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# Role of oxygen-derived metabolites in the rat gastric mucosal injury induced by nitric oxide donors

Dominique Lamarque, Brendan J.R. Whittle *

*Department of Pharmacology, Wellcome Foundation Ltd., Langley Court, Beckenham, Kent, BR3 385, UK*

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**Abstract**

Local intra-arterial infusion of high doses of the nitric oxide (NO) donor, nitroprusside (10–40 $\mu$g kg$^{-1}$ min$^{-1}$ for 15 min) induced dose-dependent haemorrhagic injury to the rat gastric mucosa and reduced systemic arterial blood pressure, whereas intragastric nitroprusside (10–50 mg ml$^{-1}$), which caused similar falls in blood pressure, failed to induce such injury. The mucosal damage induced by nitroprusside was reduced by local concurrent infusion of superoxide dismutase (500–4000 i.u. kg$^{-1}$). Local superoxide dismutase also abolished the mucosal injury induced by local infusion of the NO donor, *S*-nitroso-*N*-acetyl-penicillamine (40 $\mu$g kg$^{-1}$ min$^{-1}$), but not that induced by local infusion of endothelin-1 (5 pmol kg$^{-1}$ min$^{-1}$) indicating specific actions. Intravenous infusion of the iron chelator and peroxyl scavenger, desferrioxamine (0.25–1 mg kg$^{-1}$ min$^{-1}$) or the hydroxyl radical scavenger, dimethylthiourea (20 mg kg$^{-1}$ min$^{-1}$) also reduced the mucosal damage induced by the local administration of the NO donors, but not that induced by endothelin-1. These findings implicate the involvement of superoxide and possibly other oxygen-derived free radicals in the injurious actions of high levels of nitric oxide generated from NO donors, and may reflect a role of the cytotoxic peroxynitrite moiety.

*Keywords:* Nitric oxide (NO) donor; Superoxide; Peroxynitrite; Free radical scavenger; Gastric damage

## 1. Introduction

The labile endogenous vasodilator, nitric oxide (NO), formed from L-arginine by a constitutive enzyme (Moncada et al., 1991) plays a modulator role in the regulation of gastric mucosal blood flow and vascular integrity (Whittle et al., 1990; Whittle, 1993). However, an excessive production of NO may lead to injury under certain pathological conditions. Thus, the microvascular permeability changes in the small and large intestine observed several hours after endotoxin administration are associated with the unregulated production of NO by the inducible NO synthase (Salter et al., 1991; Boughton-Smith et al., 1993). Furthermore, in the stomach, whereas local intra-arterial infusion of low doses of nitrovasodilators that spontaneously release NO can protect against mucosal injury, high doses of such agents can lead to mucosal injury (Lopez-Belmonte et al., 1993). This cytotoxicity of NO may result from the interaction of NO and superoxide radical, producing oxidant species such as peroxynitrite and the subsequent hydroxyl radical (Beckman et al., 1990; Radi et al., 1991).

In the present study, the mechanisms of NO-induced injury in the rat gastric mucosa have been investigated. Thus, the mucosal damaging and hypotensive effects the local intra-arterial infusion of high doses of the NO donor, sodium nitroprusside, have been compared with its effects following intragastric administration. The involvement of different oxygen-derived species in the formation of gastric lesions produced by local intra-arterial infusion of nitroprusside or the nitrosothiol NO donor, *S*-nitroso-*N*-acetyl-penicillamine were also investigated. Thus, the actions of superoxide dismutase, a scavenger of superoxide radicals, catalase which reduces hydrogen peroxide to water and molecular oxygen, desferrioxamine which is both an iron chelator and peroxyl radical scavenger, as well as those of dimethylthiourea which is a hydroxyl radical scavenger, on mucosal injury induced by local administration of these NO donors were determined (Parks et al.,

* Corresponding author. Tel. 081-658-2211, fax 081-658-6176.

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1982; Itoh and Guth, 1985; Morgan-Smith et al., 1987; Zimmerman et al., 1990; Andrews et al., 1992). To evaluate the specificity of any protective actions of these agents, their effects on the mucosal damage induced by the endogenous pro-ulcerogenic peptide, endothelin-1, which induces a comparable degree of haemorrhagic injury following local intra-arterial infusion (Whittle and Esplugues, 1988) as found with NO donors, was also determined.

## 2. Materials and methods

### 2.1. Animal preparation

Male Wistar rats, weighing 200–250 g, were fasted overnight but allowed free access to water. The animals were anaesthetised with sodium pentobarbitone (60 mg $kg^{-1}$ i.p.), the abdomen opened by a mid-line incision and the oesophagus and pylorus were ligated. The left gastric artery was exposed and cannulated with a tubing of 0.6 mm diameter (PE-10, Clay Adams, Parsippany, NJ, USA) as previously described (Esplugues and Whittle, 1989). 2 ml of 0.1 M hydrochloric acid (HCl) or isotonic saline were instilled into the gastric lumen via a 25 g needle inserted through the forestomach. Body temperature was monitored by a rectal thermometer and maintained at 36.5°C by a heating blanket.

Mean systemic arterial blood pressure (blood pressure) was measured continuously via a catheter (PE-50, Clay Adams) ligated in the right carotid artery and connected to a pressure transducer and chart recorder system (model 7D polygraph, Grass instrument Co., Quincy, MA).

### 2.2. Gastric damage induced by NO donors

In studies on the intragastric actions of the NO donor, 1 ml of nitroprusside (1.25–50 mg $ml^{-1}$) and 1 ml of isotonic saline was instilled into the gastric lumen and the stomachs removed 35 min later. In control experiments, 2 ml of isotonic saline was instilled into the lumen for a 35 min period. The use of intragastric acid was avoided in this group of studies to prevent any potential deleterious effect on the stability of the compound under investigation. In comparative studies, nitroprusside (10–40 $\mu g\ kg^{-1}\ min^{-1}$) or isotonic saline was infused through the left gastric artery at a rate of 13 $\mu l\ min^{-1}$. After 20 min following the termination of the infusion, the stomachs were removed for assessment of macroscopic damage.

### 2.3. Effects of superoxide dismutase

In all further studies, 2 ml of 0.1 M HCl was instilled into the gastric lumen. Superoxide dismutase (500–4000 i.u. $kg^{-1}$ over 15 min) or isotonic saline was infused concurrently with nitroprusside (40 $\mu g\ kg^{-1}\ min^{-1}$) through a bifurcated catheter connected to the intra-arterial tubing. In control studies, the effect of superoxide dismutase that had been denatured by boiling for 10 min in isotonic saline was also evaluated. The doses of superoxide dismutase for local infusion were taken from previous dose-response studies by Esplugues and Whittle (1989) on the prevention of gastric mucosal injury induced by local administration of a superoxide-generating system.

### 2.4. Effects of catalase alone or with superoxide dismutase

Catalase (50000 i.u. $kg^{-1}$) was administered as a bolus intravenous injection followed by local intra-arteral infusion (2000 i.u. $kg^{-1}\ min^{-1}$) for 20 min, commencing 5 min prior to the close-arterial infusion or nitroprusside (40 $\mu g\ kg^{-1}\ min^{-1}$). For their concomitant intra-arterial infusion, superoxide dismutase and catalase were mixed in the same syringe and then infused locally as described, following the intravenous initial bolus administration of catalase. The dose of catalase was derived from earlier studies where inhibition of the rat gastric mucosal injury induced by local infusion of hydrogen peroxide was demonstrated (Esplugues and Whittle, 1989).

### 2.5. Effects of peroxynitrite and hydroxyl scavengers

Desferrioxamine (0.25–1 $mg^{-1}\ kg^{-1}\ min^{-1}$) or dimethylthiourea (20 mg $kg^{-1}\ min^{-1}$) were infused for 30 min through a tail vein cannulated with a 25 g needle. Infusion of these agents was commenced 15 min prior to the close intra-arterial infusion of nitroprusside (40 $\mu g\ kg^{-1}\ min^{-1}$) or *S*-nitroso-*N*-acetylpencillamine (40 $\mu g\ kg^{-1}\ min^{-1}$) for 15 min. Control rats received intravenous isotonic saline, followed by the local intra-arterial infusion of nitroprusside or *S*-nitroso-*N*-acetyl-penicillamine. The doses of these agents were derived from previous studies (Morgan-Smith et al., 1987; Zimmerman et al., 1990; Andrews et al., 1992; Parks et al., 1982; Itoh and Guth, 1985).

### 2.6. Effects on the mucosal injury induced by endothelin-1

Endothelin-1 (5 pmol $kg^{-1}\ min^{-1}$ in 0.1% bovine serum albumin in saline) was administered by close-arterial infusion for 15 min at a rate of 13 $\mu l\ min^{-1}$, in a dose shown previously to provoke macroscopically detectable mucosal haemorrhagic damage (Whittle and Esplugues, 1988). The effects of superoxide dismutase (4000 i.u. $kg^{-1}$) administered by concomitant close intra-arterial infusion, over 15 min, as well as those of desferrioxamine (1 mg $kg^{-1}\ min^{-1}$) or dimethylth-

iourea (20 mg $kg^{-1}$ $min^{-1}$), administered by intravenous infusion, on the mucosal injury induced by endothelin-1 infusion were evaluated.

### 2.7. Assessment of mucosal damage

20 min after terminating the local intra-arterial infusion, the stomachs were removed and opened along the great curvature. They were then pinned, mucosal side up, to a wax block, immersed in neutral buffered formalin and photographed on colour transparency film. The extent of macroscopically visible damage was determined from these coded projected transparencies in a randomised manner via computerised planimetry, and expressed as the percentage of the total mucosal area.

For histological evaluation of the damage induced by nitroprusside, and the protective actions of superoxide dismutase, two samples of the corpus mucosa were excised from comparable regions in each coded stomach regardless of the macroscopic appearance of the mucosa and were processed by routine techniques before embedding in paraffin. The sections (4 $\mu$m) were stained with haematoxylin and eosin and examined under a light microscope and the degree of damage assessed as previously described (Esplugues and Whittle, 1989). The 1 cm length of each histological section was divided into four fields. Each field was histologically scored on a 0–4 scale according to the following criteria: 0 = normal; 1 = epithelial cell damage; 2 = glandular disruption, vasocongestion or oedema in the upper mucosa; 3 = mucosal disruption, vasocongestion or oedema in the mid-lower mucosa; 4 = extensive mucosal disruption involving the full thickness of the mucosa. Each subsection was evaluated on a cumulative basis, the maximum score for each subsection being thus 10. The overall mean value of the scores for each of the four fields was taken as the histological index for that section. All determinations were performed in a randomised manner with the histological sections coded to eliminate observer bias.

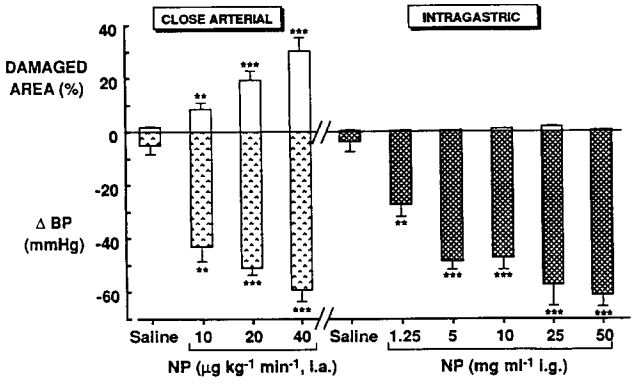


Fig. 1. Effect of close-arterial infusion (10–40 $\mu$g $kg^{-1}$ $min^{-1}$ for 15 min) or intragastric administration (1.25–50 mg $ml^{-1}$ for 35 min) of nitroprusside (NP) on rat systemic arterial blood pressure (BP) and gastric mucosal injury. Macroscopic damage, assessed 35 min after commencing the treatment, is shown as % of the total mucosal area, while the changes in BP from resting values are given as $\Delta$BP (mm Hg). Results are shown as means ± S.E.M. of at least five experiments in each group. Statistical significance from the change in resting BP ($\Delta$31 ± 1 mm Hg) or from the damage area (4 ± 1% total area) in control studies following saline infusion is given as $^{**}P < 0.01$, $^{***}P < 0.001$.

### 2.8. Statistical analysis

All data are expressed as the means ± S.E.M. of ($n$) experiments. Comparisons between groups of parametric data were made by Student's $t$-test for non-paired data or for non-parametric data by the Mann-Witney U-test. $P$ values of less than 0.05 were taken as significant.

## 3. Results

### 3.1. Gastric mucosal damage induced by local infusion or intragastric administration of NO donors

Following intragastric instillation of isotonic saline (2 ml), local intra-arterial infusion of nitroprusside (10–40 $\mu$g $kg^{-1}$ $min^{-1}$) for 15 min induced dose-dependent gastric mucosal damage, when assessed macroscopically 20 min after termination of the infusion (Fig. 1). Such intra-arterial doses of nitroprusside also induced a dose-dependent fall in blood pressure (Fig. 1), with the response to a dose of 40 $\mu$g $kg^{-1}$ $min^{-1}$ reaching its plateau value within 82 ± 38 s ($n$ = 6), which was well maintained for the duration of the infusion. Intragastric administration of nitroprusside (1.25–50 mg $ml^{-1}$) in isotonic saline induced a similar dose-dependent and sustained fall in blood pressure (reaching a plateau value within 43 ± 8 s, $n$ = 4, with the highest dose, which was not significantly different from the value following local infusion). However, intragastric administration of nitroprusside in these doses did not provoke any macroscopically detectable gastric mucosal damage ($n$ = 5 for each dose) as shown in Fig. 1. Intra-arterial infusion of vehicle, isotonic saline, did not induce any significant mucosal damage or fall in blood pressure (Fig. 1).

### 3.2. Effects of superoxide dismutase

Following intragastric instillation of 0.1 M HCl, close-arterial infusion of nitroprusside (10–40 $\mu$g $kg^{-1}$ $min^{-1}$) for 15 min provoked mucosal injury involving 45 ± 2% ($n$ = 68) of the total mucosal area with the highest dose used. Higher doses of nitroprusside were not investigated because of the anticipated profound hypotensive actions. The damage was characterized

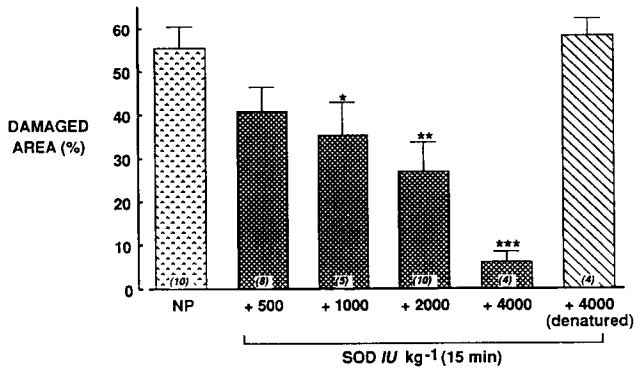


Fig. 2. Effects on concurrent close-arterial administration of superoxide dismutase (SOD; 500–4000 i.u. $kg^{-1}$ over 15 min) or denatured SOD on the rat gastric mucosal damage induced by a 15 min close-arterial infusion of nitroprusside (NP; 40 $\mu$g $kg^{-1}$ $min^{-1}$). Macroscopic damage, assessed 20 min after termination of the NP infusion, is shown as % of the total mucosal area. Results are expressed as means ± S.E.M. of ($n$) studies, where significant inhibition is shown as $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

macroscopically as areas of vasocongestion, epithelial sloughing and haemorrhagic necrosis.

Concurrent local intra-arterial infusion of superoxide dismutase (500–4000 i.u. $kg^{-1}$ over 15 min) caused a significant dose-dependent reduction in the gastric mucosal damage induced by local infusion of nitroprusside (40 $\mu$g $kg^{-1}$ $min^{-1}$ for 15 min). Denatured superoxide dismutase did not, however, affect the extent of mucosal damage induced by nitroprusside (Fig. 2).

Local infusion of superoxide dismutase (4000 i.u. $kg^{-1}$ over 15 min) likewise significantly reduced the macroscopic mucosal injury elicited by local intra-arterial infusion of *S*-nitroso-*N*-acetyl-penicillamine (40 $\mu$g $kg^{-1}$ $min^{-1}$) as shown in Fig. 3.

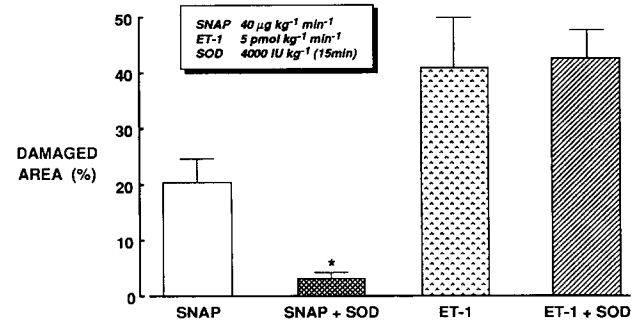


Fig. 3. Effect on concurrent close-arterial infusion of superoxide dismutase (SOD; 4000 i.u. $kg^{-1}$ over 15 min) on the rat gastric mucosal injury induced by a 15 min close-arterial infusion of *S*-nitroso-*N*-acetyl penicillamine (SNAP, 40 $\mu$g $kg^{-1}$ $min^{-1}$) or endothelin-1 (ET-1, 5 pmol $kg^{-1}$ $min^{-1}$). Macroscopic damage, assessed 20 min after termination of the SNAP or ET-1 infusion, is shown as % of the total mucosal area. Results are expressed as mean ± S.E.M. of at least five experiments in each group, where significant inhibition from the corresponding control group is shown as $^{*}P < 0.05$.

On histological evaluation, the mucosal injury provoked by close-arterial infusion of nitroprusside (40 $\mu$g $kg^{-1}$ min for 15 min) was characterized as glandular disruption, with vasocongestion or oedema in the upper regions of the mucosa, and with more severe haemorrhagic damage being located in the lower mucosa, similar to that shown previously in this model following local infusion of *S*-nitroso-*N*-acetyl-penicillamine (Lopez-Belmonte et al., 1993). These indications of cellular damage in the upper and lower regions of the mucosa were attenuated by concurrent infusion of superoxide dismutase (4000 i.u. $kg^{-1}$) the histological damage index with nitroprusside of 4.6 ± 0.6 ($n = 14$) being significantly reduced to 1 ± 0.4 ($n = 4$, $P < 0.05$), comparable to that in the control group (0.7 ± 0.3, $n = 4$) following infusion of isotonic saline alone.

### 3.3. Effects of catalase alone or with superoxide dismutase

Intravenous bolus injection of catalase (50000 i.u. $kg^{-1}$), followed by its local intra-arterial infusion (2000 i.u. $kg^{-1}$ $min^{-1}$) did not reduce the extent of mucosal damage induced by concurrent local infusion of nitroprusside (Table 1). Simultaneous infusion of catalase (2000 i.u. $kg^{-1}$ $min^{-1}$) and superoxide dismutase (2000 i.u. $kg^{-1}$ total dose over 15 min) did, however, reduce such damage, but the observed inhibition of mucosal injury was no greater than that achieved with superoxide dismutase alone (Table 1).

### 3.4. Effects of peroxyl or hydroxyl scavengers

Concurrent systemic intravenous infusion of desferrioxamine (0.25–1 mg $kg^{-1}$ $min^{-1}$) caused a dose-dependent significant reduction in the extent of mucosal damage induced by local infusion of nitroprusside (Fig.

Table 1
Effects of local intra-arterial infusion of superoxide dismutase or catalase alone, or in combination, on the gastric mucosal damage induced by concurrent local intra-arterial infusion of sodium nitroprusside

| | % damage area |
|---|---|
| Nitroprusside | 57 ± 4 (5) |
| + catalase | 66 ± 3 (10) |
| + superoxide dismutase | 27 ± 7 [a] (10) |
| + catalase + superoxide dismutase | 35 ± 5 [a] (5) |

Results are expressed as the percentage of total mucosal area exhibiting macroscopic damage determined 20 min after termination of the infusion of nitroprusside (40 $\mu$g $kg^{-1}$ $min^{-1}$). Superoxide dismutase (133 i.u. $kg^{-1}$ $min^{-1}$) or catalase (50000 i.u. $kg^{-1}$ bolus followed by infusion of 2000 i.u. $kg^{-1}$ $min^{-1}$) alone or in combination were administered concurrently with nitroprusside for 15 min. Data are shown as means ± S.E.M. of ($n$) studies, where statistical significant difference from the nitroprusside alone group is shown as [a] $P < 0.05$.

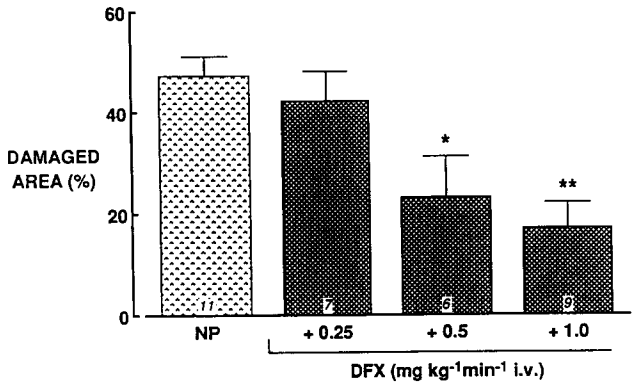


Fig. 4. Effect of intravenous infusion of desferrioxamine (DFX; 0.25–1 mg $kg^{-1}$ $min^{-1}$ for 30 min) on the rat gastric mucosal damage induced by close-arterial infusion of nitroprusside (NP; 40 $\mu g$ $kg^{-1}$ $min^{-1}$ for 15 min). Macroscopic damage, assessed 20 min after termination of the NP infusion, is shown as % of the total mucosal area. Results are expressed as means ± S.E.M. of ($n$) studies, where significant inhibition is shown as $^{*}P < 0.05$, $^{**}P < 0.01$.

4) or *S*-nitroso-*N*-acetyl-penicillamine (Table 2). Dimethylthiourea (20 mg $kg^{-1}$ $min^{-1}$), also reduced the mucosal damaged provoked by local infusion of these NO donors (Table 2).

### 3.5. Effects of superoxide dismutase and scavengers on endothelin-1 induced gastric damage

Close-arterial infusion of endothelin-1 (5 pmol $kg^{-1}$ $min^{-1}$ for 15 min) induced haemorrhagic injury in the gastric mucosa involving 25 ± 5% of the total mucosal area ($n = 5$), when assessed 20 min later. Concurrent local infusion of superoxide dismutase (4000 i.u. $kg^{-1}$ over 15 min) did not significantly alter the extent of this damage (Fig. 3).

Intravenous administration of desferrioxamine (1 mg $kg^{-1}$ $min^{-1}$) for 15 min did not significantly reduce the extent of mucosal damage induced by this dose of endothelin-1, being in this series of studies, 29 ± 1% and 26 ± 5% of the total area ($n = 8$ and 13) respectively. Likewise, intravenous administration of dimethylthiourea (20 mg $kg^{-1}$ $min^{-1}$ for 15 min) did not significantly affect the extent of mucosal injury induced by endothlein-1 ($n = 6$, $P > 0.05$).

Table 2
Effects of intravenous infusion of hydroxyl and peroxyl scavengers on gastric mucosal damage induced by concurrent local intra-arterial infusion of nitroprusside (40 $\mu g$ $kg^{-1}$ $min^{-1}$) or *S*-nitroso-*N*-acetyl penicillamine (SNAP; 40 $\mu g$ $kg^{-1}$ $min^{-1}$) for 15 min

| | % damage area | |
|---|---|---|
| | Nitroprusside | SNAP |
| Control | 40 ± 4 (23) | 28 ± 4 (17) |
| + dimethylthiourea | 25 ± 5 [a] (9) | 18 ± 5 [a] (6) |
| + desferrioxamine | 17 ± 5 [b] (9) | 14 ± 5 [a] (10) |

Results are expressed as the percentage of total mucosal area exhibiting macroscopic damage determined 20 min after termination of the infusion of the NO donor. Infusion of dimethylthiourea (20 mg $kg^{-1}$ $min^{-1}$) or desferrioxamine (1 mg $kg^{-1}$ $min^{-1}$), was commenced 15 min prior to the NO donors and infused throughout the period of administration of the NO donor. Data are expressed as means ± S.E.M. of ($n$) studies, where statistical significant changes from the corresponding control NO-donor group is given as $^{a}P < 0.01$, $^{b}P < 0.001$.

## 4. Discussion

In the present study, we have demonstrated that whereas both intragastric and intra-arterial administration of the NO donor, nitroprusside substantially lowered systemic arterial blood pressure, gastric mucosal injury in the rat was only observed following its local intra-arterial infusion. These findings thus support the suggestion of a previous study that such mucosal injury caused by local intravascular administration of high doses of nitrovasodilators is not the direct consequence of systemic hypotension (Lopez-Belmonte et al., 1993). The findings of the present study also indicate that mucosal damage can be provoked by these agents in the absence of intragastric instillation of exogenous acid.

It is possible, that by limiting mucosal blood flow, systemic hypotension may augment the direct injurious actions of the NO donors, although any such effects may be compensated by the local vasodilator actions of these compounds. Blood flow measurements will be useful to determine the time course of microcirculatory events and any contribution of changes in systemic arterial blood pressure or local ischaemia to NO-donor provoked mucosal damage. However, evaluation of the findings from such studies with a nitrovasodilator agent will be complex, and any initial injury to the microvascular endothelium will release a number of local vasoactive mediators which could also confound interpretation (Whittle, 1993).

Early studies have implicated the superoxide and the hydroxyl radical in the vascular injury that follows ischaemia-reperfusion in the intestine (Parks et al., 1982; Parks and Granger, 1983). In addition, administration of superoxide dismutase decreases macroscopic gastric damage and blood loss after haemorrhagic shock or ischaemia (Itoh and Guth, 1985), while local infusion of a superoxide generating system can provoke gastric mucosal injury (Esplugues and Whittle, 1989). The demonstration in the present study of the ability of superoxide dismutase to also prevent the mucosal injury provoked by high doses of both nitroprusside and the nitrosothiol, *S*-nitroso-*N*-acetyl-penicillamine that spontaneously liberates NO (Lopez-Belmonte et al., 1993), also implicates the involvement of the superoxide anion in the mechanisms underlying such tissue damage. The specificity of these actions of superoxide

dismutase on NO donor-induced gastric injury was demonstrated by the lack of effect of denatured superoxide dismutase. Furthermore, superoxide dismutase failed to reduce mucosal damage provoked by endothelin-1, which caused a comparable degree mucosal haemorrhagic injury following close-arterial infusion as the NO donors. This also suggests that the high intra-arterial doses of NO donors do not bring about mucosal injury by the local release of endothelin-1 in the microcirculation. The mechanism by which local infusion of these low doses of endothelin-1 provokes gastric injury does not appear to be related to mucosal vasoconstriction and may reflect changes in endothelial cell continuity (Lopez-Belmonte and Whittle, 1994).

The protective actions of systemically administered dimethylthiourea against the mucosal injury induced by the NO donors also implicates the involvement of the highly cytotoxic hydroxyl radicals (Zimmerman et al., 1990), again the specificity of action of dimethylthiourea being suggested by its failure to affect the mucosal damage observed following endothelin-1 administration. However, the inability of the local administration of catalase, in doses previously demonstrated to reduce the mucosal injury induced by hydrogen peroxide, to prevent NO donor induced mucosal injury, or to augment superoxide dismutase-induced protection, implies that the hydroxyl radicals are not derived from the iron-catalysed Haber-Weiss reaction involving superoxide and hydrogen peroxide (Morgan-Smith et al., 1987).

Another source of hydroxyl radicals could be from the peroxynitrite moiety that forms peroxynitrous acid under acidic conditions, which can subsequently decompose to the hydroxyl radical and nitrogen dioxide, peroxynitrite being formed by the interaction of NO with superoxide (Beckman et al., 1990; Radi et al., 1991). Thus, systemic administration of desferrioxamine which as well as being an iron chelator (Morgan-Smith et al., 1987; Zimmerman et al., 1990; Andrews et al., 1992) can scavenge the peroxyl-containing moieties peroxynitrite and peroxynitrous acid (Beckman et al., 1990; Radi et al., 1991; Darley-Usmar et al., 1989), was also found to reduce the mucosal injury associated with local infusion of the NO donors. Desferrioxamine has previously been shown to attenuate rat gastric mucosal injury following ischaemia-reperfusion (Morgan-Smith et al., 1987), under which conditions the agent was considered to act as an iron chelator, preventing hydroxyl radical production from hydrogen peroxide. Because of the lack of effect of catalase in preventing the mucosal injury associated with the NO donors, such a mechanism involving hydrogen peroxide products is unlikely to underlie the protective actions of desferrioxamine in the current model. Furthermore, the findings of the present study could also implicate a contribution of desferrioxamine as a peroxynitrite scavenger in its protection against ischemia-reperfusion-induced mucosal damage in some situations.

In contrast to the current findings of the cytotoxic actions of high intravascular concentrations of NO donors on the rat gastric mucosa, local infusion of lower concentrations protect against mucosal injury (Lopez-Belmonte et al., 1993), as does their intragastric application (Kitawaga et al., 1990; McNaughton et al., 1989). Intravenous infusion of an NO donor can also prevent acute intestinal microvascular injury induced by the inflammatory mediator, platelet activating factor (Boughton-Smith et al., 1992). Furthermore, local tissue superfusion of NO donors can reduce vascular permeability following ischaemia-reperfusion in the mesenteric vascular bed (Kurose et al., 1994). Since superoxide is known to be released under this latter condition (Parks et al., 1982; Parks and Granger, 1983; Perry et al., 1986; Wadhwa and Perry, 1987; Zimmerman et al., 1990; Andrews et al., 1992), such findings could suggest a lack of involvement of the peroxynitrite moiety in the vascular injury induced by ischaemia-reperfusion. However, this could also reflect the high local levels of superoxide so produced, preferentially accelerating the breakdown of NO to other products. The actions of higher concentrations of NO donors in models of ischaemia-reperfusion injury would therefore be of interest. Such observations may also suggest that ischaemia-reperfusion, leading to superoxide production, is not a key feature of the injury provoked by NO donors, but this will require experimental verification. In the present study, the cellular source or local levels of superoxide in the gastric mucosa are not known, but an endothelial origin is feasible (Gryglewski et al., 1986), which would thereby readily promote interactions with intravascular NO. Whether superoxide is released under resting conditions or whether the NO donors directly or through any initial microvascular injury resulting in local transient ischaemia, can promote superoxide biosynthesis and release, thus warrants further investigation.

These present findings give support to the formation in vivo of peroxynitrite from endogenous superoxide and NO liberated from the NO donors and its role in the genesis of gastric mucosal injury provoked by local administration of high local doses of nitroprusside and *S*-nitroso-*N*-acetyl-penicillamine. In other studies, incubation of colonic epithelial cells in vitro with NO donors leads to cellular damage (Tepperman et al., 1994), while direct application of exogenously generated peroxynitrite to the colonic mucosa has been shown to provoke haemorrhagic damage in that tissue (Rachmilewitz et al., 1993). Conditions that involve the generation of high local concentrations of NO may thus be detrimental to the integrity of the gastro-intestinal mucosal tissue. In the gut, expression of inducible NO synthase enzyme stimulated by endotoxin

is correlated with microvascular injury (Boughton-Smith et al., 1993a; Laszio et al., 1994), as well as epithelial cytoxicity (Tepperman et al., 1993; Tepperman et al., 1994). The inducible NO synthase, which is found in inflammatory cells (Hibbs et al., 1988; Marietta et al., 1989; Stuehr et al., 1989) and in the colonic mucosa of patients with ulcerative colitis (Boughton-Smith et al., 1993b) can be also detected in gastric mucosal cells (Brown et al., 1994). Induction of NO synthase in the gastric mucosa may thus occur in gastritis or other inflammatory conditions, or as the result of local toxin release from *Helicobacter pylori* in peptic ulceration. The excessive levels of NO that would be so produced could be involved, through the formation of peroxynitrite or other products (Radi et al., 1991; Lipton et al., 1993), in the associated cellular injury. It is relevant, therefore, that stimulation of the production of reactive oxygen metabolites in antral mucosa by *H. pylori* has recently been described (Davies et al., 1994).

Thus, NO may be considered not only as an important protective physiological mediator that maintains gastric mucosal integrity (Whittle, 1993), but also, in high local concentrations, as a potential cytotoxic mediator involved in mucosal damage under pathological conditions.

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