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Gastric mucosal cytoprotection in the rat by cysteine

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The free radical scavengers methyl-methionine sulphonium bromide and cysteine (5%) protected the rat gastric mucosa against ischaemic injury produced by reserpine (5 mg kg^{-1} i.p.). Pretreatment with 1 mL of 5% cysteine by gavage completely protected against injury rats with no ligation and 70% of rats with pyloric ligation after they had been given aspirin (200 mg kg^{-1} by gavage), a dose that produced gastric mucosal injury in 40% of rats with no ligation and 70% of rats with pyloric ligation after 4 h. Ethanol (1 mL of 40% solution by gavage) after 1 h produced gastric mucosal injury in all animals and pretreatment with 1 mL of 5% cysteine by gavage completely protected 80% of non-ligated and 70% of pyloric-ligated rats against this injury. This protection was not associated with any significant effect on H^+ output. The data suggest that cysteine maintains gastric mucin by a mechanism independent of acid secretion.

Clinical and laboratory experience has confirmed the key role of ischaemia in the mechanism of acute gastric mucosal injury (Lucas et al 1971; Ritchie 1975). Oxygen-derived free radicals accumulate after ischaemic damage to cellular enzymes that normally eliminate oxygen metabolites (Repine et al 1979). These radicals are implicated in the pathogenesis of ischaemic injury of the gastrointestinal mucosa (Parks et al 1982; Itoh & Guth 1985). Since sulphydryl-containing agents increase the concentration of non-protein sulphydryls in rat gastric mucosa where they bind electrophilic radicals that mediate tissue damage (Lamont et al 1983), they could protect this mucosa against ischaemic injury.

The present study in the rat was therefore undertaken to find whether the sulphydryl-containing agents methyl-methionine (MM), methyl-methionine sulphonium bromide (MMSB), DL-methionine-methyl sulphonium chloride (MMSC) and DL-cysteine protect against reserpine-induced ischaemic injury of the gastric mucosa (Haverback & Bogdanski 1957; Blackman et al 1959; Salim 1985) and to determine the effect of DL-cysteine on acute gastric mucosal injury produced by the noxious agents, aspirin and ethanol.

Materials and methods

Animals. Food was withheld for 24 h from groups of seven (the reserpine study) or ten (the aspirin and ethanol study) Sprague-Dawley rats (200–250 g) of either sex, housed in cages with wide mesh wire bottoms to prevent coprophagy.

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Source and preparation of drugs. Drugs, except ethanol, were supplied by Sigma (St Louis, MO, USA). Reserpine (1 mg mL^{-1}) was prepared by dissolving 80 mg crystalline powder in 0·3 mL glacial acetic acid and the volume made up to 80 mL with double distilled water. A solution of each of methyl-methionine, DL-methionine-methyl sulphonium chloride, methyl-methionine sulphonium bromide and DL-cysteine (5%) was prepared in 10 mL double distilled water. Aspirin powder (2 g) was dissolved in 0·9 mL of 11·7 M HCl, and the volume made up to 50 mL with double distilled water giving a 40 mg mL^{-1} suspension maintained by a magnetic stirrer. Absolute ethanol (BDH Chemicals, Poole, UK) was diluted with double distilled water to form a 40% solution. Saline was given to control animals. Solutions were freshly prepared each day and injected intraperitoneally into the left iliac fossa. Gavage was undertaken under light ether anaesthesia.

Surgery. To ligate the pylorus, animals were anaesthetized with diethyl ether and the pyloric sphincter tied with a 2/0 silk ligature.

Experimental design

Reserpine study. The groups are presented in Table 1. One mL of a 5% solution of MM, MMSB, MMSC, cysteine or 1 mL saline was administered by gavage and 20 min later groups were injected with reserpine (5 mg kg^{-1}) or saline (5 mL kg^{-1}). After 6 h animals were killed by an ether overdose, the stomachs removed, opened along the greater curvature and examined for the presence of mucosal injury. Lesions were scored independently. Each lesion was measured in maximum length and width and the surface area (mm^2) calculated. The total lesion score was obtained for each animal and the mean lesion score calculated for each study group.

Aspirin and ethanol studies were similarly carried out in groups as set out in Table 2. At the end of the experimental period (4 h for the aspirin groups and 1 h for the ethanol ones), animals were killed by overdose of ether, then their stomachs were removed and opened along the greater curvature. Gastric contents of pylorus-ligated groups were collected then H^+ output expressed in μmol calculated by titration to pH 7·0 with 0·1 M NaOH. After washing stomachs with water the integrity of gastric mucosa was determined as described above.

The vehicle solution for reserpine (0·127 M glacial acetic acid) had no macroscopic or microscopic effects

Table 1. Effect of sulphydryl-containing agents on reserpine-induced gastric mucosal injury in rats (n = 7).

Experimental group	% Incidence of animals showing lesions	Lesion area in mm^2 after 6 h (mean ± s.e.m.)
Saline 1 mL + saline 5 mL kg^{-1} i.p.	0	0
Saline 1 mL + reserpine 5 mg kg^{-1} i.p.	100	5·1 ± 1·2
5% Methyl-methionine 1 mL + reserpine 5 mg kg^{-1} i.p.	86	3·1 ± 1
5% DL-Methionine-methyl sulphonium chloride 1 mL + reserpine 5 mg kg^{-1} i.p.	86	4·6 ± 1·9
5% Methylmethionine sulphonium bromide 1 mL + reserpine 5 mg kg^{-1} i.p.	86	2·7 ± 0·6*
5% DL-Cysteine 1 mL + reserpine 5 mg kg^{-1} i.p.	71	1·4 ± 0·8**

*$P < 0·02$, **$P < 0·01$ vs saline.

on the integrity of the rat gastric mucosa and did not influence the H^+ output of the stomach over 6 h.

To minimize day-to-day variation in response to treatment, animals were allocated to the control and all of the treatment groups within the individual experiments on each experimental day.

Statistical analysis. Results are expressed as the mean ± s.e.m. The statistical significance of observed differences between groups was assessed using the Mann-Whitney U test for non-parametric data. Statistical significance was claimed when $P < 0·05$.

Results

Reserpine study (Table 1). All rats in the reserpine alone group developed oval or circular lesions confined to the glandular stomach. Pretreatment with MM or MMSC had no significant effect on the magnitude of this injury, however, both MMSB and cysteine afforded significant protection against it.

Aspirin and ethanol studies (Table 2). No gastric lesions developed in the non-ligated control animals or those given cysteine with saline. Aspirin produced lesions confined to the glandular stomach in 40% of rats. These were black, linear, running parallel to the stomach's long axis, less than 2 mm in width and situated on top of rugal crests. Pretreatment with cysteine protected all stomachs against these lesions. The glandular mucosa in all non-ligated rats given ethanol with saline was deeply congested with red coloured lesions similar in appearance to those produced by aspirin. Pretreatment with cysteine protected all stomachs against congestion and only two had lesions.

In the pylorus-ligated rats, 70% had aspirin-induced lesions similar to those in non-ligated rats, however, there was no constant relationship to rugal crests. Cysteine afforded significant ($P < 0·001$) protection against these lesions which only developed in 30% of stomachs. Ethanol produced lesions in all stomachs similar to those in non-ligated rats and cysteine afforded significant ($P < 0·001$) protection against them where only 30% of stomachs had lesions.

Table 2. Effect of cysteine on aspirin- and ethanol-induced acute gastric mucosal injury in the rat (n = 10).

Experimental group	% Incidence of animals showing lesions	Lesion area in mm^2 (mean ± s.e.m.)	H^+ output in μmol (mean ± s.e.m.)
Experimental period 4 h			
Groups without pylorus-ligation			
Saline 1 mL + saline 1 mL	0	0	—
5% DL-cysteine 1 mL + saline 1 mL	0	0	—
Saline 1 mL + aspirin 200 mg kg^{-1}	40	2·7 ±1	—
5% DL-Cysteine 1 mL + aspirin 200 mg kg^{-1}	0	0	—
Groups with pylorus-ligation			
Saline 1 mL + saline 1 mL	20	0·5 ± 0·2	506·6 ± 86·8
5% DL-Cysteine 1 mL + saline 1 mL	0	0	462 ± 68·3
Saline 1 mL + aspirin 200 mg kg^{-1}	70	12 ± 2·9	311·1 ± 38
5% DL-Cysteine 1 mL + aspirin 200 mg kg^{-1}	30	2·5 ± 0·6*	317·6 ± 34·4
Experimental period 1 h			
Groups without pylorus-ligation			
Saline 1 mL + saline 1 mL	0	0	—
5% DL-Cysteine 1 mL + saline 1 mL	0	0	—
Saline 1 mL + 40% ethanol 1 mL	100	12 ± 2	—
5% DL-Cysteine 1 mL + 40% ethanol 1 mL	20	2 ± 0·3*	—
Groups with pylorus-ligation			
Saline 1 mL + saline 1 mL	0	0	70 ± 4·5
5% DL-Cysteine 1 mL + saline 1 mL	0	0	76 ± 7
Saline 1 mL + 40% ethanol 1mL	100	28 ± 2·5	66 ± 6
5% DL-Cysteine 1 mL + 40% ethanol 1 mL	30	5 ± 3·5*	68 ± 5·3

*$P < 0·001$ vs saline + aspirin or ethanol (ligated) or vs saline + ethanol (non-ligated).

Neither aspirin or ethanol had a significant effect on H^+ output of the pylorus-ligated rats and cysteine protected the stomachs against the lesions without significantly affecting H^+ output.

Discussion

In the rat, reserpine (5 mg kg^{-1}) produces ischaemic injury of the gastric mucosa (Salim 1985). The present study shows that both MMSB and cysteine afford significant protection against this injury and that cysteine is the more effective (Table 1). Since sulphydryl-containing agents increase the concentration of non-protein sulphydryls in the rat gastric mucosa and these sulphydryls bind the electrophilic radicals mediating tissue damage (Szabó et al 1981), it appears that cysteine protects the rat gastric mucosa against the reserpine injury by scavenging oxygen-derived free radicals.

The ability of gastric mucus to form a viscous gel on the epithelial surface and its rapid secretion in response to noxious stimuli suggest a protective function for mucin. Allen & Garner (1980), on the basis of physicochemical considerations, suggested that mucin gel would maintain a pH gradient by preventing secreted HCO_3^- from being rapidly neutralized by luminal H^+. Aspirin and ethanol affect the gastric mucosa topically, aspirin by disrupting the mucosal barrier causing H^+ back diffusion and necrosis (McGreevy & Moody 1977) and alcohol by producing necrosis directly (Robert et al 1979). In the present study cysteine protected rats with or without pylorus ligation against acute gastric mucosal injury produced by aspirin (200 mg kg^{-1}) or 40% ethanol without significantly influencing the H^+ output (Table 2). These observations suggest that cysteine maintains the integrity of the gastric mucosal barrier and sustains its physicochemical properties. Substances containing sulphydryl groups are capable of chemically binding various free radicals and so they may influence the physical and chemical properties of gastric mucus (Szabó et al 1981). On the other hand, Zalewsky & Moody (1979) reported that canine mucus cells on the surface and in gastric pits contain a highly sulphated mucin, while mucin neck cells in gastric glands contain only neutral glycoproteins suggesting that sulphydryl groups may maintain the integrity of surface mucus cells. Szabó et al (1981) suggested that the protective action of mucus is related to the presence of sulphydryl groups because it contains reduced glutathione in high concentrations. Lamont et al (1983) demonstrated in the rat that cysteamine, a sulphydryl-containing agent, conveys cytoprotection by stimulating release of gastric mucin glycoproteins. One or more of these actions may be involved in the mechanism of cysteine protection against gastric mucosal injury by noxious agents. In addition, the fact that sulphydryl-containing agents make an essential contribution to protein synthesis (Turner et al 1977) suggests that cysteine might maintain the integrity of the gastric epithelium besides that of mucus.

This investigation in the rat shows that cysteine on the one hand protects the gastric mucosa against ischaemic injury, most probably by scavenging oxygen-derived free radicals, and, on the other hand, it protects the mucosa against injury by noxious agents by maintaining the physiochemical properties of the gastric mucosal barrier. The observations that the ischaemic injury model used in this study does not require H^+ for its development and that cysteine protects the rat gastric mucosa against aspirin or ethanol injuries without significantly influencing the H^+ output points to cysteine acting as a cytoprotective agent, i.e. protecting by a means other than antisecretory activity. Such action is similar to that of prostaglandins (Robert 1979).

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