J. Pharm. Pharmacol. 1995, 47:827–832
Received January 13, 1995
Accepted July 19, 1995

Effects of Chronic Nitric Oxide Synthase Inhibition on TNB-induced Colitis in Rats

C. J. PFEIFFER AND B. S. QIU

Department of Biomedical Sciences and Pathobiology, Virginia-Maryland Regional College of Veterinary Medicine, Virginia Tech, Blacksburg, VA 24061–0442, USA

Abstract

Nitric oxide (NO) synthesis is increased in ulcerative colitis, but the role of NO in colitis is poorly understood. The present study employed N^{w}-nitro-L-arginine methyl ester (L-NAME), a nitric oxide synthase inhibitor, in rats to evaluate the effect of NO on 2,4,6-trinitrobenzenesulphonic acid (TNB)-induced colitis.

L-NAME solutions were placed in subcutaneous, osmotic mini-pumps which continuously released L-NAME at 0·042, 0·208, 0·417, or 1·667 mg kg^{-1} h^{-1}. L-NAME dose-dependently enhanced lesions in TNB-induced colitis. The two higher doses of L-NAME significantly increased colonic mucosal damage, although there was slight, nonsignificant reduced lesion formation with the lowest dose of L-NAME, 0·042 mg kg^{-1} h^{-1}. A single dose of L-NAME at 100 mg kg^{-1} subcutaneously injected daily in TNB-treated rats also increased lesions, and these ulcerogenic actions of L-NAME were reversed by L-arginine but not by D-arginine (both at 500 mg kg^{-1}, s.c.). Only the highest dose of L-NAME (mini-pump) significantly depressed myeloperoxidase (MPO) activity. Faecal occult bleeding showed a close relationship with severity of colitis.

These findings suggest that there may exist a balance between NO protective and aggressive effects. In TNB-induced colitis, antagonism of endogenous NO generation was intensified, whereas slight inhibition of NO synthesis reduced lesions. Variations in responses, related to timing or dose changes in L-NAME, may reflect the differences in inducible vs constitutive NO synthase isoforms.

Nitric oxide (NO), identified as endothelium-derived relaxing factor (Ignarro 1990; Moncada et al 1991), plays an important role in the maintenance of mucosal integrity in the gastrointestinal tract (Whittle et al 1990). Acute (Ogle & Qiu 1993) or chronic (Pfeiffer 1994) treatment with an NO inhibitor intensified stress- or ethanol-induced gastric ulceration in rats. However, there remains disagreement on the role of NO in the intestine. Hutcheson et al (1990) showed that synthesis of NO from L-arginine may have a role in maintenance of the microvascular integrity of the intestinal mucosa, and that NO inhibitor pretreatment enhanced both the macroscopic and histological intestinal damage following acute endotoxin challenge. Recently, it was reported that NO synthase activities in mucosa and muscle from the colon of patients with ulcerative colitis were significantly elevated (Boughton-Smith 1993). Middleton et al (1993) also demonstrated that NO synthesis was increased in ulcerative colitis, and they further suggested that mucosal NO biosynthesis may have a pathogenic role in this disease. Indeed, excessive production of NO, characteristic of inflamed states, may have deleterious effects through its ready conversion (in the presence of O_2) to peroxynitrite, which promotes lipid and sulphydryl oxidation (Beckman et al 1990). All of these observations suggest that NO may be involved in colitis, but the mechanism requires further elucidation. The present study attempted to clarify the relationship between NO and colitis induced experimentally by 2,4,6-trinitrobenzenesulphonic acid (TNB), by using N^{w}-nitro-L-arginine methyl ester (L-NAME), an NO-synthase inhibitor. This colitis model is not only histopathologically relevant to human inflammatory bowel disease, particularly Crohn's disease, but it also has a relatively long duration of inflammation which provides a suitable period in which potential treatments can be assessed (Morris et al 1989). The severity of acute inflammation of the colon has been closely correlated with myeloperoxidase (MPO) activity (Krawisz et al 1984) and therefore colonic MPO was also assessed in this study. Since medical management of colitis is clinically problematic and its pathophysiology is still unclear, a better understanding of effects of NO in this disease may facilitate development of therapy.

Correspondence: C. J. Pfeiffer, Department of Biomedical Sciences and Pathobiology, Virginia-Maryland Regional College of Veterinary Medicine, Virginia Tech, Blacksburg, VA 24061-0442, USA.

Materials and Methods

General

Male Sprague-Dawley rats, 240–270 g, had free access to a conventional pellet diet (Ralston Purina) and tap water and were housed in a room with controlled temperature (22 ± 1°C) and humidity (65–70%). Two animals were housed per cage.

Implantation of mini-osmotic pumps

The method was similar to that of Ogle et al (1994). L-NAME (Sigma) solutions of appropriate concentrations were placed in mini-osmotic pumps (Alzet, Model 2002) which continuously released L-NAME at 0, 0·042, 0·208, 0·417, or 1·667 mg kg^{-1} h^{-1}. The mini-osmotic pumps were surgically implanted subcutaneously in the nape of the neck

of rats lightly anaesthetized with nitrous oxide and oxygen plus halothane (Airco Medical Gases, Industrial Gas and Supply, Knoxville, TN). Mini-osmotic pumps containing a solution of only 0·9% w/v NaCl were implanted into rats and served as a control. All animals were rectally instilled with TNB 5 days after pump implantation, and were killed 3 days after TNB administration.

Induction of chronic colonic inflammation

The method of TNB-induction of colitis was similar to that of Morris et al (1989). The rats were randomized into treatment groups, then lightly anaesthetized as described above. A polyethylene tube (Fr. 5, SF-FT 0580, Terumo, Japan) was inserted rectally into the colon such that the tip was 8 cm proximal to the anus, approximately at the splenic flexure. 2,4,6-Trinitrobenzene sulphonic acid (TNB) (Wako Pure Chemical Industries Ltd, Japan) dissolved in 40% ethanol (v/v) was instilled into the lumen of colon through the polyethylene tube. Two hundred and fifty microlitres of 40% ethanol containing 40 mg TNB was instilled into each rat. The instillation was divided equally into three sites; one-third at 8 cm and the other two-thirds divided at 2·2 cm intervals distally.

Assessment of colonic inflammation and damage

Three or seven days after TNB was administered, the rats were killed by hypercapnia. The colon was removed, opened by longitudinal incision, and immediately examined under a stereomicroscope for any visible damage, which was scored on a 0–5 scale by the method of Morris et al (1989), which appraises ulceration and inflammation. However, if the ulcer index reached 4–5 on this scale, the areas of lesions varied from 10–60 mm^2; hence, we utilized an additional area index to measure the colonic damage. The ulcer areas were measured on transparent graph paper with a 1-mm^2 grid. A 4-mm^2 area of oedema, petechiae or inflammation was also considered as equivalent to 1-mm^2 area of ulcer. This index consisted of a summation of such lesion areas. Both of these systems were adopted in the present investigation to better assess the severity of colitis.

Measurement of myeloperoxidase (MPO) activity

The method of measurement of MPO was similar to that used by Krawisz et al (1984). The colonic mucosa was carefully scraped off with a glass slide and weighed on an electronic balance. The specimen was minced in a beaker containing 1 mL hexadecyltrimethylammonium bromide (HTAB; Sigma) buffer on ice (0·5% HTAB in 50 mM phosphate buffer, pH 6·0). HTAB is a detergent that releases MPO from the primary granules of the neutrophil (Schultz & Kaminker 1962; Patriarca et al 1971). The samples were then transferred to a test-tube and homogenized with a Polytron homogenizer (three times for 30 s each, on ice). After homogenization, the homogenizer was rinsed twice with 1 mL HTAB. The pooled homogenate and washes were sonicated for 10 s, freeze-thawed three times, and centrifuged at 40 000 *g* for 15 min. The supernatant was assayed for MPO activity. Myeloperoxidase activity was measured spectrophotometrically; 0·1 mL supernatant was combined with 2·9 mL 50 mM phosphate buffer, pH 6.0, containing 0·167 mg mL^{-1} *o*-dianisidine hydrochloride (Sigma) and 0·0005% hydrogen peroxide (Sigma). The change in absorbance at 560 nm was measured with a spectrophotometer (UA-160 Shimadzu, Japan). One unit of MPO activity was defined as that degrading 1 μmol peroxide min^{-1} at 25°C.

Measurement of body weight and faecal occult bleeding

Rats were weighed at 0900–1000 h before mini-pump implantation and before TNB administration as well as before necropsy. Faecal occult bleeding was examined immediately after animals were killed. Faecal samples were taken from the colon, with careful avoidance of contact with blood. The faecal occult bleeding was tested by QUIK-CULT assay (Laboratory Diagnostics Co., Inc.).

Drug administration

In the experiment without mini-pump implantation, a daily single dose of L-NAME (100 mg kg^{-1}, s.c.) was injected 1 day before and 3 or 7 days after TNB administration to evaluate possible benefit of complete NO blockade in TNB-induced colitis. One group of animals was injected subcutaneously with saline (2 mL kg^{-1}) as a control. L-Arginine (Sigma) or D-arginine (Sigma; both 500 mg kg^{-1}, s.c.) was injected with L-NAME (100 mg kg^{-1}, s.c.) in additional control groups. All of the drugs were freshly prepared in 0·9% NaCl.

Statistical analyses

Results are expressed as mean ± s.e.m. Data were analysed by the two-tailed Student's *t*-test. Differences amongst

Table 1. Effects of L-NAME on TNB-induced colitis in rats.

	0·9% NaCl 2 mL kg^{-1}	L-NAME 0·1 g kg^{-1}	L-NAME 0·1 g kg^{-1}	
			+ L-arginine 0·5 g kg^{-1}	+ D-arginine 0·5 g kg^{-1}
A. Three days after induction				
Ulcer index	$3·8 \pm 0·45^{\#}$	$4·6 \pm 0·26^{\#}$	$3·9 \pm 0·3^{\#}$	$4·4 \pm 0·32^{\#}$
Lesion area (mm^2)	$11·4 \pm 2·57^{\#}$	$19·4 \pm 2·48^{\#*}$	$12·5 \pm 2·26^{\#}$	$17·8 \pm 2·98^{\#}$
B. Seven days after induction				
Ulcer index	$2·8 \pm 0·49^{\#}$	$3·3 \pm 0·53^{\#+}$	$2·9 \pm 0·52^{\#}$	$3·4 \pm 0·50^{\#}$
Lesion area (mm^2)	$4·4 \pm 1·46^{\#+}$	$11·0 \pm 2·44^{\#*+}$	$5·8 \pm 1·75^{\#+}$	$10·9 \pm 2·25^{\#*+}$

All values indicate mean ± s.e.m. of eight rats. $^{*}P < 0·05$ when compared with its saline group in same horizontal group. $^{+}P < 0·05$ when compared with its corresponding group in A. $^{\#}P < 0·01$ when compared with its corresponding control group (before TNB) with values of zero. All treatments were given subcutaneously.

Table 2. Effects of 5-day L-NAME (mini-pump) pretreatment on TNB-induced (3 days) colitis.

	0·9% NaCl 2 μL kg^{-1} h^{-1}	L-NAME (mini-pump, mg kg^{-1} h^{-1})			
		0·042	0·208	0·417	1·667
Ulcer index	3·7 ± 0·29	3·1 ± 0·23^{+}	3·8 ± 0·31	4·5 ± 0·19*	4·6 ± 0·26*
Lesion area (mm^2)	10·8 ± 1·54	7·8 ± 1·10^{+}	11·2 ± 1·56	17·2 ± 2·29*	21·1 ± 3·68**

All values indicate mean ± s.e.m. of eight rats (saline group nine rats). $^{+}P < 0·05$, $^{**}P < 0·02$ when compared with its saline group in similar parameter. $^{+}0·05 < P < 0·1$ when compared with its saline group in similar parameter.

Table 3. Effects of L-NAME on TNB induced colonic myeloperoxidase activity (units g^{-1}), body weight gain (g/rat/day) and faecal occult bleeding in rats.

	0·9% NaCl 2 mL kg^{-1}	L-NAME 0·1 g kg^{-1}	L-NAME 0·1 g kg^{-1}	
			+ L-arginine 0·5 g kg^{-1}	+ D-arginine 0·5 g kg^{-1}
A. Before induction				
Myeloperoxidase activity	0·8 ± 0·36	0·9 ± 0·46	0·8 ± 0·37	0·9 ± 0·41
Body weight gain	4·6 ± 0·36	4·3 ± 0·48	4·3 ± 0·56	4·3 ± 0·32
Occult bleeding	0 ± 0	0 ± 0	0 ± 0	0 ± 0
B. Three days after induction				
Myeloperoxidase activity	6·5 ± 0·96$^{\#}$	6·7 ± 0·86$^{\#}$	6·3 ± 0·97$^{\#}$	6·6 ± 0·91$^{\#}$
Body weight gain	−1·5 ± 0·76$^{\#}$	−2·2 ± 0·48$^{\#}$	−1·4 ± 0·56$^{\#}$	−2·0 ± 0·52$^{\#}$
Occult bleeding	2·0 ± 0·27$^{\#}$	2·4 ± 0·18$^{\#}$	2·1 ± 0·23$^{\#}$	2·3 ± 0·25$^{\#}$
C. Seven days after induction				
Myeloperoxidase activity	4·5 ± 0·51$^{\#}$	5·2 ± 0·62$^{\#}$	4·9 ± 0·52$^{\#}$	5·4 ± 0·57$^{\#}$
Body weight gain	1·7 ± 0·33$^{\#+}$	1·4 ± 0·21$^{\#+}$	1·7 ± 0·22$^{\#+}$	1·5 ± 0·19$^{\#+}$
Occult bleeding	1·0 ± 0·33$^{\#}$	2·0 ± 0·27$^{\#}$	1·1 ± 0·35$^{\#}$	2·1 ± 0·23

All values indicate mean ± s.e.m. of eight rats. $^{+}P < 0·01$ compared with its corresponding group in B. $^{\#}P < 0·01$ when compared with its corresponding group in A. All treatments were given subcutaneously. Occult bleeding: (−) = 0, (±) = 1, (+) = 2, (++) = 3.

groups exposed to the same experimental conditions were also statistically examined by one-way analysis of variance. Both ulcer index and lesion area in the same rat were analysed by linear regression. With all statistical analyses, an associated probability (P value) of < 5% was considered significant.

Results

TNB at 40 mg in 2·5 mL 40% ethanol induced significant inflammatory colonic lesions (Table 1, all $P < 0·01$). The lesions were severe at 3 days after TNB administration and declined after 7 days.

Treatment with L-NAME at 100 mg kg^{-1} subcutaneously enhanced colonic lesions and the lesion area was significantly ($P < 0·05$) larger than in saline-treated control rats pretreated with TNB at 3 or 7 days (Table 1). L-Arginine, but not D-arginine, at 500 mg kg^{-1} significantly antagonized the adverse action of L-NAME in TNB-induced colitis (Table 1).

Chronic L-NAME pretreatment by implanted mini-pump infusion dose-dependently enhanced the colonic lesions induced by TNB (Table 2). The lesion areas in those animals pretreated with L-NAME at 0·417 or 1·667 mg kg^{-1} h^{-1} were significantly larger and, lesions were much deeper than observed in control animals (Table 2, $P < 0·05$ at 0·417 and $P < 0·02$ at 1·667 mg kg^{-1} h^{-1}). There was a slight reduction of lesion size in rats pretreated with L-NAME (mini-pump) at the lowest dose, 0·042 mg kg^{-1} h^{-1}, but this was not statistically significant (Table 2, $0·05 < P < 0·1$).

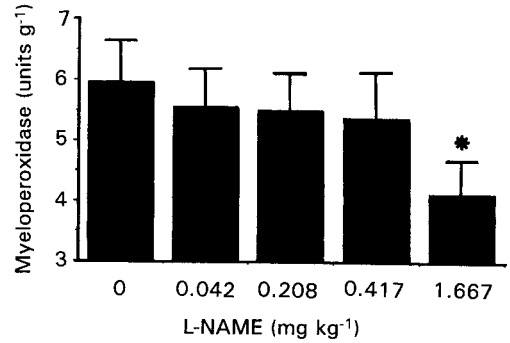

FIG. 1. The effects of 5-day L-NAME pretreatment on TNB-induced colonic myeloperoxidase activity (units g^{-1}) in rats killed 3 days post-TNB. All values indicate mean ± s.e.m. of eight rats (saline group nine rats). $^{*}P < 0·05$ when compared with its saline group for the same parameter.

Table 4. Lack of effect of 5-day L-NAME (mini-pump) pretreatment on TNB-induced faecal occult bleeding in rats killed three days after TNB administration.

0·9% NaCl 12 μL kg^{-1} h^{-1}	L-NAME (mini-pump, mg kg^{-1} h^{-1})			
	0·042	0·208	0·417	1·667
2·0 ± 0·24	1·6 ± 0·32	2·1 ± 0·30	2·3 ± 0·31	2·14 ± 0·26

All values indicate mean ± s.e.m. of eight rats (saline group nine rats). *$P < 0·05$ when compared with its saline group in similar parameter. Occult bleeding: (±) = 1, (+) = 2, (+ +) = 3.

Table 5. The effects of 5-day L-NAME pretreatment on body weight change in rats.

0·9% NaCl 12 μL kg^{-1} h^{-1}	L-NAME (mini-pump, mg kg^{-1} h^{-1})			
	0·042	0·208	0·417	1·667
Five-day mini-pump implantation, without TNB				
5·0 ± 0·58*	4·6 ± 0·53	4·6 ± 0·49	4·5 ± 0·62	4·8 ± 0·41
Five-day mini-pump implantation followed by TNB and killed three days later				
−1·6 ± 0·64	−1·5 ± 0·52	−1·4 ± 0·34	−1·6 ± 0·51	−1·8 ± 0·56

* All figures represent body weight change in g/rat/day. The values are means ± s.e.m. of eight rats (saline group nine rats).

In every group the index and the lesion area of the same rat showed a direct linear relationship ($P < 0·01$).

TNB administration also significantly enhanced colonic mucosal MPO levels (Table 3, $P < 0·01$), which paralleled the severity of colitis. At 3 days after TNB administration, there was an elevated MPO level, and by 7 days post-TNB the MPO level began to decline but still remained elevated (Table 3, $P < 0·01$). Injection of L-NAME, L-arginine, or D-arginine alone did not affect colonic mucosal MPO activity (Table 3). Chronic L-NAME pretreatment (mini-pump) with concentrations of 0·042, 0·208, or 0·417 mg kg^{-1} h^{-1} did not influence MPO, but the highest dose of L-NAME (1·667 mg kg^{-1} h^{-1}) decreased MPO significantly (Fig. 1, $P < 0·05$).

The severity of colitis showed a close direct relationship with faecal occult bleeding, and reduction of colonic lesions was associated with decreased bleeding (Tables 3, 4).

Body weight gain was about 4·56 g/rat/day for control rats. By 3 days after TNB administration, there was an initial but nonsignificant ($P > 0·05$) weight loss; however, the animals began to gain but still remained below the normal body weight by 7 days (Table 5). Five-day mini-pump L-NAME pretreatment did not affect the body weight (Table 5), and L-NAME, L-arginine, or D-arginine treatments also did not influence body weight in the animals (Table 3).

Discussion

The present results confirmed that intracolonic administration of the hapten, TNB, combined with the mucosal barrier breaker, 40% ethanol, resulted in long-lasting ulceration and inflammation of the rat colon. This prolonged inflammation, which resembles human inflammatory bowel disease, provides a suitable model with some advantages over other experimental colitis models (MacPherson & Pfeiffer 1976), in which the role of NO metabolism can be assessed. With this TNB-ethanol colitis preparation, we evaluated colonic ulceration, oedema, and inflammation by a standard ulcer scale (Morris et al 1989) as well as a lesion-area index, and these two indices both displayed a significant, linear regression which taken together accurately described the severity of colitis.

Previous findings in our laboratory had shown that subcutaneous injection of the NO synthase inhibitor, L-NAME, at 100 mg kg^{-1} significantly antagonized NO synthesis (Ogle & Qiu 1993; Pfeiffer 1994; Qiu et al 1994). Since NO had been suspected to be a pathogenic factor in colitis (Rachmilewitz et al 1993), we evaluated the possible benefit of inhibition of NO synthesis for colitis therapy. Insofar as subcutaneous L-NAME at 100 mg kg^{-1} enhanced the lesion area significantly, this suggests that inhibition of NO synthesis may not have therapeutic value in colitis. L-NAME at 100 mg kg^{-1} is a high dose which reportedly produced significant vasoconstriction and hypertension after multiple daily doses (Qiu et al 1994); thus, the possibility of ischaemia followed by reperfusion may exist with the present L-NAME pretreatment method. Accordingly, mucosal free-radical production may have been enhanced, which would have intensified the TNB-induced colitis. We therefore decided to block continuously NO synthesis by use of osmotic mini-pumps, but we still observed significant enhancement in severity of TNB-induced colitis.

NO is released by peritoneal neutrophils (McCall et al 1989), and excessive production of NO in colonic tissue associated with increased nitric oxide synthase activity may play a role in vascular damage in colitis (Boughton-Smith et al 1993, 1994; Middleton 1993). However, the role of NO or

its inhibition in colitis is complicated by factors such as origin and timing of NO synthesis and dose and timing of NO synthase-inhibitor administration. Earlier workers (Stark & Szurszewski 1992; Takeuchi et al 1995) considered NO to be protective for the gastrointestinal mucosa and endogenous NO formed from L-arginine has been hypothesized to help maintain the microvascular integrity of the intestinal mucosa following acute endotoxin challenge (Hutcheson et al 1990). Boughton-Smith et al (1994), however, reported that endotoxin increased nitric oxide synthase more in colonic mucosa than in colonic muscle and that distinct nitric oxide synthase isoforms may exist. Since citrulline concentrations were reportedly increased in rectal biopsy specimens from patients with active ulcerative colitis, and incubation of biopsy samples from patients with ulcerative colitis with N^G-monomethyl-L-arginine (L-NMMA) inhibited citrulline production, this suggested that NO synthesis was increased in colitis (Middleton et al 1993). Boughton-Smith et al (1993) also demonstrated that NO synthase activity was enhanced in ulcerative colitis but not in Crohn's disease. It was observed by reflectance spectrophotometry that mucosal blood flow was significantly increased in the affected intestinal areas of patients with ulcerative colitis or Crohn's disease. A second measurement, when the disease activity was in remittance, demonstrated normal mucosal perfusion (Su et al 1989). It has been suggested that augmented mucosal blood flow and increased NO production should not be considered as harmful to the intestinal mucosa (Guslandi 1993).

The pathophysiology of TNB-induced colitis is still poorly understood, but increased inflammatory cell infiltration has been observed (Morris et al 1989), and the present study also showed that colonic mucosal MPO increased, which parallels the number of neutrophils and macrophages (Krawisz et al 1984). Also, TNB increased MPO levels in the mucosa of guinea-pigs with ileitis (Miller et al 1993). Hence, TNB-induced colitis could produce peroxynitrite and exogenous peroxynitrite at high doses has been shown to induce colitis in rats (Rachmilewitz et al 1993). Our findings also showed that a low dose of L-NAME ($0{\cdot}042\,mg\,kg^{-1}\,h^{-1}$) reduced the lesions in TNB-induced colitis. It is possible that TNB stimulated NO synthesis and release, which could elicit a protective action via improvement of mucosal blood flow. Conversely, excessive production of NO could further damage the neutrophils and macrophages which would generate more NO; this reaction, duplicated again and again, could lead to tissue damage. Slight inhibition of NO, which would halt overproduction of NO, but not completely antagonize NO synthesis, may have some benefit in colitis therapy. Reduction of lesions by the small dose of L-NAME used in the present study, could also suggest that TNB induced excessive NO production.

In the present study, MPO activity, which is directly proportional to neutrophil number, was unchanged after mini-pump administration of L-NAME at a dose of $0{\cdot}417\,mg\,kg^{-1}\,h^{-1}$, and it was significantly lower after L-NAME at the highest dose, $1{\cdot}667\,mg\,kg^{-1}\,h^{-1}$, although both doses enhanced the lesions. Since L-NAME is well known to depress mucosal blood flow, it is possible that the lesion exacerbation was due to mucosal blood flow reduction. The higher dose of L-NAME may have strongly constricted blood vessels, which would lead to ischaemia and depression of MPO activity by decreasing inflammatory cell infiltration. This suggests that mucosal blood flow has an important role in maintaining integrity of the colonic mucosa, and the inflammatory cells are not the sole intensifying factor in colitis.

The present findings suggest that there may exist a balance between modulation of colonic mucosal blood flow by NO, and the production of free radicals. Lower levels of endogenous NO generation may play a protective role, whereas excessive NO production may, conversely, initiate an adverse reaction.

Acknowledgement

The authors greatly appreciate the kind gift of TNB from Professor Shigeaki Sato of Kobe University, Japan.

References

Beckman, J. S., Beckman, T. W., Chen, J., Marshall, P. A., Freeman, B. A. (1990) Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide. Proc. Natl. Acad. Sci. USA 87: 1620–1624

Boughton-Smith, N. K., Evans, S. M., Hawkey, C. J., Cole, A. T., Balsitis, M., Whittle, B. J. R., Moncada, S. (1993) Nitric oxide synthase activity in ulcerative colitis and Crohn's disease. The Lancet 342: 338–340

Boughton-Smith, N. K., Evans, S. M., Whittle, B. J. R. (1994) Characterization of nitric oxide synthase activity in the rat colonic mucosa and muscle after endotoxin and in a model of colitis. Agents Actions 41: C223–C225

Guslandi, M. (1993) Nitric oxide in ulcerative colitis. The Lancet 341: 905–906

Hutcheson, I. R., Whittle, B. J. R., Boughton-Smith, N. K. (1990) Role of nitric oxide in maintaining vascular integrity in endoxin-induced acute intestinal damage in rat. Br. J. Pharmacol. 101: 815–820

Ignarro, L. J. (1990) Biosynthesis and metabolism of endothelium-derived nitric oxide. Pharmacol. Toxicol. 30: 535–560

Krawisz, J. E., Sharon, P., Stenson, W. F. (1984) Quantitative assay for acute intestinal inflammation based on myeloperoxidase activity. Gastroenterology 87: 1344–1350

MacPherson, B., Pfeiffer, C. J. (1976) Experimental colitis. Digestion 1976: 424–452

McCall, T., Boughton-Smith, N., Palmer, R., Whittle, B., Moncada, S. (1989) Synthesis of nitric oxide from l-arginine by neutrophils. Biochem. J. 261: 293–296

Middleton, S. J., Shorthouse, M., Hunter, J. O. (1993) Increased nitric oxide synthesis in ulcerative colitis. The Lancet 341: 465–466

Miller, M. J. S., Sadowska-Krowicka, H., Chotinaruemol, S., Kakkis, J. L., Clark, D. A. (1993) Amelioration of chronic ileitis by nitric oxide synthase inhibition. J. Pharmacol. Exp. Ther. 264: 11–16

Moncada, S., Palmer, R. M. J., Higgs, A. (1991) Nitric oxide: physiology, pathophysiology and pharmacology. Pharmacol. Rev. 43: 109–142

Morris, G. P., Beck, P. L., Herridge, M. S., Depew, W. T., Szewczuk, M. R., Wallace, J. L. (1989) Hapten-induced model of chronic inflammation and ulceration in the rat colon. Gastroenterology 96: 795–803

Ogle, C. W., Qiu, B. S. (1993) Nitric oxide inhibition intensifies cold-restraint induced gastric ulcers in rats. Experientia 49: 304–307

Ogle, C. W., Hui, S.-C. G., Hui, G., Qiu, B. S. (1994) Further observations on the gastric ulcerogenic action of nicotine. Exp. Clin. Gastroenterol. 4: 41–45

Patriarca, P., Cramer, R., Marussi, M., Rossi, F., Romeo, D. (1971) Model of activation of granule-bound NADPH oxidase in leukocytes during phagocytosis. Biochem. Biophys. Acta 237: 335–338

Pfeiffer, C. J. (1994) Effects of chronic nitric oxide synthase inhibitor treatment in cold-restraint and ethanol induced gastric mucosal damage in rats. Digestion 55: 33

Qiu, B. S., Hui, S.-C. G., Wong, D., Ogle, C. W. (1994) Nitric oxide and stress-induced gastric ulcer formation. Exp. Clin. Gastroenterol. 4: 47–52

Rachmilewitz, D., Stamler, J. S., Karmeli, F., Mullins, M. E., Singel, D. J., Loscalzo, J., Xavier, R. J., Podolsky, D. K. (1993) Peroxynitrite-induced rat colitis-A new model of colonic inflammation. Gastroenterology 105: 1681–1688

Schultz, J., Kaminker, K. (1962) Myeloperoxidase of the leukocyte of normal human blood. I. Content and localization. Arch. Biochem. Biophys. 96: 465–467

Stark, M. E., Szurszewski, J. H. (1992) Role of nitric oxide in gastrointestinal and hepatic function and disease. Gastroenterology 103: 1928–1949

Su, K. C., Leung, F. W., Guth, P. H. (1989) Assessment of mucosal hemodynamics in normal human colon and patients with inflammatory bowel disease. Gastrointest. Endosc. 35: 22–27

Takeuchi, K., Ohuchi, T., Okabe, S. (1995) Effects of nitric oxide synthase inhibitor *N*-nitro-L-arginine methyl ester on duodenal alkaline secretory and ulcerogenic responses induced by mepirizole in rats. Dig. Dis. Sci. 40: 670–677

Whittle, B. J. R., Lopez-Belmonte, J., Moncada, S. (1990) Regulation of gastric mucosal integrity by endogenous nitric oxide: interactions with prostanoids and sensory neuropeptides in the rat. Br. J. Pharmacol. 99: 607–611