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Involvement of peroxynitrite in the lipid peroxidation induced by nitric oxide in rat gastric mucosa

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Abstract

Local intra-arterial infusion of high doses of nitric oxide (NO) donors, such as *S*-nitroso-*N*-acetyl-penicillamine or nitroprusside cause extensive gastric mucosal damage. The involvement of lipid peroxidation of mucosal tissue in the mechanism of such gastric damage has been investigated in the pentobarbitone-anaesthetised rat. Local intra-arterial infusion of nitroprusside (40 $\mu g \cdot kg^{-1} \cdot min^{-1}$) or *S*-nitroso-*N*-acetyl-penicillamine (40 $\mu g \cdot kg^{-1} \cdot min^{-1}$) induced macroscopically apparent injury and provoked a dose-dependent peroxidation of lipid in gastric tissue. By contrast, endothelin-1 infusion provoked mucosal injury of the mucosa, yet did not produce lipid peroxidation. Local co-infusion of superoxide dismutase (2000–4000 IU $\cdot kg^{-1}$) reduced both the lipid peroxidation and the mucosal damage provoked by *S*-nitroso-*N*-acetyl-penicillamine and nitroprusside. These findings indicate that lipid peroxidation accompanies the mucosal tissue damage induced by NO donors, while the action of superoxide dismutase implicates the involvement of peroxynitrite, formed from superoxide and NO, in this process.

Keywords: Nitric oxide (NO); Lipid peroxidation, stomach

High concentrations of nitric oxide (NO) released from the local intra-arterial infusion of spontaneously decomposing NO donors such as nitroprusside or the nitrosothiol, *S*-nitroso-*N*-acetyl-penicillamine, have been shown to provoke gastric mucosal injury (Lopez-Belmonte et al., 1993). It has been proposed that the cytotoxicity of NO may result from the interaction of NO with the superoxide radical, producing the highly cytotoxic moiety peroxynitrite and its subsequent derivative hydroxyl radical (Beckman et al., 1990). Both reactive species are known to be cytotoxic, and particularly, to oxidise the lipid in cellular membranes (Beckman and Crow, 1993). In the present study, we have therefore determined the extent of lipoperoxidation of gastric mucosal tissue, in vivo, after local intra-arterial infusion of nitroprusside or *S*-nitroso-*N*-acetyl-penicillamine, and investigated the actions of locally infused superoxide dismutase on both the mucosal injury and lipid peroxidation.

Male Wistar rats (200–250 g) fasted overnight but allowed free access to water, were anaesthetised with sodium pentobarbitone (60 $mg \cdot kg^{-1}$ i.p.). The oesophagus and pylorus were ligated following a mid-line incision and 2 ml of 0.1 M hydrochloric acid was instilled into the gastric lumen via a 25 gauge needle inserted through the forestomach. The left gastric artery was cannulated as previously described (Lamarque and Whittle, 1995).

Nitroprusside (40 $\mu g \cdot kg^{-1} \cdot min^{-1}$; Sigma Chemical Co., Poole, Dorset), *S*-nitroso-*N*-acetyl-penicillamine (40 $\mu g \cdot kg^{-1} \cdot min^{-1}$; Wellcome Research Laboratories), endothelin-1 (5 $pmol \cdot kg^{-1} \cdot min^{-1}$ in 0.1% bovine serum albumin-isotonic saline; Sigma) or isotonic saline was infused through the left gastric artery (13 $\mu l \cdot min^{-1}$). After 20 min following termination of the infusion, the stomachs were removed for assessment of macroscopic damage as previously described (Lamarque and Whittle, 1995).

Superoxide dismutase (2000–4000 IU $\cdot kg^{-1}$ over 15

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min; Sigma Chemical Co., Poole, Dorset) or isotonic saline was infused concurrently with *S*-nitroso-*N*-acetyl-penicillamine or nitroprusside through a bifurcated catheter connected to the intra-arterial tubing.

The amount of hydroperoxidised lipid was determined by an iodometric assay (Buege and Aust, 1978). Briefly, the corpus area of the stomach was removed 20 min after termination of the local infusion of NO donors. Each sample was homogenised (30 s, Ultra-Turrax; 5 mm blade) in 2 ml of buffered saline and 1 ml was added to 5 ml of chloroform : methanol (2 : 1). After centrifugation (1000 × *g* over 5 min), the chloroform layer was removed and evaporated under a stream of nitrogen. A solution of acetic acid-chloroform (3 : 2, 1 ml) kept at 4°C was added to the dry residue, followed by 0.05 ml of potassium iodide (1.2 $g \cdot ml^{-1}$; Sigma Chemical Co.). The assay mixture was left in the dark for 5 min and then 3 ml of cadmium acetate (5 $mg \cdot ml^{-1}$; Sigma Chemical Co.) was added. After centrifugation (1000 × *g* for 10 min) lipid hydroperoxide was measured from the spectrophotometric absorption in the upper phase at 353 nm.

All data are expressed as the mean ± S.E. Comparisons between groups of parametric data were made by Student's *t*-test for non-paired data and for non-parametric data by the Mann-Witney *U*-test. *P* values of less than 0.05 were taken as significant.

Local infusion of saline for 15 min caused minimal damage involving only 4 ± 2% ($n = 7$) of the total mucosal area, when assessed macroscopically 20 min after termination of the infusion. By contrast, local infusion for 15 min of *S*-nitroso-*N*-acetyl-penicillamine (40 $\mu g \cdot kg^{-1} \cdot min^{-1}$) or nitroprusside (40 $\mu g \cdot kg^{-1} \cdot min^{-1}$), induced substantial ($P < 0.01$) damage involving 32 ± 4% ($n = 17$) and 43 ± 7% of the mucosal area ($n = 6$) respectively.

Local intra-arterial co-infusion of administration of superoxide dismutase (2000–4000 $IU \cdot kg^{-1}$) caused a significant dose-dependent reduction in the extent of damage induced by the local intra-arterial infusion of *S*-nitroso-*N*-acetyl-penicillamine (40 $\mu g \cdot kg^{-1} \cdot min^{-1}$ over 15 min) as shown in Fig. 1. Co-infusion of superoxide dismutase (4000 $IU \cdot kg^{-1}$) also reduced the extent of the damage (to 12 ± 8% of the mucosal area, $n = 5$; $P < 0.01$) induced by local intra-arterial administration of nitroprusside (40 $\mu g \cdot kg^{-1} \cdot min^{-1}$ for 15 min).

After local intra-arterial infusion of saline, the peroxidised lipid concentration, measured in corpus mucosa, was 3 ± 2 $\mu M \cdot g^{-1}$ ($n = 5$). After local infusion of *S*-nitroso-*N*-acetyl-penicillamine (40 $\mu g \cdot kg^{-1} \cdot min^{-1}$; $n = 9$) or nitroprusside (40 $\mu g \cdot kg^{-1} \cdot min^{-1}$; $n = 5$) the peroxidised lipid concentration was increased to 283 ± 103 $\mu M \cdot g^{-1}$ and 327 ± 18 $\mu M \cdot g^{-1}$ respectively.

Local intra-arterial co-infusion of superoxide dismutase (2000–4000 $IU \cdot kg^{-1}$) significantly reduced the peroxidised lipid, as damage of the mucosa, as shown in Fig. 1. Likewise, local infusion of superoxide dismutase (4000 $IU \cdot kg^{-1}$) reduced by 90 ± 7% the amount of peroxidised lipid in the mucosa induced by the local infusion of nitroprusside (to 33 ± 23 $\mu M \cdot g^{-1}$, $n = 5$; $P < 0.001$).

In control experiments, close arterial infusion of endothelin-1 (5 $pmol \cdot kg^{-1} \cdot min^{-1}$ for 15 min) induced haemorrhagic injury of the mucosa, involving 39 ± 6% ($n = 6$) of total gastric area when assessed 20 min later. By contrast with the NO donors, this damage was not accompanied by a significant increase in the lipoperoxidised lipid (9 ± 4 $\mu M \cdot g^{-1}$; $n = 6$) compared to the vehicle control group.

In the present study, we have confirmed that intra-arterial infusion of high doses of the NO donors, nitroprusside or *S*-nitroso-*N*-acetyl-penicillamine, induced dose-dependent damage in gastric mucosa, suggesting a local cytotoxic action of high levels of NO on the microvascular endothelium (Lopez-Belmonte et al., 1993). In previous studies, superoxide dismutase prevented such damage with NO donors, suggesting the involvement of the superoxide radical (Lamarque and Whittle, 1995), as confirmed in the present study. The superoxide radical may act by combining with NO to form peroxynitrite which can subsequently decompose to the hydroxyl radical and nitrogen dioxide (Beckman et al., 1990).

Peroxynitrite cytotoxicity has been described as oxidis-

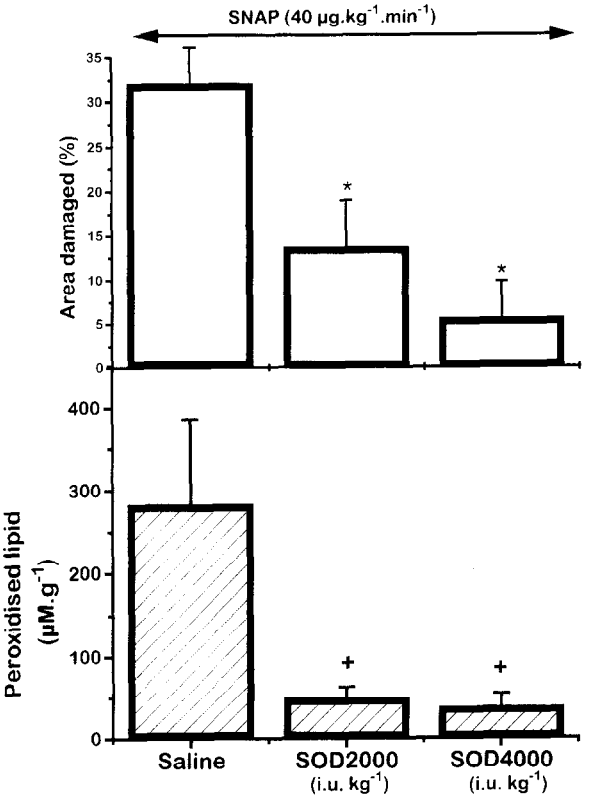

Fig. 1. Effects of local co-infusion (15 min) of superoxide dismutase (SOD; 2000–4000 $IU \cdot kg^{-1}$) on the gastric mucosal damage (open columns) and the lipid peroxidation in gastric mucosa (hatched columns) induced by local intra-arterial infusion (15 min) of *S*-nitroso-*N*-acetyl-penicillamine (SNAP; 40 $\mu g \cdot kg^{-1} \cdot min^{-1}$). Significant difference from the control group (SNAP alone) is shown as: $P < 0.05$ (*) for damage and $P < 0.01$ (+) for lipid peroxidation.

ing sulfhydryl groups and inducing scission of DNA and protein strands (King et al., 1992). Peroxynitrite can cause oxidative damage to protein, lipid, carbohydrate, DNA, subcellular organelles and cell synthesis (Beckman and Crow, 1993). Peroxynitrite also may alter the integrity of the cell membrane by the peroxidation of lipids. In the present study, peroxidised lipid was found in mucosal extracts after local infusion of nitroprusside or *S*-nitroso-*N*-acetyl-penicillamine. By contrast, local infusion of endothelin-1, which also provoked mucosal damage, failed to induce lipoperoxidation in gastric mucosa, indicating that the occurrence of lipid peroxidation is not necessarily associated with mucosal injury provoked by intra-vascular challenge. Furthermore, co-infusion of superoxide dismutase not only reduced the lesions induced by NO donors, but also inhibited the peroxidation in gastric tissue. The present study thus gives support to the involvement of lipid peroxidation and superoxide in the mechanisms by which NO donors can cause damage.

High levels of NO can be formed following expresssion of the inducible isoform of NO synthase, which can be provoked by endotoxin (Boughton-Smith et al., 1993) and by water soluble extract of *H. pylori* (Lamarque et al., 1995). Lipid peroxidation following peroxynitrite formation may hence be involved in the mucosal injury associated with *H. pylori* infection in the stomach.

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