

# Sulphydryl blocker induced gastric damage is ameliorated by scavenging of free radicals

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## Abstract

**Background**—Sulphydryl compounds and nitric oxide are essential in maintaining gastric mucosal integrity.

**Aims**—To characterise the gastric damage induced by a sulphydryl blocker, to evaluate the role of nitric oxide in its pathogenesis, and to reveal its possible prevention by scavenging of free radicals.

**Methods**—Gastritis was induced in rats by addition of iodoacetamide (0.1%) to the drinking water, with and without daily intragastric administration of TEMPOL. After death, the stomach was resected, washed, lesion area assessed, and mucosal inflammatory mediators, myeloperoxidase and nitric oxide synthase activities were determined.

**Results**—Administration of iodoacetamide induced gastric mucosal erosions present for up to two weeks. Myeloperoxidase activity was increased for up to seven days and nitric oxide synthase activity was significantly decreased for up to 14 days. Treatment for seven days with the free radical scavenger, TEMPOL, decreased by 68% the damage induced by iodoacetamide.

**Conclusions**—Gastric damage induced by iodoacetamide, a sulphydryl alkylator, accompanied by inhibition of nitric oxide synthase activity shows the important contribution of sulphydryl compounds and nitric oxide to the maintenance of gastric mucosal integrity. Nitric oxide donation and scavenging of free radicals may be a novel approach to prevent gastric damage.

(Gut 1996; 38: 826-831)

Keywords: nitric oxide, TEMPOL, iodoacetamide, free radicals, gastric damage.

Glutathione exists in a combination of its reduced form (GSH) and its oxidised dimer in almost all mammalian cells. GSH is a nucleophilic scavenger of superoxide and also acts as a cofactor in the GSH peroxidase mediated reduction of  $H_2O_2$ .<sup>1</sup> GSH has an important role in maintaining mucosal integrity in the gastrointestinal tract. GSH is essential for the protection of gastric epithelial<sup>1</sup> and chief cells<sup>2</sup> against oxidative stress. Moreover, calcium induced gastric mucosal injury is mediated by reduction in GSH content.<sup>3</sup> In rats, the sulphydryl (SH) blocker, iodoacetamide, was previously shown to induce diffuse gastritis<sup>4</sup> and, also, significant colonic injury,<sup>5</sup> indicating the important contribution of SH compounds

to the maintenance of gastrointestinal integrity. In this study the model in iodoacetamide induced gastric damage and its possible prevention by scavenging of free radicals was evaluated. This model was further characterised macroscopically and histologically, with special focus on the involvement of inflammatory mediators and nitric oxide (NO) in its pathogenesis.

NO has an important role in maintaining gastric mucosal integrity. Inhibition of gastric NO formation decreases gastric blood flow, deprives the tissue of oxygen, and increases mucosal vulnerability to intragastric administration of irritants that mildly damage the gastric mucosa. Moreover, inhibition of NO formation also increases gastric mucosal haemodynamic changes, such as those induced by ethanol administration.<sup>6</sup> Oxygen derived radicals and non-radical species have been implicated previously in the pathogenesis of gastric ulceration<sup>7,8</sup> and TEMPOL, a free radical, which is also a potent scavenger of other free radicals, was shown by us to prevent gastric mucosal lesions in rats.<sup>9</sup> In this study the role of gastric NO formation in iodoacetamide induced gastric damage and its possible prevention by scavenging of free radicals was evaluated.

## Methods

### Materials

L-arginine, L-citrulline, L-NAME, D-NAME, NADPH, valine, aminoguanidine, dithiothreitol, phenylmethylsulphonyl fluoride, EDTA, EGTA, and 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPOL) were purchased from Sigma Chemical Co, St Louis, MO, USA; Dowex AG50W-X8 (Na form) 100-200 mesh, and TRIS base (electrophoresis grade) were purchased from Bio-Rad Labs, Richmond, CA, USA; aquasol-2 was purchased from DuPont Co/NEN Research Products, Bad Hamburg, Germany.

### Animals

Male, Sprague-Dawley rats, weighing 200-250 g and fed ad libitum were used in all the studies.

### Iodoacetamide induced gastritis

Gastritis was induced by the addition of iodoacetamide (0.1%) to the drinking water for a period of 1-14 days. Control rats were provided with tap water without iodoacetamide. In several experiments, in addition to

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Accepted for publication  
7 December 1995

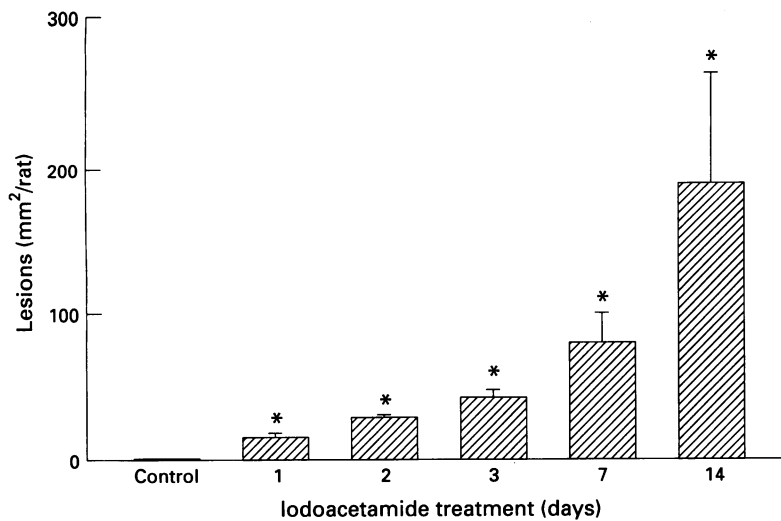


Figure 1: Gastric lesion area in iodoacetamide treated rats. Rats were treated with iodoacetamide (0.1%) added to the drinking water. Rats were killed after 1–14 days, the stomach was removed, washed, lesion area assessed, as described in Methods. Results are mean (SEM) of 4–20 rats in each time interval. \*Significantly different from control,  $p < 0.05$ .

the iodoacetamide, rats were treated with TEMPOL (0.5 g/kg) intragastrically, once daily until death after two or seven days. Rats were killed one, two, three, seven, and 14 days after iodoacetamide treatment. The stomach was isolated, removed, washed with ice cold saline, and the extent of haemorrhagic erosions was blindly assessed. Tissue sections were obtained for histological examination and the remaining mucosa was scraped and processed for determination of lipoxygenase products, prostaglandin E<sub>2</sub> (PGE<sub>2</sub>), myeloperoxidase (MPO), and NO synthase (NOS) activities.

#### Determination of mucosal damage

Mucosal damage was measured macroscopically and scored by multiplying the length (mm) and width (mm). For each rat the total score was determined by summing the ulcer scores (mm<sup>2</sup>). All measurements of damage were blindly performed by two observers using a stereomicroscope. The variability between the two observers was 7%.

#### Determination of MPO activity

Two hundred mg of gastric mucosal scrapings were homogenised three times for 30 seconds

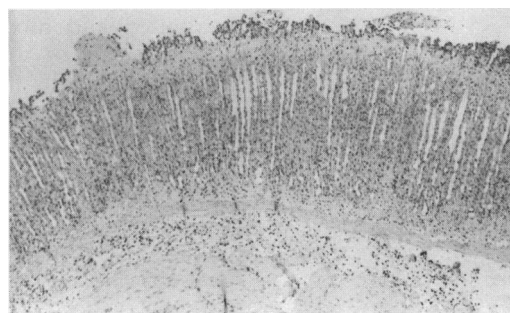


Figure 2: Histological section from the stomach of a rat treated for 48 hours with iodoacetamide (0.1%) added to the drinking water. Note the superficial ulcerations of the mucosa with a mild inflammatory cell infiltrate. Haematoxylin and eosin  $\times 73$ .

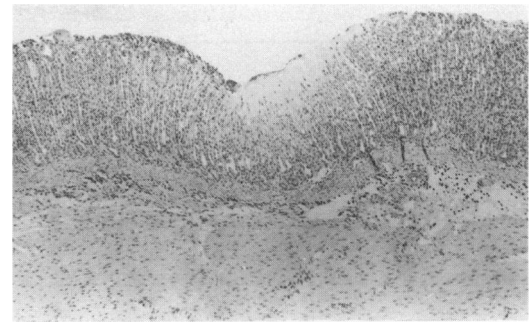


Figure 3: Histological section from the stomach of a rat treated for seven days with iodoacetamide (0.1%) added to the drinking water. Note the deep ulceration involving half of the mucosal thickness. Haematoxylin and eosin  $\times 73$ .

at 4°C with a polytron (Kinematica GmbH, Kriens-Luzern, Switzerland) in 1.0 ml of ice cold 0.5% hexadecyltrimethylammonium bromide in 50 mM phosphate buffer, pH 6.0. The polytron probe was rinsed twice with 1.0 ml of the buffer and the washings were added to the homogenate. The homogenate was then sonicated for 10 seconds, freeze thawed three times, and centrifuged for 15 minutes at 40 000 *g*. An aliquot of the supernatant was taken for determination of the enzyme activity, according to Bradley *et al.*<sup>10</sup>

#### Determination of NOS activity

NOS activity was monitored by the conversion of [<sup>3</sup>H]-L-arginine to citrulline according to Bush *et al.*<sup>11</sup> Mucosal scrapings (100 mg) were homogenised for 30 seconds at 4°C with a polytron (Kinematica GmbH, Kriens-Luzern, Switzerland) in 0.3 ml of ice cold 50 mM TRIS HCl, pH 7.4 containing 0.1 mM EDTA, 0.1 mM EGTA, 0.5 mM dithiothreitol, 1 mM phenylmethylsulphonyl fluoride. Homogenates were centrifuged at 20 000  $\times g$  for 60 minutes at 4°C and the supernatant was used as the source of NOS. Enzymatic reactions were conducted at 37°C in 50 mM TRIS HCl, pH 7.4 containing 100  $\mu$ M L-arginine, 100  $\mu$ M NADPH, 2 mM CaCl<sub>2</sub>, 0.2–0.4 mg supernatant proteins, and approximately 200 000 dpm of L-[2,3,4,5-<sup>3</sup>H]-arginine HCl (77 Ci/mmol; Amersham, UK) to a final volume of 100  $\mu$ l. In several experiments homogenates prepared from mucosal scrapings obtained from iodoacetamide treated and normal rats were incubated in the absence of Ca<sup>++</sup> or in the presence of 1–100 mM L-NAME, 10 mM aminoguanidine or 60 mM valine in the assay mixture. Enzymatic reactions were terminated by addition of 2.0 ml of ice cold 'stop buffer': 20 mM sodium acetate, pH 5.5, 1 mM L-citrulline, 2 mM EDTA, and 0.2 mM EDTA. Citrulline was determined by applying the samples (2.0 ml) prepared as described above, to columns (1 cm diameter) containing 1 ml of Dowex AG50W-X8, Na form, that had been pre-equilibrated with stop buffer. Columns were eluted with 4  $\times$  1.0 ml of water collected into scintillation vials. Opti-fluor (10 ml) was added to each vial and samples were counted in a Packard Tri-Carb liquid scintillation spectrometer. Citrulline was recovered in the

*Effect of TEMPOL on iodoacetamide induced gastritis*

Treatment	IA		IA+TEMPOL	
Days of treatment	2	7	2	7
Number	10	9-19	5	10
Lesions (mm <sup>2</sup> /rat)	27.0 (2.2)	71.0 (14.7)	36.8 (9.0)	22.8 (3.2)*
MPO (U/g)	2.5 (0.4)	1.8 (0.2)	1.0 (0.2)	1.6 (0.3)
NOS (nmol/g/min)	3.2 (0.4)	4.2 (0.4)	4.2 (0.1)	4.0 (0.1)

Rats were treated with iodoacetamide (0.1%) added to the drinking water with or without daily intragastric treatment with TEMPOL (50 mg/100 g). Rats were killed after two and seven days, the stomach removed, lesion area assessed, and mucosal MPO and NOS activities determined, as described in Methods. Results are mean (SEM). IA=iodoacetamide. \*Significantly different from rats treated with iodoacetamide,  $p < 0.05$ .

first 4.0 ml of the Dowex column eluate to the extent of  $96 \pm 2\%$ .

*Determination of eicosanoid generation*

One hundred and fifty mg of mucosa were placed in preweighed tubes containing 1.0 ml of phosphate buffer (50 mM, pH 7.4). The mucosa was minced with scissors and centrifuged in an Eppendorf centrifuge for 10 seconds. The pellet was resuspended in 1.0 ml of the above buffer, incubated for one minute in a vortex mixer, 10  $\mu$ g indomethacin added, and the tubes centrifuged for 60 seconds. The supernatants were kept at  $-20^\circ\text{C}$  until radioimmunoassays were performed. The capability of the mucosa to generate leukotriene B<sub>4</sub> (LTB<sub>4</sub>) and leukotriene C<sub>4</sub> (LTC<sub>4</sub>) was expressed as ng/g wet tissue weight.

*Measurement of LTB<sub>4</sub>*

LTB<sub>4</sub> immunoreactivity was determined by a radioimmunoassay kit (Amersham, TRK 940). The assay combines the use of a high specific activity LTB<sub>4</sub> tracer, an antiserum specific for LTB<sub>4</sub> (cross reactivity 100%), and a leukotriene standard (range 1.6 to 200 pg/tube). The specific binding of tracer is 42.5%, non-specific binding 2.4%. Fifty per cent B/Bo displacement is obtained with 15 pg/tube and 90% B/Bo displacement with 2.2 pg/tube of LTB<sub>4</sub>.

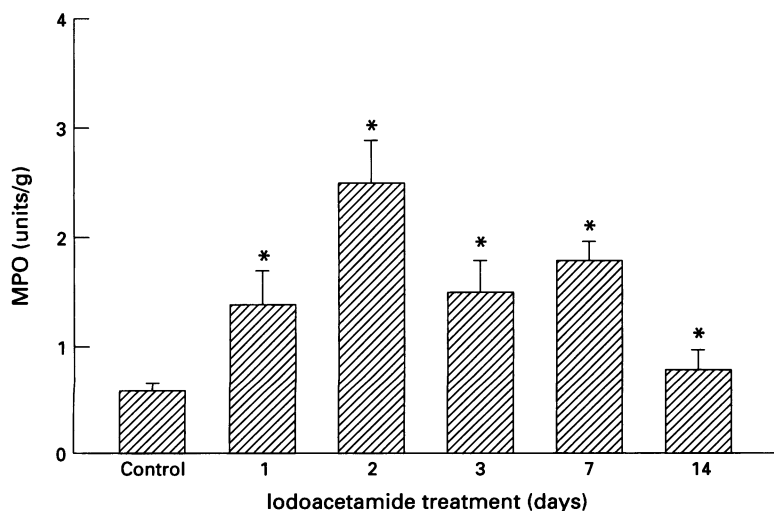


Figure 4: Gastric MPO activity in iodoacetamide treated rats. Rats were treated with iodoacetamide (0.1%) added to the drinking water. Rats were killed after 1-14 days, the stomach was removed, washed, the mucosa scraped, and MPO activity determined, as described in Methods. Results are mean (SEM) of 4-19 rats in each time interval. \*Significantly different from control,  $p < 0.05$ .

*Measurement of LTC<sub>4</sub>*

LTC<sub>4</sub> immunoreactivity was determined by radioimmunoassay. The assay combines the use of a high specific activity LTC<sub>4</sub> tritiated tracer with a monoclonal antibody specific for LTC<sub>4</sub> and LTC<sub>4</sub> standard (8-500 pg/tube). The assay uses highly specific LTC<sub>4</sub> antiserum (cross reactivity=100%) and has low cross reactivity with leukotriene D<sub>4</sub> (LTD<sub>4</sub>) (<5%). The specific binding of tracer is 40%-45% and non-specific binding is 1%-3%. Fifty per cent B/Bo displacement is obtained with 34 pg/tube, and 80% B/Bo displacement with 9.5 pg/tube of LTC<sub>4</sub>. The percentage coefficient of variation for within assay precision ranges from 7.68 (low) to 3.94 (high). The percentage coefficient of variation for the precision profile of the assay ranges from 1.64 to 3.49 (n=9).

*Measurement of PGE<sub>2</sub>*

PGE<sub>2</sub> was determined by radioimmunoassay, as previously described.<sup>12</sup>

*Morphological studies*

Sections were obtained from four representative animals in each of the treatment groups. They were fixed in phosphate buffered formaldehyde, embedded in paraffin wax, and routine 5  $\mu$ m sections were prepared. Tissues were routinely stained with haematoxylin and eosin and were evaluated by light microscopy by a pathologist unaware of the experiments being performed.

*Statistical analysis*

Data are expressed as mean (SEM). Statistical analysis for significant differences was performed according to the Student's *t* test for paired and unpaired data and the non-parametric Mann-Whitney U test.

**Results**

The addition of iodoacetamide (0.1%) to the drinking water induced gastric mucosal damage, which was apparent already after 24 hours and lasted for the entire 14 days of the experiment (Fig 1). At 24 and 48 hours the damage was observed adjacent to the fore-stomach. With prolongation of iodoacetamide treatment the damage affected the glandular part of the stomach. At 14 days lesions were widely distributed and most of the surface of the glandular part of the stomach was affected. Histologically, after the first 48 hours exposure to iodoacetamide the ulcerations were superficial and were accompanied by mild inflammatory cell infiltrate (Fig 2). After seven days of iodoacetamide treatment the ulcerations were deeper and affected half of the mucosal thickness (Fig 3). At 14 days the mucosal involvement was similar to that seen after seven days of treatment. Seven days after treatment with iodoacetamide and TEMPOL gastric lesions were significantly decreased by 68%, compared with the lesions in rats treated with iodoacetamide only. The effective protection

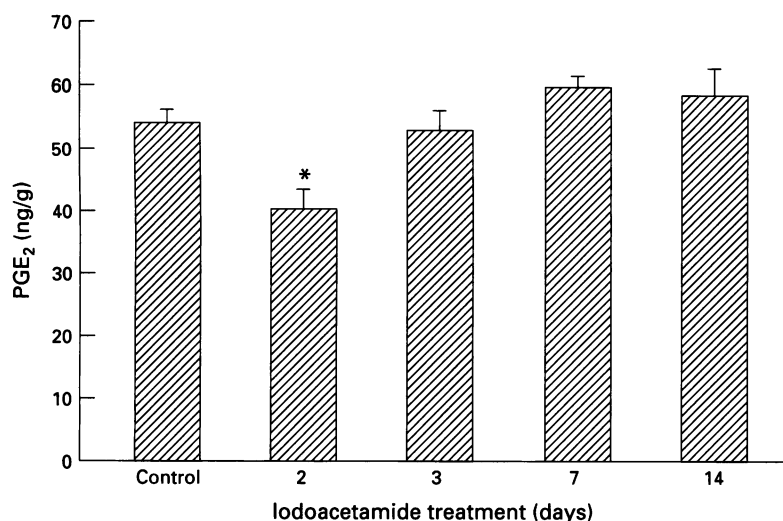


Figure 5: Gastric PGE<sub>2</sub> generation in iodoacetamide treated rats. Rats were treated with iodoacetamide (0.1%) added to the drinking water. Rats were killed after 1–14 days, the stomach was removed, washed, the mucosa scraped, and mucosal PGE<sub>2</sub> generation was determined, as described in Methods. Results are mean (SEM) of 9–20 rats in each time interval. \*Significantly different from control,  $p < 0.05$ .

provided by TEMPOL was not accompanied by significant effect on gastric MPO or NOS activities (Table).

Gastric mucosal MPO activity was significantly increased during the first seven days of iodoacetamide treatment. At 14 days gastric MPO activity was not stimulated when compared with its activity in normal gastric mucosa (Fig 4).

Iodoacetamide treatment induced a significant ( $p < 0.05$ ) 25% decrease in mucosal PGE<sub>2</sub> generation but only after 48 hours. At all other time intervals mucosal PGE<sub>2</sub> generation was similar in iodoacetamide treated and control rats (Fig 5). Iodoacetamide induced a twofold and 3.5-fold increase in mucosal LTC<sub>4</sub> generation after seven and 14 days of treatment but not beforehand. At all time intervals mucosal LTB<sub>4</sub> generation was similar in iodoacetamide and control rats (Fig 6).

Gastric mucosal NOS activity in control rats was 5.7 (0.2) nmol/g/min. At all time intervals after iodoacetamide treatment, gastric NOS

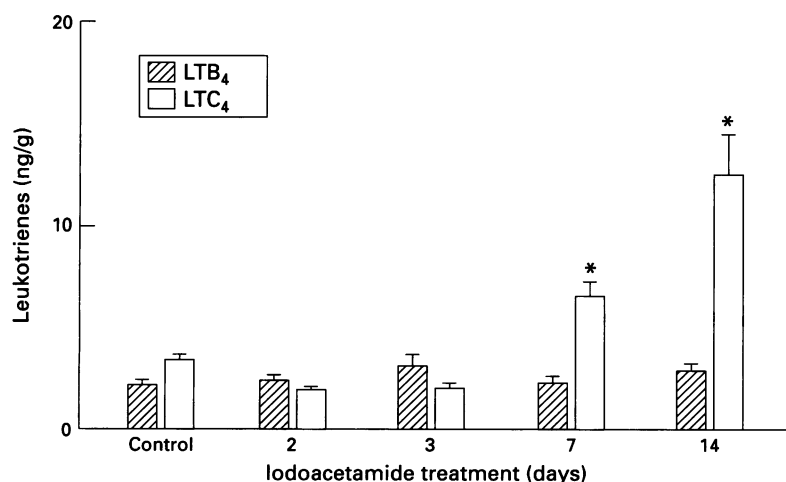


Figure 6: Gastric leukotriene generation in iodoacetamide treated rats. Rats were treated with iodoacetamide (0.1%) added to the drinking water. Rats were killed after 1–14 days, the stomach was removed, washed, the mucosa scraped, and mucosal LTB<sub>4</sub> and LTC<sub>4</sub> generation was determined, as described in Methods. Results are mean (SEM) of 8–16 rats in each time interval. \*Significantly different from control,  $p < 0.05$ .

activity was significantly reduced when compared with its activity in normal rats (Fig 7). Both in control and in iodoacetamide treated rats deletion of calcium reduced gastric NOS activity by almost 50% and the addition of valine reduced the enzyme activity by 30%. Aminoguanidine induced a 65.8 (2.3)% and 55.0 (4.0)% decrease in the gastric enzyme activity of control and iodoacetamide treated rats, whereas in both groups L-NAME induced a dose dependent inhibition of the enzyme activity (Fig 8).

## Discussion

In this study gastric damage induced by an SH blocker was found to be accompanied by decrease in gastric NOS activity, indicating the importance of both SH groups and NO to the maintenance of gastric mucosal integrity.

Endogenous SH compounds have an important role in maintaining mucosal integrity in the gastrointestinal tract. SH drugs prevent whereas SH blockers were shown to aggravate ethanol induced gastric injury.<sup>4</sup> SH blockers were also found to counteract the protective effect of prostaglandins against ethanol induced gastric damage.<sup>13</sup> The SH blockers, iodoacetamide and ethylmaleimide, when added to the drinking water, were found to induce gastric mucosal injury, which was diminished by the sulphur containing amino acid, methionine.<sup>4</sup> This study confirms the preliminary results reported by Szabo *et al*<sup>4</sup> on the deleterious gastric effects of the SH alkylator, iodoacetamide, characterises in detail, the histological features of the model, the involvement of inflammatory mediators with specific evaluation of the involvement of NO in its pathogenesis. The results reported in this study also confirm the recent finding that iodoacetamide induces colonic damage and inflammation.<sup>5</sup>

Gastric injury induced by iodoacetamide was apparent within 24 hours and increased with time for up to 14 days. Mucosal ulceration with inflammatory cell infiltrate characterises the damage. After seven days of treatment the ulcers were deep, affected half of the mucosal thickness and, at 14 days, most of the gastric mucosa was affected. Usually, tissue injury is accompanied by increased generation of inflammatory mediators. It is of interest that, in iodoacetamide induced gastric injury LTB<sub>4</sub> generation was not increased whereas LTC<sub>4</sub> generation was increased, but only after seven and 14 days of treatment. It is, therefore, suggested that leukotriene contribution to the pathogenesis of tissue damage is limited and specific to each type of injury. We have previously shown that in iodoacetamide induced colitis mucosal leukotriene generation also did not correlate with the severity of tissue injury.<sup>5</sup> Decrease in mucosal PGE<sub>2</sub> generation could have also contributed to the pathogenesis of iodoacetamide induced damage. However, in this study only two days after iodoacetamide treatment gastric PGE<sub>2</sub> generation was decreased and it is, therefore, unlikely that prostaglandins have an important role in

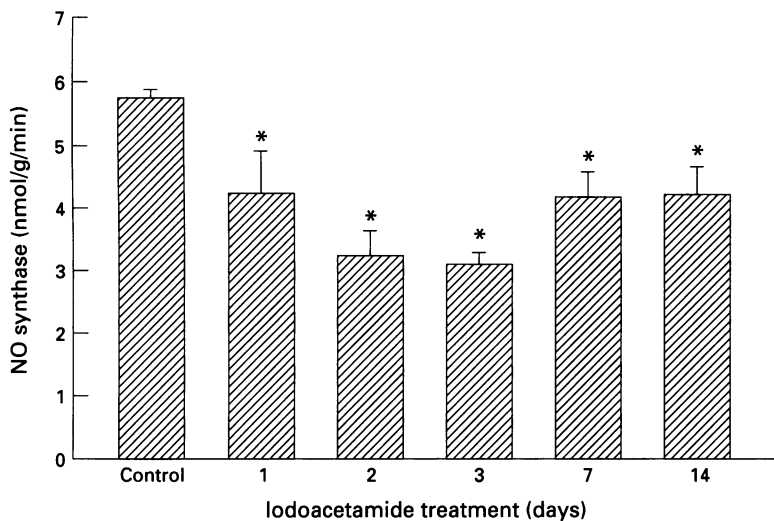


Figure 7: Gastric NOS activity in iodoacetamide treated rats. Rats were treated with iodoacetamide (0.1%) added to the drinking water. Rats were killed after 1–14 days, the stomach was removed, washed, gastric NOS activity was determined as described in Methods. Results are mean (SEM) of 7–16 rats in each time interval. \*Significantly different from control,  $p < 0.05$ .

the pathogenesis of this type of tissue injury. In a similar fashion Hauser *et al* concluded that prostaglandins are minimally involved in the mechanism of gastric protection, whereas endogenous SHs are important mediators.<sup>14</sup>

In iodoacetamide induced gastritis mucosal MPO activity was found to be stimulated during the first seven days of treatment but not thereafter. The stimulated MPO activity may indicate the contribution of granulocytes to the inflammatory response at the beginning of the process. Similarly, in iodoacetamide induced colitis mucosal MPO activity was found to be stimulated also only during the first three days after damage induction.<sup>5</sup> In a recent study the reliability of MPO as an indicator of neutrophil infiltration was questioned.<sup>15</sup> However, it is also possible that during the second week of iodoacetamide treatment inflammatory cells

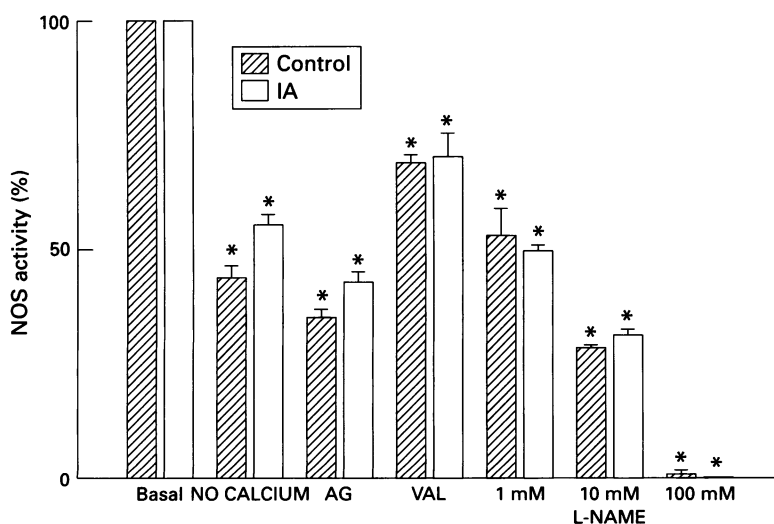


Figure 8: Effect of calcium sequestration, L-NAME, aminoguanidine, and valine on gastric NOS activity. Gastric mucosal scrapings were obtained from normal rats and from rats treated for 72 hours with iodoacetamide (0.1%) added to their drinking water. NOS activity was determined in the absence of calcium and after addition of L-NAME (1–100 mM), valine (60 mM) or aminoguanidine (10 mM), as described in Methods. Basal activity in normal and iodoacetamide treated rats was 4.80 (0.08) ( $n=4$ ) and 2.59 (0.17) ( $n=4$ ) nmol/g/min, respectively, and was regarded as 100%. Results are mean (SEM) of determinations performed with tissue obtained from four rats in each group. \*Significantly different from basal,  $p < 0.05$ .

other than granulocytes were responsible for the perpetuation of the inflammatory response.

In models of colonic inflammation increased  $\text{NO}_x$  generation by stimulated NOS activity was reported<sup>16</sup> and inhibition of colonic NOS activity was found to ameliorate the injury in several models of experimental colitis.<sup>17</sup> In the model of iodoacetamide induced gastric injury herewith presented, gastric NOS activity was found to be decreased, showing the important contribution of NO to the maintenance of gastric mucosal integrity. In a similar way inhibition of gastric NOS activity was shown to amplify gastric damage induced by ethanol.<sup>6</sup>

Gastric NOS activity determined in this study by the conversion of L-arginine to citrulline is specific, as indicated by its effective inhibition by L-NAME. The comparatively small decrease in the conversion of L-arginine to citrulline in the presence of valine, which inhibits arginase activity<sup>18</sup> rules out a significant contribution of arginase to the detected conversion of L-arginine to citrulline. Sequestration of calcium decreased by more than 50% gastric NOS activity in control rats and almost to the same extent also NOS activity in iodoacetamide treated rats. These results suggest that in the rat stomach at least half of the NOS activity represents the constitutive NOS isoform, which is calcium dependent. In the rat colon, on the other hand, only 17% of the total NOS activity is derived from the constitutive isoform.<sup>16</sup> The relatively high representation of the constitutive NOS isoform in the stomach may suggest the importance of NO for the maintenance of gastric integrity and its vulnerability to reduction in its availability, such as that induced by L-NAME.

It is possible that the decrease in gastric NOS activity after treatment with iodoacetamide is a mechanism contributing to the induction of tissue injury in addition to its alkylation of SH compounds. The different representation of the constitutive and inducible NOS isoforms in rat colonic and gastric mucosa may explain, in part, their different vulnerability to NO depletion due to inhibition of NOS activity. Gastric mucosa may be dependent on higher concentrations of NO usually furnished by the relatively high representation of the constitutive isoform, whereas the integrity of colonic mucosa is less dependent on NO. In experimental colitis the increased NO generation is mainly derived from stimulation of the inducible isoform and inhibition of its activity is, therefore, beneficial.<sup>16</sup>

Acute NO synthesis inhibition causes an immediate rise in arterial blood pressure because of widespread vascular constriction.<sup>19</sup> The addition of L-NAME to the drinking water induced in rats an increase of 38 mm Hg in the mean arterial blood pressure.<sup>17</sup> This rise in mean arterial blood pressure, associated with significant reduction in mesenteric blood flow,<sup>19</sup> may reduce the extent of injury, thereby reducing the formation of reactive oxygen radicals. In the colon inhibition of NO generation was found to protect against injury, however, this was shown to be not only because of

its vascular effect.<sup>17</sup> On the other hand, in the stomach NO regulation of the mucosal haemodynamics, including blood flow and haemoglobin oxygen saturation, was shown to be responsible for its important contribution to the maintenance of mucosal integrity.<sup>6</sup> These aspects of NO deprivation may certainly contribute to the severity of the gastric injury induced by iodoacetamide. Inhibition of NO formation was shown to aggravate gastric mucosal injury induced by ethanol. Decrease in the availability of NO induces a decrease in the resting mucosal blood flow, resulting in tissue hypoxia.<sup>6</sup> The cumulative data thus show that endogenous NO is an essential protective factor in the pathogenesis of gastric injury induced by agents such as ethanol and iodoacetamide.

TEMPOL, the potent free radical scavenger, was found to effectively decrease the severity of gastric mucosal injury induced by iodoacetamide. We have reported that TEMPOL is a powerful protective agent against injury induced by various other irritants, indicating the important contribution of free radicals to the pathogenesis of gastric injury.<sup>9</sup> These results suggest that scavenging of free radicals counteracts the deleterious effects of NO depletion, which contributes to the increased free radical generation by the hypoxic tissue. TEMPOL was found to have no effect on gastric NOS activity. However, as TEMPOL removes free radicals, which rapidly interact with NO and lower its concentration, TEMPOL may indirectly induce an increase of tissue NO concentrations. Increase in tissue NO concentrations may be an additional mechanism to explain the protective effect of TEMPOL. Potent scavengers, like TEMPOL, may therefore be useful for prevention of gastric mucosal injury induced by any irritant and, especially, by those that induce generation of free radicals. The importance of SH compounds in counteracting the deleterious effect of free radicals is also indicated by *N*-ethylmaleimide enhancement of gastric mucosal cell injury induced by oxygen free radicals generated by the xanthine oxidase-xanthine system, an effect which is reduced by glutathione and cystamine.<sup>20</sup>

In conclusion, the results reported in this study show the importance of SH compounds

and of NO to the maintenance of gastric mucosal integrity. NO donation, scavenging of free radicals, and provision of SH compounds may, therefore, be useful for prevention of gastric injury.

The authors are grateful to Dr S Szabo, who inspired the study.

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