ELSEVIER

European Journal of Pharmacology 280 (1995) 63–68

ejp

Reduction of gastrointestinal injury in acute endotoxic shock by flurbiprofen nitroxybutylester

John L. Wallace [a,*], G. Cirino [b], G. Webb McKnight [a], Susan N. Elliott [a]

[a] *Intestinal Disease Research Unit, Faculty of Medicine, University of Calgary, Calgary, Alberta, T2N 4N1, Canada*
[b] *Department of Experimental Pharmacology, University of Naples, Naples, 80131, Italy*

Received 24 November 1994; revised 13 February 1995; accepted 21 March 1995

Abstract

Nitric oxide has been reported to have paradoxical effects in experimental endotoxic shock, contributing to the hemodynamic consequences of endotoxin administration, but apparently protecting the gastrointestinal mucosa. A novel class of nitric oxide-releasing nonsteroidal anti-inflammatory drug (NSAID) derivatives has recently been described which exert anti-inflammatory activities but produce significantly less gastrointestinal injury than the parent nonsteroidal anti-inflammatory drugs from which they are derived. Thus, the present study was performed to determine the effects of one of these derivatives, flurbiprofen 4-nitroxybutylester, compared to the native nonsteroidal anti-inflammatory drug, flurbiprofen, in an experimental model of endotoxic shock. Intravenous administration of endotoxin from *Salmonella typhosa* to rats pretreated with flurbiprofen produced a profound decrease in systemic arterial blood pressure, an increase in hematocrit and extensive gastric and small intestinal damage. In rats pretreated with flurbiprofen 4-nitroxybutylester, endotoxin produced comparable changes in blood pressure and hematocrit to those seen in rats treated with flurbiprofen; however, the severity of gastrointestinal damage was significantly reduced. Gastric blood flow was profoundly decreased following endotoxin administration, but was significantly higher in rats pretreated with flurbiprofen 4-nitroxybutylester than in rats pretreated with flurbiprofen. These results demonstrate that despite not affecting the acute systemic effects of endotoxin administration, flurbiprofen 4-nitroxybutylester is capable of protecting the gastrointestinal mucosa from injury, possibly through preservation of mucosal blood flow.

Keywords: Non-steroidal anti-inflammatory drug; Nitric oxide (NO); Endotoxic shock, acute; Gastrointestinal; Ulcer; Prostaglandin; Cardiovascular

1. Introduction

Nitric oxide has been suggested to be a critical mediator of the cardiovascular consequences of endotoxemia (Wright et al., 1992; Thiemermann and Vane, 1990; Rees et al., 1990). There have been conflicting reports on the effects of administration of inhibitors of nitric oxide synthase on the hypotension caused by endotoxin administration (Thiemermann and Vane, 1990; Wright et al., 1992), possibly a consequence of differences in the species or the nitric oxide synthase inhibitors used in these studies. While there is evidence that nitric oxide participates in the pathogenesis of endotoxic shock, nitric oxide has also been suggested to play a role in protecting the gastrointestinal tract during acute endotoxemia. Such damage is believed to be attributable, at least in part, to the reduced mucosal blood flow as a consequence of systemic hypotension, and to the local generation of ulcerogenic mediators such as platelet-activating factor and thromboxane (Wallace et al., 1987, 1990; Whittle et al., 1987; Boughton-Smith et al., 1989). Boughton-Smith et al. (1990) demonstrated that administration of a nitric oxide synthase inhibitor to rats prior to administration of endotoxin resulted in a marked exacerbation of intestinal injury, suggesting an important protective role for nitric oxide in the gut. Their demonstration that infusion of a nitric oxide donor, *S*-nitroso-*N*-penicillamine, resulted in a significant attenuation of endotoxin-induced intestinal injury further supported this hypothesis. This nitric oxide donor also reversed the detrimental effects of administration of a nitric oxide synthase inhibitor (Boughton-Smith et al., 1990).

* Corresponding author. Department of Pharmacology and Therapeutics, University of Calgary, 3330 University Avenue NW, Calgary, Alberta, T2N 4N1, Canada. Tel. 403-220-4539, fax 403-270-3353.

Elsevier Science B.V.
SSDI 0014-2999(95)00184-0

Nonsteroidal anti-inflammatory drugs (NSAIDs) have been shown to improve survival and attenuate some of the cardiovascular alterations which characterize endotoxic shock (Wise et al., 1980; Jacobs et al., 1982; Beck and Abel, 1987). These effects have been attributed to the ability of these drugs to suppress the synthesis of prostaglandins and thromboxane, which appear to contribute to many of the sequelae of endotoxin administration. We recently described a novel class of nonsteroidal anti-inflammatory drug derivatives which spare the gastrointestinal tract, despite significantly suppressing prostaglandin synthesis (Wallace et al., 1994a,b; Reuter et al., 1994). We hypothesized that the lack of gastrointestinal injury with these compounds was a consequence of their ability to release nitric oxide, which would maintain gastrointestinal blood flow and prevent neutrophil adherence to the vascular endothelium in the gastrointestinal microcirculation. This hypothesis is supported by a number of observations, including the significant increase in plasma nitrate/nitrite levels observed within an hour of their administration (Wallace et al., 1994a,b).

The purpose of the present study was to determine the effects of a nitric oxide-releasing nonsteroidal anti-inflammatory drug, flurbiprofen 4-nitroxybutylester, compared to its parent drug, flurbiprofen, on the systemic and gastrointestinal effects of endotoxin in the rat. Since nitric oxide donors have been shown to protect the gastrointestinal tract in endotoxic shock (Boughton-Smith et al., 1990) and in other models of injury (MacNaughton et al., 1989; Kitagawa et al., 1990; Lopez-Belmonte et al., 1993), we tested the hypothesis that the nitric oxide-releasing NSAID would exert protective effects in the gastrointestinal tract in experimental endotoxic shock.

2. Materials and methods

2.1. Animals

Male, Sprague-Dawley rats weighing 200–225 g were obtained from Charles River Breeding Farms (Montreal, Canada) and were housed in rack-mounted, wire mesh cages and fed standard laboratory chow and water ad libitum. The rats were deprived of food, but not water, for 18–22 h prior to an experiment. All experimental procedures described in this paper were approved by the Animal Care Committee of the University of Calgary.

2.2. Endotoxic shock

Rats were anesthetized with sodium pentobarbital (65 mg/kg i.p.) and a carotid artery and femoral vein were cannulated for recording of systemic arterial blood pressure and administering drugs, respectively. A blood sample was taken from the carotid cannula and the initial hematocrit was determined. Blood pressure was recorded for 15 min, then either flurbiprofen (10 mg/kg; $n = 9$), flurbiprofen 4-nitroxybutylester (15 mg/kg; equimolar dose to that of flurbiprofen; $n = 11$) or vehicle (50% dimethyl sulfoxide; 50% isotonic saline; v/v; $n = 8$) were administered intravenously. Dose-response studies of these compounds on gastrointestinal damage and prostaglandin synthesis have been performed previously (Wallace et al., 1994a; Reuter et al., 1994). It was based on the results of the previous studies that the doses used in the present study were selected. At these doses, flurbiprofen 4-nitroxybutylester and flurbiprofen were found to markedly suppress gastric prostaglandin synthesis, and to exert anti-inflammatory properties. We did, however, carry out a series of experiments with a lower dose of each drug (flurbiprofen at 5 mg/kg and flurbiprofen 4-nitroxybutylester at 7.5 mg/kg; $n = 4$ per group). Invariably the administration of the test drugs or vehicle caused a transient fall in blood pressure (~ 40 mm Hg), which had returned to basal levels within 1 min. Fifteen minutes later, endotoxin (lipopolysaccharide from *Salmonella typhosa*) was administered intravenously at a dose of 25 mg/kg. Ninety minutes later a blood sample was taken from the carotid cannula and the final hematocrit was determined. The rat was then killed and the stomach and small intestine removed, opened by an incision along the greater curvature/anti-mesenteric border and pinned out on a wax platform. The tissue was then covered with neutral-buffered formalin. An observer who was unaware of the treatment groups then scored the macroscopically visible gastric and intestinal injury on a 0–3 scale using the following criteria: 0, normal; 1, diffuse, superficial hyperemia; 2, extensive hyperemia with patches of overt hemorrhage; 3, extensive hyperemia and overt hemorrhage. After the macroscopic scoring was completed, samples of the corpus region were excised and processed by routine techniques for histological evaluation by light microscopy. Again, this evaluation was performed by an observer unaware of the treatment groups. The severity of damage was scored on a 0–3 scale using criteria previously described by Hutcheson et al. (1990), as follows: 0, normal; 1, focal regions of vasocongestion; 2, extensive vasocongestion of the subepithelial vessels and focal congestion of deeper vessels; 3, extensive vasocongestion of the entire depth of the mucosa and submucosal hemorrhage.

2.3. Gastric blood flow

As an index of the effects of endotoxin, flurbiprofen and flurbiprofen 4-nitroxybutylester on splanchnic

blood flow, gastric blood flow was measured by laser-Doppler flowmetry as described previously (Wallace et al., 1990). Following induction of anesthesia with sodium pentobarbital (65 mg/kg i.p.), an ex vivo gastric chamber was prepared (Wallace et al., 1990). The exposed mucosa was bathed with isotonic saline throughout the experiment. Basal gastric blood flow was measured for 15 min, after which flurbiprofen, flurbiprofen 4-nitroxybutylester or vehicle were administered intravenously, as in the experiments described above. Fifteen minutes later endotoxin was administered, as above, and gastric blood flow was monitored for the ensuing 90 min. As laser-Doppler flowmetry is not suitable for measuring absolute rates of gastric blood flow, all data are expressed as the percent blood flow relative to basal flow in each experiment. Each group consisted of 5–6 rats.

2.4. Statistical analysis

All data are presented as the mean ± S.E.M. Comparisons of the gastrointestinal damage scores among groups were made using a Mann-Whitney *U*-test, while comparisons of all other data among treatment groups were made using a two-way analysis of variance followed by a Newman-Keuls test. In all cases, an associated probability (*P* value) of less than 5% was considered significant.

2.5. Materials

Flurbiprofen 4-nitroxybutylester was kindly provided by Nicox (London, UK). Flurbiprofen, dimethyl sulfoxide and endotoxin (*S. typhosa*) were obtained from Sigma Chemical Company (St. Louis, MO, USA). Endotoxin was dissolved in isotonic saline at a concentration of 12.5 mg/ml.

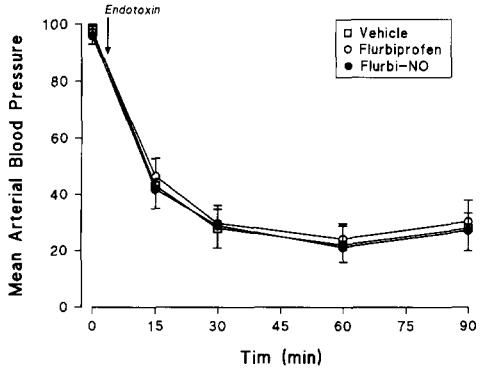

Fig. 1. Effects of intravenous flurbiprofen (10 mg/kg; $n = 9$), an equimolar dose of flurbiprofen 4-nitroxybutylester (Flurbi-NO; 15 mg/kg; $n = 11$) or vehicle ($n = 4$) on systemic arterial blood pressure responses to intravenously administered endotoxin.

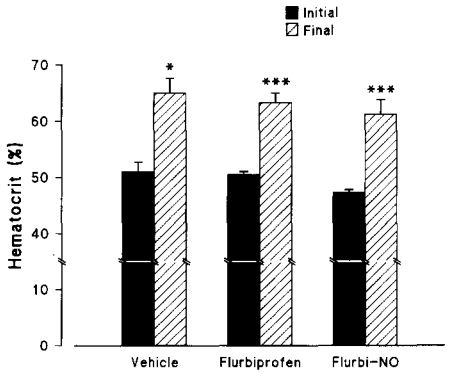

Fig. 2. Effects of intravenous flurbiprofen (10 mg/kg; $n = 9$), an equimolar dose of flurbiprofen 4-nitroxybutylester (Flurbi-NO; 15 mg/kg; $n = 11$) or vehicle ($n = 4$) on hematocrit before and after intravenous administration of endotoxin.

3. Results

3.1. Systemic effects of endotoxin

Administration of endotoxin resulted in a marked decrease in blood pressure which did not recover during the 90 min of the experiment (Fig. 1). The blood pressure responses in the flurbiprofen- and flurbiprofen 4-nitroxybutylester-treated groups were indistinguishable. In the group pretreated with vehicle, 4 of the 8 rats died within 10 min of endotoxin administration, while such mortality was not observed in the rats pretreated with flurbiprofen or flurbiprofen 4-nitroxybutylester. The blood pressure responses in the 3 vehicle-treated rats that survived were comparable to those shown in Fig. 1 for the other two groups.

In the 4 vehicle-treated rats that survived until the end of the experiment, the hematocrit increased from 50.7 ± 1.6 to 65.4 ± 2.2 ($P < 0.05$). Similar increases in the hematocrit were observed in the rats pretreated with flurbiprofen or flurbiprofen 4-nitroxybutylester (Fig. 2).

In rats pretreated with a lower dose of flurbiprofen (5 mg/kg) or flurbiprofen 4-nitroxybutylester (7.5 mg/kg), the systemic effects of endotoxin were also not significantly affected relative to vehicle-treated controls (e.g. hematocrit increased from approximately 53 to 65 in both groups).

3.2. Gastrointestinal damage

In the 4 vehicle-treated rats that survived until the end of the experiment, extensive hemorrhagic damage was observed in both the stomach and small intestine (mean damage scores of 2.72 ± 0.32 and 2.33 ± 0.31, respectively). Extensive gastrointestinal damage was

also observed in the group pretreated with flurbiprofen (Fig. 3). However, in the group pretreated with flurbiprofen 4-nitroxybutylester, the severity of both gastric and small intestinal damage was significantly reduced ($P < 0.01$). These results were confirmed histologically.

In rats pretreated with a lower dose of flurbiprofen (5 mg/kg) or flurbiprofen 4-nitroxybutylester (7.5 mg/kg), gastric damage scores were not significantly different from those in vehicle-treated rats (mean scores for flurbiprofen were 3.0 ± 0 for both the stomach and small intestine; mean scores for flurbiprofen 4-nitroxybutylester were 2.5 ± 0.3 for both the stomach and small intestine).

3.3. Gastric blood flow

Of the 6 vehicle-treated rats, 2 died shortly after endotoxin administration. In the 4 surviving rats, gastric blood flow was unaffected by vehicle administration, but declined profoundly following endotoxin administration. Within 15 min of endotoxin administration the gastric blood flow was only $14 \pm 2\%$ of basal levels, and by the end of the experiment the blood flow was only $12 \pm 3\%$ of basal levels. The mucosa appeared pale throughout the post-endotoxin portion of the experiment. All rats pretreated with flurbiprofen survived the entire experiment. Very similar effects of endotoxin on gastric blood flow were observed in these rats (Fig. 4). Indeed, gastric blood flow in the fluribiprofen-treated rats did not differ significantly from that in the vehicle-treated group. On the contrary, pretreatment with flurbiprofen 4-nitroxybutylester resulted in a significant preservation of gastric blood flow following endotoxin administration (Fig. 4), and all 5 rats survived until the end of the experiment. The gastric mucosa appeared to be much better perfused (pink colour) than those in the other two groups. Basal rates of gastric blood flow did not significantly differ among the three groups, and was not affected by administration of either of the test drugs or the vehicle.

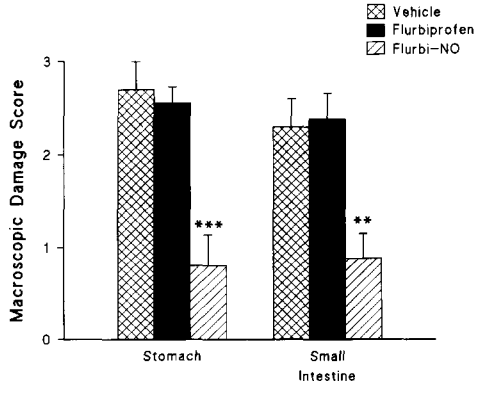

Fig. 3. Macroscopic damage scores for the stomach and small intestine of rats pretreated with either flurbiprofen (10 mg/kg; $n = 9$), flurbiprofen 4-nitroxybutylester (Flurbi-NO; 15 mg/kg; $n = 11$), or vehicle ($n = 4$) then given endotoxin intravenously. Damage was scored on a 0–3 scale by an observer unaware of the treatment. ** $P < 0.01$; *** $P < 0.001$ compared to the other two groups.

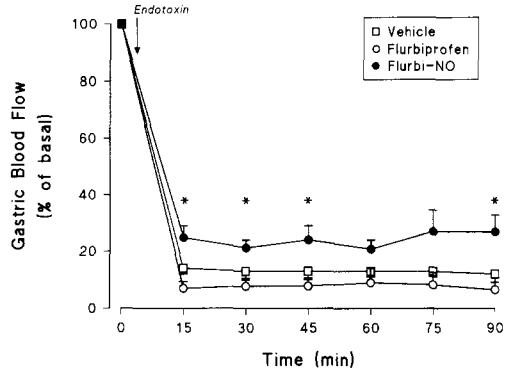

Fig. 4. Effects of intravenous flurbiprofen (10 mg/kg; $n = 5$), an equimolar dose of flurbiprofen 4-nitroxybutylester (Flurbi-NO; 15 mg/kg; $n = 5$) or vehicle ($n = 4$) on gastric blood flow responses to intravenously administered endotoxin. Results are expressed as a percentage of basal flow rates in each experiment. * $P < 0.05$ compared to the other two groups.

4. Discussion

As well characterized in numerous earlier studies (Wallace et al., 1987; Boughton-Smith et al., 1989; Hutcheson et al., 1990), administration of a high dose of endotoxin resulted in profound hypotension, hemoconcentration and, within 90 min, severe gastrointestinal damage. In half of the vehicle-treated rats, death occurred within 10 min of endotoxin administration. Pretreatment with flurbiprofen or the nitroxybutylester derivative, flurbiprofen 4-nitroxybutylester, did not significantly affect endotoxin-induced hypotension or hemoconcentration, but did improve survival. The latter effect has been reported previously for other nonsteroidal anti-inflammatory drugs (Wise et al., 1980; Jacobs et al., 1982; Beck and Abel, 1987). While the gastrointestinal damage observed in rats pretreated with flurbiprofen was comparable in terms of severity to that seen in vehicle-treated rats, pretreatment with flurbiprofen 4-nitroxybutylester significantly reduced the severity of gastrointestinal damage. Flurbiprofen and flurbiprofen 4-nitroxybutylester exert comparable effects on cyclo-oxygenase activity, both in vivo and in vitro (Wallace et al., 1994a; Mitchell et al., 1994), so it seems likely that the nitroxybutyl moiety on flurbipro-

fen 4-nitroxybutlyester accounted for the beneficial effects of this compound in the gastrointestinal tract.

The gastrointestinal damage following administration of endotoxin is likely attributable in part to reduced vascular perfusion as a consequence of systemic hypotension and in part to local generation of mediators, such as platelet-activating factor and thromboxane, which alter endothelial permeability, promote leukocyte adherence and activation and further reduce mucosal perfusion (Wallace et al., 1987; Whittle et al., 1987; Boughton-Smith et al., 1989; Hutcheson et al., 1990). Thus, blockade of the production and/or action of these vasoactive mediators have been shown to reduce the severity of endotoxin-induced damage (Wallace et al., 1987; Boughton-Smith et al., 1989). These interventions also resulted in attenuation of some of the systemic effects of endotoxin administation (e.g. hypotension, hemoconcentration). On the other hand, gastrointestinal damage induced by high doses of endotoxin can be markedly reduced by administration of an NO donor, or exacerbated by blockade of NO synthesis (Boughton-Smith et al., 1990; Hutcheson et al., 1990). These observations led to the hypothesis that NO plays an important role in maintaining intestinal vascular integrity following acute endotoxin challenge (Hutcheson et al., 1990). As flurbiprofen 4-nitroxybutylester has been suggested to release NO in vivo (Wallace et al., 1994a), it is possible that this accounts for its ability to reduce endotoxin-induced gastrointestinal damage. Several observations support the hypothesis that this class of compounds releases NO. For example, these compounds: (1) significantly increase plasma nitrate/nitrite concentrations (Wallace et al., 1994a,b); (2) exhibit enhanced inhibitory activity on platelet aggregation in vivo (Wallace et al., 1994a,b); (3) prevent leukocyte adherence (Wallace et al., 1994a) and the decrease in gastric mucosal blood flow (Wallace et al., 1994b) observed with comparable doses of the parent NSAID; (4) significantly elevate intracellular cGMP levels in cultured endothelial cells (Baydoun et al., 1995).

In order to assess if flurbiprofen 4-nitroxybutylester pretreatment resulted in maintenance of splanchnic blood flow during endotoxic shock, gastric blood flow was measured in rats treated with this drug, flurbiprofen or vehicle. These studies demonstrated that gastric blood flow was profoundly reduced following endotoxin administration in both the vehicle- and flurbiprofen-pretreated groups. However, flurbiprofen pretreatment 4-nitroxybutylester resulted in a significant preservation of blood flow to the tissue. We have previously observed that a similar preservation of gastric blood flow in a hemorrhagic shock model, achieved by pretreatment with a platelet-activating factor antagonist, was sufficient to markedly reduce the extent of gastric damage (Wallace et al., 1990). As in the present study, the antagonist used in that study preserved gastric mucosal blood flow without altering the systemic blood pressure response. While the elevation of gastric blood flow in rats treated with flurbiprofen 4-nitroxybutylester may appear to be small, we previously demonstrated that elevating gastric blood flow in a rat model of hemorrhagic shock by a similar extent was sufficient to prevent gastric mucosal damage (Wallace et al., 1990). It is possible that nitric oxide released from flurbiprofen 4-nitroxybutylester exerted protective effects on the vasculature, as has been suggested previously by Whittle, Boughton-Smith and co-workers (Whittle et al., 1990; Boughton-Smith et al., 1990; Hutcheson et al., 1990). It is also possible that flurbiprofen 4-nitroxybutylester reduced the release of other mediators which might contribute to endotoxin-induced gastrointestinal hypoperfusion and injury. Nitric oxide has been shown to inhibit release of various mediators, including platelet-activating factor and histamine, from mast cells (Salvemini et al., 1991; Hogaboam et al., 1993; Kanwar et al., 1994), so it is conceivable that flurbiprofen 4-nitroxybutylester produced similar effects, although this remains to be directly examined. Another possible mechanism through which flurbiprofen 4-nitroxybutylester may have exerted protective effects in the gut is through inhibition of leukocyte adherence to the vascular endothelium. Leukocytes, in particular neutrophils, have been suggested to play a role in the gastrointestinal damage associated with various forms of shock and ischemia-reperfusion injury (Hernandez et al., 1987). As mentioned above, flurbiprofen 4-nitroxybutylester has previously been found to exert inhibitory effects on leukocyte adherence (Wallace et al., 1994a).

The role of nitric oxide in endotoxin-induced gastrointestinal damage is complex. NO synthase has been shown to be induced in the jejunum and colon following administration of a low dose of endotoxin to rats (Boughton-Smith et al., 1993). In the present study, in which the experiments were carried out over a period of only 90 min, it is unlikely that there would have been significant expression of the inducible isoform of NO synthease. The acute injury to the gastrointestinal tract following administration of higher doses of endotoxin has been shown to be attenuated by NO donors and exacerbated by inhibitors of NO synthase (Boughton-Smith et al., 1990; Hutcheson et al., 1990). In the present study, the nitric oxide-releasing derivative of flurbiprofen was found to reduce the severity of gastrointestinal damage induced by endotoxin, without altering the associated systemic cardiovascular alterations. These observations are therefore consistent with the previous observation of reduced gastrointestinal injury by pretreatment with an NO donor. The mechanism responsible for the reduced level of gastrointestinal injury in rats pretreated with flurbiprofen 4-nitroxybu-

tylester is not completely clear, although the results of this study suggest that preservation of splanchnic blood flow during the shock period is likely to have been an important factor.

Acknowledgements

This work was supported by a grant from the Medical Research Council of Canada. J.L.W. is an MRC Scientist and an Alberta Heritage Foundation for Medical Research Scientist. The authors would like to thank Dr. Piero Del Soldato of Nicox Ltd. (London, UK) for kindly providing the flurbiprofen 4-nitroxybutylester.

References

Baydoun, A.R., G. Cirino and J.L. Wallace, 1995, Elevation of cGMP in human endothelial cells by flurbiprofen-nitroxybutylester, a novel nonsteroidal anti-inflammatory drug with reduced ulcerogenic actions in vivo, Br. J. Pharmacol. 114, 71.

Beck, R.R. and F.L. Abel, 1987, Effect of ibuprofen on the course of canine endotoxin shock, Circ. Shock 23, 59.

Boughton-Smith, N.K., I. Hutcheson and B.J.R. Whittle, 1989, Relationship between PAF-acether and thromboxane A_2 biosynthesis in endotoxin-induced intestinal damage in the rat, Prostaglandins 38, 319.

Boughton-Smith, N.K., I.R. Hutcheson, A.M. Deakin, B.J.R. Whittle and S. Moncada, 1990, Protective effect of *S*-nitroso-*N*-acetylpenicillamine in endotoxin-induced acute intestinal damage

Boughton-Smith, N.K., S.M. Evans, F. Laszlo and B.J.R. Whittle, 1993, The induction of nitric oxide synthase and intestinal vascular permeability by endotoxin in the rat, Br. J. Pharmacol. 110, 1189.in the rat, Eur. J. Pharmacol. 191, 485.

Hernandez, L.A., M.B. Grisham, B. Twohig, K.E. Arfors, J.M. Harlan and D.N. Granger, 1987, Granulocytes: the culprit in ischemic damage to the intestine, Am. J. Physiol. 253, H699.

Hogaboam, C.M., A.D. Befus and J.L. Wallace, 1993, Modulation of rat mast cell reactivity by interleukin 1β: divergent effects on nitric oxide and platelet-activating factor release, J. Immunol. 151, 3767.

Hutcheson, I.R., B.J.R. Whittle and N.K. Boughton-Smith, 1990, Role of nitric oxide in maintaining vascular integrity in endotoxin-induced acute intestinal damage in the rat, Br. J. Pharmacol. 101, 815.

Jacobs, E.R., M.E. Soulsby, R.C. Bone, F.J. Wilson and F.C. Hiller, 1982, Ibuprofen in canine endotoxic shock, J. Clin. Invest. 70, 536.

Kanwar, S., J.L. Wallace, D. Befus and P. Kubes, 1994, Nitric oxide synthesis inhibition increases epithelial permeability via mast cells, Am. J. Physiol. 266, G222.

Kitagawa, H., F. Takeda and H. Kohei, 1990, Effect of endothelium-derived relaxing factor on the gastric lesion induced by HCl in rats, J. Pharmacol. Exp. Ther, 253, 1133.

Lopez-Belmonte, J., B.J.R. Whittle and S. Moncada, 1993, The actions of nitric oxide donors in the prevention of induction of injury to the rat gastric mucosa, Br. J. Pharmacol. 108, 73.

MacNaughton, W.K., G. Cirino and J.L. Wallace, 1989, Endothelium-derived relaxing factor (nitric oxide) has protective effects in the stomach, Life Sci. 45, 1869.

Mitchell, J.A., G. Cirino, P. Akarasereenont, J.L. Wallace, R.J. Flower and J.R. Vane, 1994, Flurbinitrobutylester: a novel anti-inflammatory drug devoid of ulcerogenic activity, inhibits cyclo-oxygenase-1 and cyclo-oxygenase-2, Can. J. Physiol. Pharmacol. 72 (Suppl. 1), 273.

Rees, D.D., S. Cellek, R.M.J. Palmer and S. Moncada, 1990, Dexamethasone prevents the induction by endotoxin of a nitric oxide synthase and the associated effects on vacular tone: an insight into endotoxin shock, Biochem. Biophys. Res. Commun. 173, 541.

Reuter, B.K., G. Cirino and J.L. Wallace, 1994, Markedly reduced intestinal toxicity of a diclofenac derivative, Life Sci. 55, PL1.

Salvemini, D., E. Masini, A. Pistelli, P.F. Mannaioni and J.R. Vane, 1991, Nitric oxide: a regulatory mediator of mast cell reactivity, J. Cardiovasc. Pharmacol. 17 (Suppl. 3), S258.

Thiemermann, C. and J.R. Vane, 1990, Inhibition of nitric oxide synthesis reduces the hypotension induced by bacterial lipopolysaccharides in the rat in vivo, Eur. J. Pharmacol. 182, 591.

Wallace, J.L., G. Steel, B.J.R. Whittle, V. Lagente and B. Vargaftig, 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.

Wallace, J.L., C.M. Hogaboam and G.W. McKnight, 1990, Platelet-activating factor mediates gastric damage induced by hemorrhagic shock, Am. J. Physiol. 259, G140.

Wallace, J.L., B. Reuter, C. Cicala, W. McKnight, M.B. Grisham and G. Cirino, 1994a, Novel NSAID derivatives with markedly reduced ulcerogenic properties, Gastroenterology 107, 173.

Wallace, J.L., B. Reuter, C. Cicala, W. McKnight, M.B. Grisham and G. Cirino, 1994b, A diclofenac derivative without ulcerogenic properties, Eur. J. Pharmacol., 257, 249.

Whittle, B.J.R., N.K. Boughton-Smith, I.R. Hutcheson, J.V. Esplugues and J.L. Wallace, 1987, Increased formation of PAF in endotoxin-induced damage in the rat, Br. J. Pharmacol. 92, 3.

Whittle, B.J.R., J. Lopez-Belmonte and S. Moncada, 1990, Regulation of gastric mucosal integrity by endogenous nitric oxide: interactions with prostanoids and sensory neuropeptides in the rat, Br. J. Pharmacol. 99, 607.

Wise, W.C., J.A. Cook, T. Eller and P.V. Halushka, 1980, Ibuprofen improves survival from endotoxic shock in the rat, J. Pharmacol. Exp. Ther. 215, 160.

Wright, C.E., D.D. Rees and S. Moncada, 1992, Protective and pathological roles of nitric oxide in endotoxin shock, Cardiovasc. Res. 26, 48.