The actions of nitric oxide donors in the prevention or induction of injury to the rat gastric mucosa

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1 The protective or damaging actions on the gastric mucosa, of locally infused nitrovasodilators that donate nitric oxide (NO), have been investigated in the pentobarbitone-anaesthetized rat.

2 Local intra-arterial infusion of endothelin-1 (ET-1; 5 pmol kg⁻¹ min⁻¹ for 10 min) induced extensive, macroscopically apparent, haemorrhagic injury to the rat gastric mucosa. This damage was dose-dependently reduced by concurrent local intra-arterial infusion of glyceryl trinitrate (GTN; 10–40 µg kg⁻¹ min⁻¹) which liberates NO on metabolic transformation, or the nitrosothiol, S-nitroso-N-acetyl-penicillamine (SNAP, 2.5–10 µg kg⁻¹ min⁻¹) which spontaneously liberates NO.

3 Local infusion of higher doses of SNAP (20 and 40 µg kg⁻¹ min⁻¹, i.a.) did not, however, significantly protect against mucosal injury induced by ET-1.

4 Furthermore, local infusion alone of these higher doses of SNAP, as well as sodium nitroprusside (10–40 µg kg⁻¹ min⁻¹, i.a.) which also spontaneously liberates NO, induced significant mucosal injury, as assessed macroscopically and confirmed by histology.

5 Local infusion of these higher doses of SNAP and nitroprusside reduced systemic arterial blood pressure (BP), but this was not correlated with the extent of mucosal injury.

6 Furthermore, local infusion of GTN (10–40 µg kg⁻¹ min⁻¹, i.a.) alone, which also reduced BP, failed to induce gastric mucosal damage.

7 These findings suggest that exogenous NO can protect the rat gastric mucosa from damage induced by the vasoconstrictor peptide ET-1, which may reflect local microcirculatory interactions. However, the unregulated release of high levels of NO within the microvasculature induces mucosal injury.

Keywords: Nitric oxide; NO-donors; nitrovasodilators; nitrosothiols; glyceryl trinitrate; nitroprusside; S-nitroso-N-acetyl-penicillamine; gastric damage; endothelin-1

Introduction

The regulation of the mucosal microcirculation is intimately involved in the maintenance of gastric integrity and hence the local release of vasoactive mediators from endothelial cells of the microvasculature has a significant modulatory role. Endothelin-1 (ET-1), a 21 residue vasoconstrictor peptide formed by the endothelium (Yanagisawa *et al.*, 1988; Inoue *et al.*, 1989), induces extensive mucosal injury following local intra-arterial infusion to the rat stomach (Whittle & Esplugues, 1988; Whittle & Lopez-Belmonte, 1991) and can augment mucosal damage induced by intragastric ethanol (Wallace *et al.*, 1989). Endothelial cells also release a labile vasodilator substance (Furchgott & Zawadzki, 1980) now characterized as nitric oxide (NO) biosynthesized from L-arginine by a constitutive NO synthase (Palmer *et al.*, 1987; 1988; Ignarro *et al.*, 1987; Khan & Furchgott, 1987). By the use of inhibitors of NO biosynthesis such as N^G-monomethyl-L-arginine and N^G-nitro-L-arginine methyl ester, a role of endogenous NO in the regulation of the rat gastric microcirculation under both resting and stimulated conditions has been established (Pique *et al.*, 1989; 1992; Walder *et al.*, 1990; Tepperman & Whittle, 1992). Furthermore, an interaction of endogenous NO with prostanoids and sensory neuropeptides in the regulation of mucosal integrity has also been demonstrated (Whittle *et al.*, 1990).

The release of NO from nitrovasodilator agents either following metabolic transformation as with glyceryl trinitrate (GTN) and isoamyl nitrite, or spontaneously as with nitroprusside, is responsible for their ability to activate guanylate cyclase and relax vascular smooth muscle (Gruetter *et al.*, 1979; Ignarro *et al.*, 1981; Feelisch & Noack, 1987; Feelisch, 1991). Following intragastric application, these nitro-

vasodilators can protect against acute haemorrhagic mucosal injury induced by topical irritants (MacNaughton *et al.*, 1989; Kitagawa *et al.*, 1990). The nitrosothiol, S-nitroso-N-acetyl-penicillamine (SNAP), which spontaneously liberates NO (Ignarro *et al.*, 1981) has also been demonstrated to protect against acute microvascular injury in the stomach and small intestine induced by platelet-activating factor (PAF) or endotoxin following intravenous administration (Boughton-Smith *et al.*, 1990; 1992b).

To explore further the interaction between endothelium-derived vasodilator and vasoconstrictor mediators in the mucosal microcirculation, the influence of the local intravascular administration of the NO donors, GTN and SNAP on the gastric damage induced by local intra-arterial administration of ET-1 has been evaluated. In addition, the effects of close-arterial infusion of these nitrovasodilators alone and of nitroprusside, on gastric mucosal integrity have also been investigated.

A preliminary account of this study has been presented to the British Pharmacological Society (Lopez-Belmonte *et al.*, 1992).

Methods

Gastric damage induced by local infusion of endothelin-1

Male Wistar rats (230–260 g body weight) were deprived of food but not water for 18–20 h before the experiments. The animals were anaesthetized with sodium pentobarbitone (60 mg kg⁻¹, i.p.) and the stomach exposed by a mid-line incision. The left gastric artery was cannulated with a short 24 gauge teflon cannula (Esplugues & Whittle, 1988). The oesophagus and pylorus were ligated and 2 ml of 0.1 M hy-

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drochloric acid (HCl) were instilled into the gastric lumen via the forestomach. ET-1 ($2.5\text{--}20\text{ pmol kg}^{-1}\text{ min}^{-1}$) or its vehicle, 0.1% bovine serum albumin in saline was infused locally for 10 min ($13\text{ }\mu\text{l min}^{-1}$), and the stomachs removed 20 min later.

Effect of nitrovasodilators on endothelin-1 damage

In these studies, the short gastric intra-arterial 24 gauge teflon cannula was attached to a bifurcated catheter which allowed the concurrent administration of two substances under study. Each substance was infused at a rate of $13\text{ }\mu\text{l min}^{-1}$, reaching the vasculature within 5 s of entering the cannula. The local intra-arterial infusion of the nitrovasodilators, SNAP ($2.5\text{--}40\text{ }\mu\text{g kg}^{-1}\text{ min}^{-1}$) or GTN ($5\text{--}40\text{ }\mu\text{g kg}^{-1}\text{ min}^{-1}$) or isotonic saline, was started 5 min before the infusion of ET-1 and then continued for a further 10 min together with ET-1, being thus administered for a total of 15 min. After a further 20 min following termination of the infusions, the stomachs were removed for assessment of macroscopic damage.

Gastric damage induced by local infusion of nitrovasodilators

In these studies, following instillation of 2 ml 0.1 M HCl into the gastric lumen, GTN ($10\text{--}40\text{ }\mu\text{g kg}^{-1}\text{ min}^{-1}$), nitroprusside ($10\text{--}40\text{ }\mu\text{g kg}^{-1}\text{ min}^{-1}$), SNAP ($5\text{--}40\text{ }\mu\text{g kg}^{-1}\text{ min}^{-1}$) or isotonic saline were infused close-arterially for 15 min ($13\text{ }\mu\text{l min}^{-1}$) and the stomachs removed 20 min later.

Measurement of systemic blood pressure

Mean systemic arterial blood pressure (BP) was measured continuously from a cannula in the right carotid artery, connected to a pressure transducer (Elcomatic) and a chart recorder (Grass model 7D polygraph).

Assessment of mucosal damage

Twenty minutes after terminating local infusion of the drugs, the stomachs were removed from the abdominal cavity, cut open along the greater curvature and gently rinsed with isotonic saline. They were then pinned, mucosal side up, to a wax block and immersed in neutral buffered formalin. Each stomach was photographed on colour transparency film (Kodak EPY 50). Each photograph was then coded to allow for subsequent unbiased assessment. The transparencies were projected and both the overall areas and the damage areas (observed as vasocongestion, haemorrhagic necrosis and epithelial desquamation with mucus precipitation) assessed in a randomized manner by computerised planimetry (Apple IIe) and expressed as % of the total mucosal area.

For histological confirmation of the nature of the mucosal injury, samples of the corpus mucosa were excised from standardized regions of the dorsal and ventral sides of the mucosa. These sections were processed by routine techniques before being embedded in paraffin. Sections ($4\text{ }\mu\text{m}$) were stained with haematoxylin and eosin and examined under a light microscope.

Statistical analysis

All data are expressed as mean $\pm$ s.e.mean. Comparisons between groups were made by Student's *t* test for paired or unpaired data as appropriate, where *P* values of less than 0.05 were taken as significant.

Materials

Endothelin-1 (human-porcine; Sigma Chemical Co., Poole, Dorset) was dissolved in sterile distilled water and kept frozen (-20°C) in aliquots. Samples were freshly diluted in

0.1% bovine serum albumin in saline.

Glyceryl trinitrate (Du Pont Ltd., U.K., Hertfordshire) was obtained as 0.5 mg ml^{-1} vials, and prepared freshly for infusion by dilution with isotonic saline. Sodium nitroprusside (Sigma Chemical Co., Poole, Dorset) and S-nitroso-N-acetyl-penicillamine (Wellcome Research Laboratories) were dissolved freshly in isotonic saline and kept on ice protected from the light.

Results

Effect of local endothelin-1

In dose-ranging studies, local intra-arterial infusion of ET-1 ($2.5\text{--}20\text{ pmol kg}^{-1}\text{ min}^{-1}$ in 0.1% bovine serum albumin) for 10 min induced dose-dependent mucosal damage, assessed macroscopically 20 min later involving $28 \pm 4\%$ and $86 \pm 3\%$ of the total mucosal area with the lower and high doses respectively ($n=4$ for each $P<0.01$), while ET-1 ($5\text{ pmol kg}^{-1}\text{ min}^{-1}$ i.a.) induced a submaximal degree of injury involving $35 \pm 5\%$ of the total mucosal area ($n=7$; $P<0.01$). This damage was characterized macroscopically as regions of vasocongestion, epithelial sloughing, haemorrhage and necrosis, which was confirmed on histological evaluation. At the dose chosen for subsequent studies, local infusion of ET-1 ($5\text{ pmol kg}^{-1}\text{ min}^{-1}$) had no significant effect on BP ($\Delta 3 \pm 2\text{ mmHg}$, $n=4$). Intra-arterial infusion of the vehicle, 0.1% bovine serum albumin in saline, did not significantly damage the mucosa (Figure 2) or affect BP ($\Delta 2 \pm 1\text{ mmHg}$, $n=4$).

Effect of glyceryl trinitrate co-administration

Concurrent close-arterial administration of GTN ($5\text{--}40\text{ }\mu\text{g kg}^{-1}\text{ min}^{-1}$ for 15 min) caused a significant and dose-dependent reduction in the extent of damage induced by ET-1 ($5\text{ pmol kg}^{-1}\text{ min}^{-1}$) as shown in Figure 1. Thus, GTN (10 , 20 and $40\text{ }\mu\text{g kg}^{-1}\text{ min}^{-1}$) inhibited mucosal damage by $53 \pm 10\%$ ($n=5$, $P<0.05$), $69 \pm 7\%$ ($n=7$, $P<0.001$) and $72 \pm 6\%$ ($n=8$, $P<0.001$) respectively.

Figure 1 Inhibition by the local intra-arterial infusion (15 min) of glyceryl trinitrate (GTN; $5\text{--}40\text{ }\mu\text{g kg}^{-1}\text{ min}^{-1}$), of rat gastric mucosal damage induced by concurrent local intra-arterial infusion (10 min) of endothelin-1 (ET-1, $5\text{ pmol kg}^{-1}\text{ min}^{-1}$). Results, shown as the area of damage, % of total mucosal area assessed macroscopically 20 min after termination of the ET-1 infusion, are the mean of 5–8 experiments in each group; vertical bars show s.e.mean. Significant difference from the control group (ET-1 alone, first column) is shown as: * $P<0.05$; *** $P<0.001$.

Figure 2 Effects of local intra-arterial infusion (15 min) of S-nitroso-N-acetylpenicillamine (SNAP; 2.5–40 $\mu\text{g kg}^{-1} \text{min}^{-1}$), on rat gastric mucosal damage induced by concurrent local intra-arterial infusion (10 min) of endothelin-1 (ET-1, 5 $\text{pmol kg}^{-1} \text{min}^{-1}$). Results, shown as the area of damage, % of total mucosal area assessed macroscopically 20 min after termination of the ET-1 infusion, are the mean of 5–8 experiments in each group; vertical bars show s.e.mean.

Significant difference from the control group (ET-1 alone) is shown as: ** $P < 0.01$; *** $P < 0.001$.

Effect of S-nitroso-N-penicillamine co-administration

Co-administration of SNAP (2.5–10 $\mu\text{g kg}^{-1} \text{min}^{-1}$, i.a. for 15 min) caused a significant and dose-dependent inhibition of ET-1-induced damage, reaching a maximum degree of inhibition at a dose of 10 $\mu\text{g kg}^{-1} \text{min}^{-1}$ (Figure 2). Thus SNAP (2.5, 5 and 10 $\mu\text{g kg}^{-1} \text{min}^{-1}$) inhibited mucosal damage by $37 \pm 10\%$ ($n = 8$), $54 \pm 11\%$ ($n = 8$, $P < 0.01$) and $79 \pm 7\%$ ($n = 8$, $P < 0.001$) respectively.

Higher close-arterial doses of SNAP (20 and 40 $\mu\text{g kg}^{-1} \text{min}^{-1}$, i.a.), failed to protect significantly against the mucosal injury induced by ET-1 and with the highest dose of SNAP (40 $\mu\text{g kg}^{-1} \text{min}^{-1}$, i.a.), the damage was comparable to that induced by ET-1 alone (Figure 2).

Effect of close-arterial infusion of S-nitroso-N-penicillamine, nitroprusside and glyceryl trinitrate alone

Local intra-arterial infusion of SNAP (5–40 $\mu\text{g kg}^{-1} \text{min}^{-1}$) alone for a period of 15 min resulted in a dose-dependent and significant induction of mucosal injury (Figure 3). This damage was macroscopically characterized by extensive areas of vasocongestion and sloughing of epithelium and focal sites of haemorrhage, findings confirmed on histological evaluation (Figure 4). By contrast, local infusion of a solution of SNAP at a dose of 40 $\mu\text{g kg}^{-1} \text{min}^{-1}$, that had been incubated at 37°C in ambient light for 48 h, failed to induce any significant mucosal injury ($3 \pm 2\%$ of total mucosal area, $n = 7$), being comparable to that induced by intra-arterial saline ($3 \pm 1\%$ of total mucosal area, $n = 4$).

Close-arterial infusion of nitroprusside (10–40 $\mu\text{g kg}^{-1} \text{min}^{-1}$, 15 min) alone, also induced significant gastric mucosal damage (Figure 3). By contrast, close-arterial infusion of potassium ferricyanide (40 $\mu\text{g kg}^{-1} \text{min}^{-1}$ for 15 min) did not induce any significant macroscopic mucosal damage ($4 \pm 2\%$ of total mucosal area, $n = 5$). Close-arterial infusion of GTN failed to induce any significant gastric mucosal damage at the doses used (10–40 $\mu\text{g kg}^{-1} \text{min}^{-1}$ for 15 min) as shown in Figure 3. Administration of higher doses of GTN was hindered by solubility of the commercially available stock solutions.

Effect on mean systemic arterial blood pressure

Local intra-arterial administration of SNAP alone (5–40 $\mu\text{g kg}^{-1} \text{min}^{-1}$) for 15 min induced a dose-dependent significant fall in BP, from the resting BP of $110 \pm 5 \text{ mmHg}$ ($n = 20$) as shown in Figure 3. By contrast, infusion of a solution of SNAP (at 40 $\mu\text{g kg}^{-1} \text{min}^{-1}$) that had been incubated for 48 h at 37°C in ambient light had no significant effect on BP ($n = 5$).

Local intra-arterial infusion of nitroprusside (10–40 $\mu\text{g kg}^{-1} \text{min}^{-1}$) likewise induced dose-dependent and significant reductions in BP (Figure 3). Furthermore, GTN (20 and

Figure 3 Effect of local intra-arterial infusion (15 min) of (a) S-nitroso-N-acetylpenicillamine (SNAP; 5–40 $\mu\text{g kg}^{-1} \text{min}^{-1}$) (b) sodium nitroprusside (10–40 $\mu\text{g kg}^{-1} \text{min}^{-1}$) or (c) glyceryl trinitrate (GTN; 10–40 $\mu\text{g kg}^{-1} \text{min}^{-1}$) on rat systemic arterial blood pressure (BP) and the induction of gastric mucosal injury. Macroscopic damage, assessed 20 min after infusion, is shown as % of the total mucosal area, while the change in BP from resting values is given as ΔBP (mmHg). Results are shown as mean of 5–8 experiments; vertical bars represent s.e.mean. Statistical significance from the change in resting BP ($\Delta 2 \pm 1 \text{ mmHg}$) or from damage area ($3 \pm 1\%$ total area) in control studies following saline infusion is given as: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Figure 4 Histological appearance of the rat gastric mucosa, 20 min following local intra-arterial infusion of S-nitroso-N-acetylpenicillamine (SNAP; $20 \mu\text{g kg}^{-1} \text{min}^{-1}$). Sections ($4 \mu\text{m}$; wax embedded) were stained with haematoxylin and eosin; marker bar represents $100 \mu\text{m}$. Arrows represent areas of vasocongestion and epithelial disruption.

$40 \mu\text{g kg}^{-1} \text{min}^{-1}$, i.a.) also significantly reduced BP (Figure 3). The fall in BP induced by the highest local dose of GTN ($40 \mu\text{g kg}^{-1} \text{min}^{-1}$, i.a.) was comparable to that induced by nitroprusside ($20 \mu\text{g kg}^{-1} \text{min}^{-1}$, i.a.) and by SNAP ($20 \mu\text{g kg}^{-1} \text{min}^{-1}$) being 30 ± 2 , 29 ± 5 and 37 ± 3 mmHg respectively ($n = 6-8$), yet as can be seen in Figure 3, GTN unlike SNAP and nitroprusside in these doses, did not induce mucosal damage. Furthermore, the fall in BP induced by the highest dose of nitroprusside ($40 \mu\text{g kg}^{-1} \text{min}^{-1}$, i.a.) was less than that induced by SNAP ($40 \mu\text{g kg}^{-1} \text{min}^{-1}$, i.a.) being 42 ± 6 mmHg compared with 53 ± 2 mmHg respectively, yet nitroprusside caused two fold greater ($P < 0.05$) damage than SNAP at these doses (Figure 3).

Discussion

In the present study, close-arterial infusion of the NO donor, GTN, dose-dependently prevented the mucosal injury induced by local intra-arterial infusion of ET-1. Likewise at low doses, intra-arterial infusion of the nitrosothiol, SNAP, caused a dose-dependent inhibition of ET-1-induced mucosal damage. These findings thus extend the observations that intragastric instillation of the NO donor, nitroprusside can reduce the haemorrhagic injury induced by a combination of topical ethanol and intravenous infusion of ET-1 (Wallace *et al.*, 1989). The mucosal injury induced by topical ethanol alone has also been shown to be reduced by intragastric GTN or nitroprusside, while that provoked by strong luminal acid was inhibited by intragastric acidified sodium nitrite which liberates NO, and the metabolically activated nitrovasodilator, isoamyl nitrite (MacNaughton *et al.*, 1989; Kitagawa *et al.*, 1990). Intravenous infusion of SNAP likewise protects the stomach from microvascular damage

induced by intravascular PAF (Boughton-Smith *et al.*, 1992b).

However, with local intra-arterial doses higher than $10 \mu\text{g kg}^{-1} \text{min}^{-1}$, SNAP did not significantly inhibit the mucosal injury induced by ET-1. This lack of protection appears to reflect the injurious action of SNAP at these higher doses, since its local intra-arterial infusion alone at these doses induced mucosal damage. This damage was macroscopically apparent as sloughing of the epithelium with areas of vasocongestion and focal sites of haemorrhage, findings confirmed on histological evaluation. Such characteristics of mucosal injury have previously been observed with intravascular agents that act by disrupting the microcirculation (Esplugues & Whittle, 1988; 1989), as well as by topical irritants such as ethanol thought to initiate mucosal injury by disruption of endothelial cell continuity (Guth *et al.*, 1984; Szabo *et al.*, 1985). This effect of SNAP appeared to be related to the release of NO and not to the penicillamine moiety of the compound, since incubation of this thermodynamically unstable and photosensitive NO donor for 48 h in ambient light, to deplete its NO content, abolished both its systemic vasoactive effects and its ability to induce mucosal injury.

As found with SNAP, local infusion of nitroprusside which also spontaneously liberates NO, likewise induced extensive mucosal injury following local intra-arterial infusion. The contribution of the concomitant local release of cyanide from nitroprusside to this cytotoxicity cannot be excluded, but it is considered that any such cyanide production *in vivo* will not normally exceed the capacity for its rapid metabolism to the much less toxic thiocyanate (Michenfelder & Tinker, 1977; Kruszyna *et al.*, 1985). Furthermore, close-arterial infusion of potassium ferricyanide, in which the nitroso moiety of nitroprusside is substituted by another cyano group, failed to induce any macroscopically detectable damage, strongly suggesting that NO release accounts for the observed toxicity of nitroprusside.

By contrast to both SNAP and nitroprusside, no mucosal injury was observed following local intra-arterial infusion of GTN. This may reflect the requirement of GTN to be enzymatically activated by a mechanism involving sulphydryls to release NO (Ignarro *et al.*, 1981; Feelisch & Noack, 1987), a process that would be regulated and limited by the metabolic capacity of the microvascular tissue. Gastric intra-arterial infusion of these three NO donors reduced systemic BP, indicating their escape into the systemic circulation following their passage through the mucosal microcirculation. However, such a hypotensive effect on systemic BP, which could possibly reduce microvascular perfusion in the gastric mucosa, does not appear to be the prime mechanism by which tissue injury was initiated. Thus, GTN did not produce mucosal damage in doses producing a comparable fall in systemic BP as injurious doses of nitroprusside, while nitroprusside was substantially more active than SNAP in inducing damage at comparable hypotensive doses.

The mucosal injury associated with the spontaneous release of NO from these agents may indicate cytotoxic actions of high levels of NO on the microvascular endothelium. Indeed, the excessive production of NO by an inducible NO synthase in endothelial cells is considered to underlie the reduction in viability of these cells in culture following exposure over a 48 h period to endotoxin and the cytokine, interferon- γ (Palmer *et al.*, 1992). Furthermore, the microvascular permeability changes, an index of endothelial injury, seen in the rat small and large intestine 4–6 h after endotoxin administration are correlated with the induction of a calcium-independent NO synthase over this period (Boughton-Smith *et al.*, 1992a). The substantial synthesis of NO by the immunologically-induced NO synthase in macrophages also accounts for the cytotoxic effects against tumour cells (Hibbs *et al.*, 1987; 1988, Marletta *et al.*, 1988; Stuehr *et al.*, 1989). In addition, activated macrophages exert cytostatic or cytotoxic actions on fungal and bacterial and protozoal

pathogens by events involving NO (Granger *et al.*, 1990; Adams *et al.*, 1990; Liew *et al.*, 1990), while induction of NO synthesis is also implicated in damage to adenocarcinoma cells (O'Connor & Moncada, 1991). Whether NO is cytotoxic to a cell type may relate to the importance of iron-sulphur-centred enzymes in that target cell (Draper & Hibbs, 1988), and the current findings may thus reflect a significant presence of such cellular enzymes in the gastric mucosa.

The induction of NO synthase has also been implicated in the cardiovascular crisis and collapse seen over several hours in both animals and man in endotoxaemia (Nava *et al.*, 1991; Petros *et al.*, 1991). However, it is also clear that the physiological formation of NO by the constitutive enzyme has importance in protecting against the initial detrimental cardiovascular changes and the intestinal vascular injury seen in the acute phases of endotoxin shock (Wright *et al.*, 1992; Hutcheson *et al.*, 1990). The present results similarly suggest a dual role for NO in the regulation of gastric mucosal

integrity. Thus, the physiological formation of NO plays a role in modulating both mucosal blood flow and tissue integrity (Pique *et al.*, 1989; 1992; Whittle *et al.*, 1990). The protection by exogenously generated NO observed in the current study may reflect microcirculatory interactions with the vasoconstrictor, ET-1, both on changes of vascular tone and on endothelial cell continuity. However, the inappropriate release of NO, mimicked in the present study by the use of high doses of spontaneously decomposing NO donors, can lead to extensive tissue injury. This could result from direct cytotoxicity of the NO radical or a subsequent metabolite such as the peroxynitrite radical which generates the highly reactive hydroxyl radical (Beckman *et al.*, 1990). Thus, while a reduction in the physiological formation of NO may compromise gastric integrity leading to ulceration (Whittle *et al.*, 1990), the unregulated excessive formation of NO may also be involved in the pathology of gastro-intestinal mucosal injury.

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