 50 mM, $MgCl_2$ 1 mM, $CaCl_2$ 0.2 mM, valine 50 mM, dithiothreitol 1 mM, L-arginine 15 nM, citrulline 1 mM, NADPH 0.3 mM, FAD 3 μM, FMN 3 μM, and [^{14}C]-L-arginine 157 pM (110,000 d.p.m. ml^{-1}). The reaction was arrested via the removal of the substrate L-arginine by the addition (0.5 ml) of a 1 : 1 suspension of Dowex (AG 50W-8) in water. The mixture was dispersed and diluted with 0.85 ml of distilled water (total volume 1.5 ml). After allowing the resin to settle, the supernatant was removed for estimation of the radiolabelled products by scintillation counting (2 ml Pico-Fluor). Sample protein content was estimated via spectrophotometric assay (Bio-Rad Protein Assay), and NOS activity was expressed as pmol min^{-1} mg^{-1} protein.

NOS activity was defined as citrulline formation that was abolished by incubation *in vitro* with L-NMMA (700 μM), and was further characterized by the effects of incubation *in vitro* with EGTA (1 mM). Thus, basal L-NMMA-sensitive activity, that was abolished by EGTA, was taken as calcium-dependent constitutive NOS, while that not inhibited by EGTA incubation was taken as calcium-independent inducible NOS activity.

Effects of dexamethasone

Dexamethasone (1 mg kg^{-1}) in a dose that prevents the induction of NOS (Salter *et al.*, 1991; Boughton-Smith *et al.*, 1993b) was injected s.c. concurrently with and 24 h after administration of indomethacin (10 mg kg^{-1}, s.c.). Jejunal plasma leakage and Ca^{2+}-independent NOS activity were determined 24 and 48 h after indomethacin administration.

Effects of ampicillin

Ampicillin (800 mg kg^{-1}), in a dose known to eliminate substantially gut bacteria such as *E. coli* (Jones *et al.*, 1990), was administered orally via a gastric tube, 24 h before, concurrently with and 24 h after the administration of indomethacin (10 mg kg^{-1}, s.c.). Plasma leakage and NOS activity in the jejunum were determined 24 and 48 h after indomethacin administration.

To evaluate the effects of ampicillin on the processes leading to the induction of NOS, rats were treated twice with ampicillin (800 mg kg^{-1} p.o.) or saline (0.5 ml kg^{-1}), 24 h and 2 h prior to administration of endotoxin lipopolysaccharide (LPS) from *E. coli* (3 mg kg^{-1}, i.v.), a dose known to lead to expression of inducible NOS within 3 h (Boughton-Smith *et al.*, 1993b). Segments of jejunum were removed 5 h after challenge and NOS activity determined as described above. To evaluate the effects of ampicillin on inducible NOS activity *in vitro*, segments of jejunum were removed 5 h following challenge with LPS (3 mg kg^{-1}, i.v.) and homogenized as described above. Aliquots of the supernatant (40 μl) were incubated with ampicillin (1 mM) for 10 min prior to determination of NOS activity.

Effects of L-NAME

L-NAME (2–10 mg kg^{-1}, s.c.) was injected 18 h after the administration of indomethacin (10 mg kg^{-1}, s.c.). Plasma leakage in the jejunum was determined 6 h later, i.e. 24 h after indomethacin administration.

L-Arginine reversal of the effects of L-NAME

In additional groups of rats, where L-NAME (10 mg kg^{-1}, s.c.) was administered 18 h after indomethacin (10 mg kg^{-1}, s.c.), L-arginine (300 mg kg^{-1}, s.c.) was injected 15 min before L-NAME. Plasma leakage in the jejunum was measured 6 h later.

Materials

^{125}I-labelled human serum albumin and L-[U-^{14}C]-arginine monohydrochloride were obtained from Amersham International (U.K.). Dexamethasone was supplied as the injectable form (Decadron, Merck Sharp & Dohme, Herts). All the other compounds were from the Sigma Chemical Company (Poole, Dorset, U.K.).

Statistical evaluation

The data are expressed as mean ± s.e.mean from (*n*) rats per experimental group. For statistical comparisons, Student's *t* test for unpaired data or analysis of variance with the Bonferroni test were used, where $P<0.05$ was taken as significant.

Results

Effects of indomethacin on intestinal plasma leakage and nitric oxide synthase activity

Administration of indomethacin (10 mg kg^{-1}, s.c.) provoked a significant time-dependent increase in plasma leakage into the jejunal tissue, starting 18 h after indomethacin injection (Figure 1). After 48 h, the increase of plasma leakage of $\Delta 441 \pm 43$ μl g^{-1} tissue ($n=20$, $P<0.01$) was 10 fold that observed under resting conditions. In control studies following saline administration (0.2 ml kg^{-1}, s.c.), plasma leakage determined over 48 h was not significantly different at any time point from that observed for the initial 2 h period (49 ± 4 μl g^{-1} tissue, $n=14$). Intravascular volume in the jejunum (69 ± 6 μl g^{-1} tissue; $n=4$) was not significantly altered following indomethacin administration, being 61 ± 7, 75 ± 11 and 72 ± 5 μl g^{-1} tissue ($n=4$ for each) determined after 18, 24 and 48 h.

Basal NOS activity was detected in the supernatants of homogenates of segments of jejunum from control rats, being 33 ± 6 pmol min^{-1} mg^{-1} protein ($n=12$). This basal activity was abolished by incubation with EGTA (1 mM), with no Ca^{2+}-independent activity thus being detected. Although there was no significant change in NOS activity from this value 3, 6 or 12 h after administration of indomethacin, NOS activity

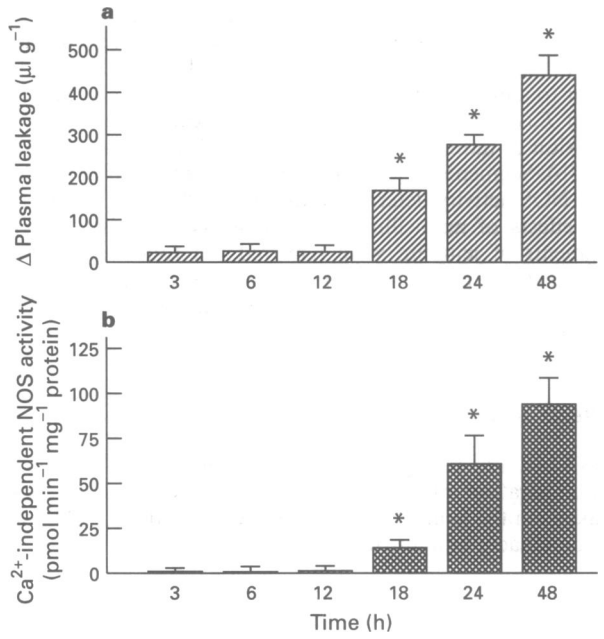

Figure 1 Time-dependent enhancement of vascular leakage of radiolabelled albumin (expressed as Δ plasma leakage, $\mu l\,g^{-1}$ tissue, a) and induction of Ca^{2+}-independent nitric oxide synthase (NOS, expressed as $pmol\,min^{-1}\,mg^{-1}$ protein, b) in the rat jejunum following the administration of indomethacin ($10\,mg\,kg^{-1}$, s.c.). Plasma leakage and NOS were determined at each time point in separate groups of rats. Data are shown as the mean ± s.e.mean, where (n) is at least 4 for each group, and where statistical significance is given as $^*P<0.01$ compared to control (untreated) groups.

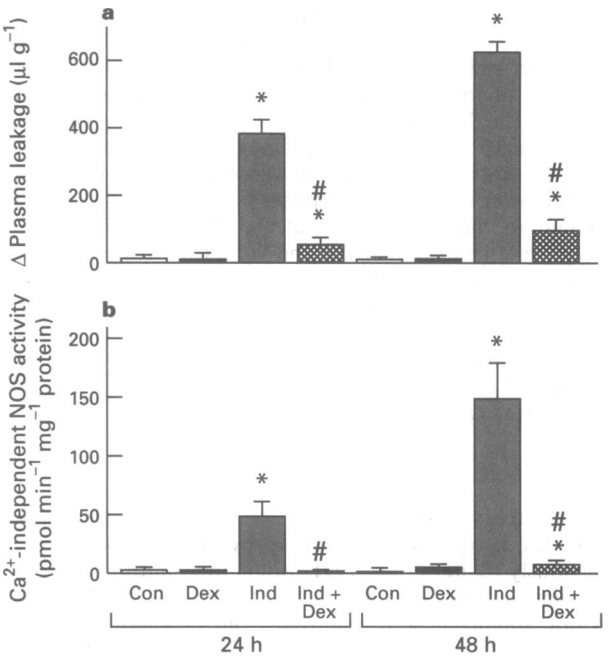

Figure 2 Inhibition by the administration of dexamethasone (Dex $1\,mg\,kg^{-1}$, s.c., concurrently with and 24 h after indomethacin) of the provocation of vascular leakage of radiolabelled albumin (expressed as Δ plasma leakage, $\mu l\,g^{-1}$ tissue, a) and induction of Ca^{2+}-independent nitric oxide synthase (NOS, expressed as pmol $min^{-1}\,mg^{-1}$ protein, b) in the rat jejunum 24 and 48 h following the administration of indomethacin (Ind, $10\,mg\,kg^{-1}$, s.c.). Data are shown as the mean ± s.e.mean, where (n) is at least 4 for each group, and where statistical significance is given as $^*P<0.01$ compared to control (Con, untreated) groups and $^*P<0.01$ compared to indomethacin-treated groups.

was significantly elevated in tissue taken from rats after 18, 24 and 48 h (Figure 1). This elevated NOS activity following indomethacin administration was not significantly inhibited by incubation with EGTA (1 mM; data not shown).

Effects of dexamethasone on jejunal plasma leakage and nitric oxide synthase activity

Treatment with dexamethasone ($1\,mg\,kg^{-1}\,day^{-1}$, s.c.) inhibited plasma leakage determined 24 and 48 h after indomethacin administration by 85 ± 5% and 86 ± 5% respectively, ($n=6$ for each, $P<0.01$), as shown in Figure 2.

The indomethacin-induced increase in Ca^{2+}-independent NOS activity was likewise reduced by 98 ± 2% and 96 ± 3% ($n=6$ respectively $P<0.01$), 24 and 48 h following dexamethasone treatment (Figure 2).

Effects of ampicillin on jejunal plasma leakage and nitric oxide synthase activity

Treatment with ampicillin ($800\,mg\,kg^{-1}$ p.o., 24 h before, concurrently with and 24 h after indomethacin) significantly reduced the plasma leakage and the induction of NOS activity, determined 24 and 48 h after indomethacin. Thus, plasma leakage was reduced by 74 ± 5% ($n=6$, $P<0.01$) and the appearance of the Ca^{2+}-independent NOS activity was reduced by 73 ± 8% ($n=4$, $P<0.01$), 24 h after indomethacin (Figure 3). Administration of ampicillin significantly inhibited plasma leakage by 82 ± 6% ($n=6$, $P<0.01$) and the appearance of the Ca^{2+}-independent NOS activity by 92 ± 2% ($n=10$, $P<0.01$), 48 h after indomethacin (Figure 3).

Treatment with ampicillin ($800\,mg\,kg^{-1}$, 24 h and 2 h prior to challenge) did not affect the induction of NO synthase by LPS ($3\,mg\,kg^{-1}$ i.v.) in the jejunum; thus the Ca^{2+}-independent activity in the jejunum, 5 h after challenge with LPS (263 ± 11 pmol min^{-1} mg^{-1} protein; $n=5$), was not significantly changed (4 ± 3%, $n=5$) by administration of ampicillin. Furthermore, the Ca^{2+}-independent NOS activity, from jejunal tissue of LPS-treated rats, was not significantly altered (5 ± 2%; $n=5$) by 10 min incubation with ampicillin (1 mM).

Effects of L-NAME on jejunal plasma leakage

Treatment with L-NAME ($2-10\,mg\,kg^{-1}$, s.c.) 18 h after indomethacin administration, dose-dependently inhibited plasma leakage, with a maximum reduction of 74 ± 5% ($n=6$, $P<0.001$) with the highest dose, when determined 24 h later (Figure 4). With a dose of L-NAME of $10\,mg\,kg^{-1}$, plasma leakage at 24 h was significantly lower than that observed with indomethacin alone at 18 h (increase of Δ97 ± 17 and Δ169 ± 19 $\mu l\,g^{-1}$ tissue respectively; $n=4$, $P<0.05$).

Pretreatment with L-arginine ($300\,mg\,kg^{-1}$, s.c.), 15 min before administration of L-NAME ($10\,mg\,kg^{-1}$, s.c., 18 h after indomethacin), abolished the L-NAME-induced reduction in plasma leakage (Figure 4).

Discussion

In the present study, indomethacin provoked a time-dependent increase in vascular leakage of albumin in the rat jejunum, as an index of microvascular injury, that became apparent 18 h after administration. This microvascular leakage was associated with the expression of a Ca^{2+}-independent NOS activity in the rat jejunum, that also became apparent 18 h after indomethacin administration. Such Ca^{2+}-independent NOS activity in the intestine is most likely to be due to the expression of the inducible isoform, (Salter *et al.*, 1991; Boughton-Smith *et al.*, 1993b; Bandaletova *et al.*, 1993). The temporal re-

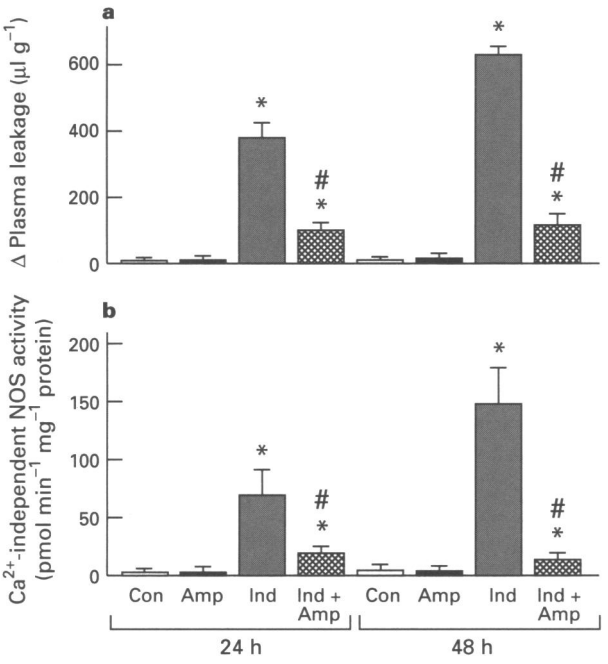

Figure 3 Inhibition by the administration of ampicillin (Amp, 800 mg kg^{-1}, p.o. 24 h before, concurrently with and 24 h after indomethacin) of provocation of vascular leakage of radiolabelled albumin (expressed as Δ plasma leakage, μl g^{-1} tissue, a) and induction of Ca^{2+}-independent nitric oxide synthase (NOS, expressed as pmol min^{-1} mg^{-1} protein, b) in the rat jejunum, 24 and 48 h after the administration of indomethacin (Ind, 10 mg kg^{-1}, s.c.). Data are shown as the mean ± s.e.mean, where (n) is at least 4 for each group, and where statistical significance is given as $*P<0.01$ compared to control (Con, untreated) groups and $\#P<0.01$ compared to indomethacin-treated groups.

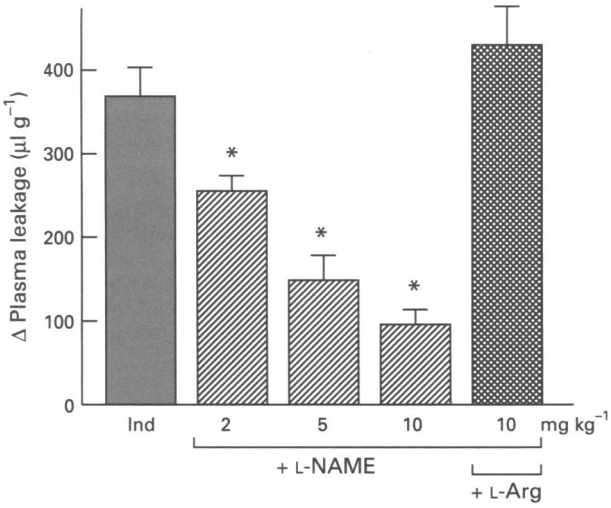

Figure 4 Inhibition of indomethacin (Ind, 10 mg kg^{-1}, s.c.)-induced vascular leakage of radiolabelled albumin in the rat jejunum (expressed as Δ plasma leakage, μl g^{-1} tissue) by the administration of L-NAME (2–10 mg kg^{-1}, s.c., 18 h after indomethacin) and its reversal by L-arginine (L-Arg, 300 mg kg^{-1}, s.c., 15 min before L-NAME (10 mg kg^{-1}, s.c.). Plasma leakage was determined 24 h after indomethacin administration, i.e. 6 h after the injection of L-NAME. Data are shown as the mean ± s.e.mean, where (n) is 6–8 for each group, and where statistical significance is given as $*P<0.05$ compared to the indomethacin-treated group.

lationship between microvascular injury and NOS induction suggests a role of the excessive local generation of NO in the intestinal inflammatory response that follows indomethacin administration. Moreover, the reduction of albumin extravasation by L-NAME, administered at the time of detection of the Ca^{2+}-independent NOS, gives further support to the involvement of NO in this jejunal damage.

Treatment with the broad-spectrum antibiotic, ampicillin, was shown in the present study to prevent the indomethacin-provoked microvascular leakage in the jejunum. Such findings support several previous observations that antibiotics inhibit the intestinal inflammation induced by indomethacin in rats (Fang *et al.*, 1977; Yamada *et al.*, 1993a). Furthermore, a significant increase in intestinal luminal bacterial concentration following indomethacin administration has been recently demonstrated (Yamada *et al.*, 1993a), which may promote the local invasion of bacteria or the release of bacterial products into the intestinal mucosa, thus initiating an inflammatory response. In previous studies, the time-dependent enhancement of intestinal vascular permeability provoked by bacterial endotoxin from *E. coli*, a predominant member of the rat gut flora, was associated with the expression of the Ca^{2+}-independent NOS (Boughton-Smith *et al.*, 1993b). A role for local toxins produced by the gut bacterial flora in the processes underlying the induction of NOS following indomethacin is supported by the finding that following ampicillin administration, the Ca^{2+}-independent NOS activity in the jejunum was virtually abolished. In control experiments, administration of ampicillin did not affect the induction of NOS in the jejunum provoked by endotoxin challenge *in vivo*, nor did it inhibit the activity of the inducible NOS from jejunal homogenates when incubated *in vitro*, supporting an indirect action through its antibacterial properties in the gut.

The induction of NOS has been described in models of inflammatory bowel disease in rats (Boughton-Smith *et al.*, 1992) and elevated levels of the Ca^{2+}-independent isoform have been found in the inflamed mucosa of ulcerative colitis patients (Boughton-Smith *et al.*, 1993a). The overproduction of NO has also been suggested as a pathogenic factor in the generation of experimental chronic ileitis and in inflammatory bowel diseases (Yamada *et al.*, 1993b; Miller *et al.*, 1993a,b; Middleton *et al.*, 1993). Glucocorticoids, such as dexamethasone have been shown both *in vivo* and *in vitro* to inhibit the induction of NOS in vascular tissue (Radomski *et al.*, 1990; Knowles *et al.*, 1990; Palmer *et al.*, 1992) and in intestinal epithelial cells (Tepperman *et al.*, 1993) following endotoxin or cytokine challenge. Moreover, dexamethasone inhibits the expression of the inducible NOS and the associated vascular leakage in the jejunum and colon following the administration of endotoxin (Boughton-Smith *et al.*, 1993b).

In the present study, dexamethasone substantially reduced the indomethacin-provoked appearance of the Ca^{2+}-independent NOS and the associated vascular leakage in the jejunum. Such findings, along with the observations that L-NAME can also attenuate the extravasation, thus further support the involvement of the inducible NOS in this microvascular injury promoted by indomethacin. Recent studies have also shown that dexamethasone can inhibit indomethacin-induced haemorrhagic ulceration in the rat jejunum, as evaluated macroscopically and by histochemical techniques (Anthony *et al.*, 1994). The present findings therefore indicate that the therapeutic effect of corticosteroids in the treatment of inflammatory conditions of the intestine may relate, in part, to the inhibition of the expression of the inducible NOS. The cellular distribution of inducible NOS within the jejunum and the contribution of invading inflammatory cells following indomethacin challenge awaits further biochemical, immunohistochemical and molecular studies.

The results of the present study thus suggest the involvement of bacteria in the mechanism leading to the induction of NOS in the rat intestine following indomethacin administration, and to the associated increase in microvascular leakage. Clinical studies also suggest that the anti-bacterial agent, metronidazole can improve the chronic intestinal enteropathy induced by non-steroid anti-inflammatory drugs (Bjarnason *et al.*, 1991). It is feasible that the early inhibition of cyclo-oxygenase by indomethacin and similar agents (Vane, 1971) results

in a breach in the physiological regulation of intestinal mucosal barrier function (Whittle, 1981; 1992), allowing the eventual ingress of the gut bacterial flora or their toxins. The local release of mediators, such as cytokines, provoked by such bacterial products or following tissue injury, may also be involved in the subsequent priming or activation of inflammatory cells, as well as epithelial and vascular cells, leading to induction of NOS. If similar mechanisms underlie the pathological processes in the human gut, then inhibitors of the inducible NOS may have potential therapeutic benefit in the treatment of intestinal inflammation provoked by non-steroid anti-inflammatory drugs.

References

ANTHONY, A., DHILLON, A.P., SIM, R., POUNDER, R.E. & WAKEFIELD, A.J. (1994). Dexamethasone promotes ulcer plugging in experimental enteritis. *Aliment. Pharmacol. Ther.*, **8**, 597–602.

BANDALETOVA, T., BROUET, I., BARTSCH, H., SUGIMURA, T., ESUMI, H. & OHSHIMA, H. (1993). Immunohistochemical localization of an inducible form of nitric oxide synthase in various organs of rats treated with *Propionibacterium acnes* and lipopolysaccharide. *APMIS*, **101**, 330–336.

BECKMAN, J.S., BECKMAN, T.W., CHEN, J., MARSHALL, P.A. & FREEMAN, B.A. (1990). Apparent hydroxyl radical production by peroxynitrate: implications for endothelial injury from nitric oxide and superoxide. *Proc. Natl. Acad. Sci. U.S.A.*, **87**, 1620–1624.

BJARNASON, I., ZANELLI, G., SMITH, T., PROUSE, P., WILLIAMS, P., SMETHURST, P., DELACEY, G., GUMPEL, M.J. & LEVI, A.J. (1987). Nonsteroidal anti-inflammatory drug-induced intestinal inflammation in humans. *Gastroenterology*, **93**, 480–489.

BJARNASON, I., HOPKINSON, N., ZANELLI, P., PROUSE, P., SMETHURST, P. & LEVI, A.J. (1991). Treatment of non-steroidal anti-inflammatory drug induced enteropathy in man. *Gut*, **31**, 777–780.

BOUGHTON-SMITH, N.K., EVANS, S.M., HAWKEY, C.J., COLE, A.T., BALSITIS, M., WHITTLE, B.J.R. & MONCADA, S. (1993a). Nitric oxide synthase activity in ulcerative colitis and Crohn's disease. *Lancet*, **342**, 338–340.

BOUGHTON-SMITH, N.K., EVANS, S.M., LASZLO, F., WHITTLE, B.J.R. & MONCADA, S. (1993b). The induction of nitric oxide synthase and intestinal vascular permeability by endotoxin in the rat. *Br. J. Pharmacol.*, **110**, 1189–1195.

BOUGHTON-SMITH, N.K., EVANS, S.M., WHITTLE, B.J.R. & MONCADA, S. (1992). Induction of colonic nitric oxide synthase in a rat model of colitis. *Gastroenterology*, **102**, A598.

FANG, W.-F., BROUGHTON, A. & JACOBSON, E.D. (1977). Indomethacin induced intestinal inflammation. *Am. J. Dig. Dis.*, **22**, 749–760.

HOGG, N., DARLEY-USMAR, V.M., WILSON, M.T. & MONCADA, S. (1992). Production of hydroxyl radicals from the simultaneous generation of superoxide and nitric oxide. *Biochem. J.*, **281**, 419–424.

HUCKER, H.B., ZACCHEI, A.G., COX, S.V., BRODIE, D.A & CANTWELL, N.H.R. (1966). Studies on the absorption, distribution and excretion of indomethacin in various species. *J. Pharmacol. Exp. Ther.*, **153**, 237–249.

HUTCHESON, I.R., WHITTLE, B.J.R. & BOUGHTON-SMITH, N.K. (1990). Role of nitric oxide in maintaining vascular integrity in endotoxin-induced acute intestinal damage in the rat. *Br. J. Pharmacol.*, **101**, 815–820.

JONES, W.G., BARBER, A.E., MINEI, J.P., FAHEY, T.J., SHIRES, G.T. & SHIRES, G.T. (1990). Antibiotic prophylaxis diminishes bacterial translocation but not mortality in experimental burn wound sepsis. *J. Trauma*, **30**, 737–740.

KNOWLES, R.G., SALTER, M., BROOKS, S.L. & MONCADA, S. (1990). Anti-inflammatory glucocorticoids inhibit the induction by endotoxin of nitric oxide synthase in the lung, liver and aorta of the rat. *Biochem. Biophys. Res. Commun.*, **172**, 1042–1048.

LASZLO, F., EVANS, S.M. & WHITTLE, B.J.R. (1995). Aminoguanidine inhibits both constitutive and inducible nitric oxide synthase isoforms in rat intestinal microvasculature in vivo. *Eur. J. Pharmacol.*, **272**, 169–175.

LASZLO, F., WHITTLE, B.J.R. & MONCADA, S. (1994). Time-dependent enhancement and inhibition of endotoxin-induced vascular injury in rat intestine by nitric oxide synthase inhibitors. *Br. J. Pharmacol.*, **111**, 1309–1315.

MIDDLETON, S.J., SHORTHOUSE, M. & HUNTER, J.D. (1993). Increased nitric oxide synthesis in ulcerative colitis. *Lancet*, **341**, 465–466.

MILLER, M.J.S., SADOWSKA-KROWICKA, H., CHOTINARUEMOL, S., KAKKIS, J.L. & CLARK, D.A. (1993a). Ameloriation of chronic ileitis by nitric oxide synthase inhibition. *J. Pharmacol. Exp. Ther.*, **264**, 11–16.

MILLER, M.J.S., ZHANG, Z.-J., SADOWSKA-KROWICKA, H., CHOTINARUEMOL, S., MCINTYRE, J.A., CLARK, D.A. & BUSTAMANTE, S.A. (1993b). Nitric oxide release in response to gut injury. *Scand. J. Gastroenterol.*, **28**, 149–154.

MONCADA, S., PALMER, R.M.J. & HIGGS, E.A. (1991). Nitric oxide: physiology, pathophysiology, and pharmacology. *Pharmacol. Rev.*, **43**, 109–141.

PALMER, R.M.J., BRIDGE, L., FOXWELL, N.A. & MONCADA, S. (1992). The role of nitric oxide in endothelial cell damage and its inhibition by glucocorticoids. *Br. J. Pharmacol.*, **105**, 11–12.

RADOMSKI, M.W., PALMER, R.M.J. & MONCADA, S. (1990). Glucocorticoids inhibit the expression of an inducible, but not the constitutive nitric oxide synthase in vascular endothelial cells. *Proc. Natl. Acad. Sci. U.S.A.*, **87**, 10043–10047.

ROBERT, A. & ASANO, T. (1977). Resistance of germ-free rats to indomethacin-induced intestinal inflammation. *Prostaglandins*, **14**, 333–341.

SALTER, M., KNOWLES, R.G. & MONCADA, S. (1991). Widespread tissue distribution, species distribution and changes in activity of Ca^{2+}-dependent and Ca^{2+}-independent nitric oxide synthases. *FEBS Lett.*, **291**, 145–149.

SOMOGYI, A., KOVACS, K. & SELYE, H. (1969). Jejunal ulcers produced by indomethacin. *J. Pharm. Pharmacol.*, **21**, 122–123.

TEPPERMAN, B.L., BROWN, J.F. & WHITTLE, B.J.R. (1993). Nitric oxide synthase induction and intestinal epithelial cell viability in rats. *Am. J. Physiol.*, **265**, G214–G218.

VANE, J.R. (1971). Inhibition of prostaglandin synthesis as a mechanism of action of aspirin-like drugs. *Nature (New Biol)*, **231**, 232–235.

WALLACE, J.L., STEEL, G., WHITTLE, B.J.R., LAGENTE, V. & VARGAFTIG, B. (1987). Evidence for platelet-activating factor as a mediator of endotoxin induced gastrointestinal damage in the rat. Effects of three platelet-activating factor antagonists. *Gastroenterology*, **93**, 765–773.

WEISSENBORN, U., MAEDGE, S., BUETTNER, D. & SEWING, K.F. (1985). Indomethacin-induced gastrointestinal lesions in relation to tissue concentration, food intake and bacterial invasion in the rat. *Pharmacology*, **30**, 32–39.

WHITTLE, B.J.R. (1981). Temporal relationship between cyclooxygenase inhibition, as measured by prostacyclin biosynthesis, and the gastrointestinal damage induced by indomethacin in the rat. *Gastroenterology*, **80**, 94–98.

WHITTLE, B.J.R. (1992). Unwanted effects of aspirin and related agents in the gastrointestinal tract. In *Aspirin and Other Salicylates*. ed. Vane, J.R. & Botting, R.M. pp.465–509. London: Chapman & Hall.

WHITTLE, B.J.R. (1994). Nitric oxide in gastrointestinal physiology and pathology. In *Physiology of the Gastrointestinal Tract*. Third edition. ed. Johnson, L.R. pp 267–294. New York: Raven Press.

YAMADA, T., DEITCH, E., SPECIAN, R.D., PERRY, M.A., SARTOR, R.B. & GRISHAM, M.B. (1993a). Mechanisms of acute and chronic intestinal inflammation induced by indomethacin. *Inflammation*, **17**, 641–662.

YAMADA, T., SARTOR, R.B., MARSHALL, S., SPECIAN, R.D. & GRISHAM, M.B. (1993b). Mucosal injury and inflammation in a granulomatous colitis in rats. *Gastroenterology*, **104**, 759–771.

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