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The role of the gastric mucosal sulphydryls in the ulcer-protecting effects of sulphasalazine

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Abstract—The role of gastric mucosal sulphydryls in gastric ulceration, produced by restraint at 4°C (stress) for 2 h, and in the ulcer-protecting effects of sulphasalazine and its constituents (sulphapyridine and 5-aminosalicylic acid), have been studied in rats. Stress significantly raised the mucosal sulphydryl content, but sulphasalazine and sulphapyridine did not influence these changes; only 5-aminosalicylic acid decreased the mucosal sulphydryl concentration. These results indicate that depletion of mucosal sulphydryls does not occur in stress-induced ulceration, in contrast to what has been shown in other experimental ulcer models. The antiulcer effects of sulphasalazine or of any of its constituents may, therefore, not involve the sulphydryl mechanism.

The role of sulphydryls in gastric mucosal protection is unclear. Decreased stomach wall sulphydryls accompany ethanol-induced gastric mucosal damage (Szabo et al 1981); paradoxically, their depletion by diethylmaleate antagonizes ethanol-evoked lesions (Robert et al 1984). Thus, evidence for a direct relationship between gastric sulphydryls and mucosal susceptibility to ethanol-induced lesions is equivocal. We have studied the participation of mucosal sulphydryls in stomach ulcers produced by cold-restraint stress, and we have also looked at their relationship to the antiulcer action of sulphasalazine and its constituents (sulphapyridine and 5-aminosalicylic acid) (Garg et al 1990), which might be expected to be mediated through the sulphydryl mechanism because the drug possesses a sulphur atom in the side chain.

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Materials and methods

Female Sprague-Dawley rats, 150–190 g, were starved for 48 h before use but were allowed to drink a solution of sucrose 8% in NaCl 0·2% w/v which was removed 1 h before the experiment. Sulphasalazine (Sigma), sulphapyridine (Sigma) or 5-aminosalicylic acid (Sigma), freshly dissolved in 0·1 M NaOH, was injected s.c. 30 min before the animals were restrained in individual wire-mesh tubular cages at 4°C (stress) for 2 h; controls received a similar volume (5 mL kg^{-1}) of vehicle. Non-stressed rats were left in their cages (temperature 23 ± 1°C, humidity 65–70%). All animals were killed by a sharp blow on the head after 2 h. Their stomachs were removed, opened, rinsed in ice-cold sodium phosphate buffer (0·2 M, pH 8·0), and quickly placed on an ice-cold surface for scraping off the glandular mucosa. The scrapings were then suspended in 1 mL sodium phosphate buffer, homogenized, made up to 2 mL with buffer, and centrifuged at 5000 rev min^{-1} for 10 min at 4°C. The supernatant sulphydryl content was determined (Sedlak & Lindsay 1968); protein sulphydryl levels were obtained by subtracting non-protein sulphydryl values from that of the total sulphydryls. Light absorbance at 412 nm, against a reagent blank, was measured with a spectrophotometer (Varian, Cary 219). Sul-

Table 1. Effects of sulphasalazine, sulphapyridine or 5-aminosalicylic acid (injected s.c. 30 min beforehand) on gastric mucosal sulphydryls in stressed rats. Values are the means ± s.e.m.

Pretreatment	Dose (mg kg^{-1})	No. of rats	Mucosal sulphydryls (μmol (mg protein)$^{-1}$) Total	Non-protein	Protein
A. No stress (unrestrained at room temperature for 2 h)					
0·1 M NaOH	5 mL	9	169·0 ± 8·1	56·2 ± 3·1	112·8 ± 6·4
Sulphasalazine	200	7	215·2 ± 7·1****	68·7 ± 3·3*	146·5 ± 5·4***
Sulphapyridine	125	6	192·4 ± 15·2	79·0 ± 5·2***	113·4 ± 10·5
5-Aminosalicylic acid	75	7	160·3 ± 6·1	59·4 ± 1·6	100·9 ± 5·5
B. Stress (restrained at 4°C for 2 h)					
0·1 M NaOH	5 mL	9	207·4 ± 16·3†	60·8 ± 5·2	146·6 ± 12·1†
Sulphasalazine	200	7	181·1 ± 7·0†††	57·4 ± 2·3††	123·7 ± 5·0††
Sulphapyridine	125	7	239·3 ± 11·7†	69·0 ± 8·9	170·3 ± 6·9†††
5-Aminosalicylic acid	75	7	157·8 ± 6·0*	55·4 ± 1·6	102·4 ± 5·1**

*$P < 0.05$; **$P < 0.02$; ***$P < 0.01$; ****$P < 0.002$, compared with its own control. †$P < 0.05$; ††$P < 0.02$; †††$P < 0.01$ compared with the corresponding non-stressed group in A.

phydryl concentrations, calculated from freshly prepared standard curves of glutathione (Sigma), were expressed as μmol (mg tissue protein)$^{-1}$. Data were analysed by the two-tailed Student's *t*-test.

Results and discussion

Sulphasalazine (50, 100 or 200 mg kg^{-1}), sulphapyridine (31·25, 62·5 or 125 mg kg^{-1}) or 5-aminosalicylic acid (18·75, 37·5 or 75 mg kg^{-1}) have been shown to lower the severity of stress-induced gastric ulceration (Garg et al 1990); the results were statistically significant with all the three doses of sulphasalazine and the highest doses of its constituents. Hence, dose levels of sulphasalazine 200 mg kg^{-1}, sulphapyridine 125 mg kg^{-1} and 5-aminosalicylic acid 75 mg kg^{-1} were used in the present study.

All the animals subjected to stress showed varying degrees of gastric ulceration; ulcer size in the drug-pretreated animals was lower than that in their vehicle-injected controls. The reductions in ulceration, by the doses selected for this study, were similar to those observed previously (Garg et al 1990). However, in the current investigation, the ulcers were not measured because it has been found that the ratio between the non-protein and protein-bound sulphydryls is altered by handling the mucosal tissue during lesion measurements (unpublished data); thus, the gastric mucosa was scraped off immediately after stomach removal.

Sulphasalazine pretreatment significantly increased the total sulphydryls in non-stressed rats, as reflected in the non-protein and protein fractions (Table 1A). Sulphapyridine also elevated the sulphydryl levels in non-stressed animals, statistical significance being reached with the non-protein fraction. 5-Aminosalicylic acid had no effect.

Stress significantly raised the total and the protein fraction sulphydryl levels (Table 1B). Rats pretreated with sulphasalazine showed a fall in the total sulphydryl level in both fractions (Table 1B); these changes were significant only when compared with the non-stressed controls (Table 1A). However, sulphapyridine pretreatment increased the total and the protein fraction sulphydryls in stressed rats, but the changes only reached statistical significance when compared with the corresponding non-stressed groups (Table 1A). 5-Aminosalicylic acid markedly lowered the total and non-protein fraction sulphydryls, when compared with the stressed vehicle-pretreated controls.

It is concluded that cold-restraint stress does not reduce gastric sulphydryl levels, contrary to what has been observed in ethanol-induced gastric mucosal damage in rats (Szabo et al 1981); instead, it increases the protein sulphydryl content (Table 1). 5-Aminosalicylic acid lowers the total and protein sulphydryl levels; however, it also, like sulphapyridine, has the ability to antagonize (Garg et al 1990) stress-induced gastric mucosal lesion formation. These results indicate that depletion of mucosal sulphydryls does not occur in stress-evoked ulceration and that the antiulcer effects of sulphasalazine or of any of its constituents may not involve the sulphydryl mechanism. Garg et al (1990) have shown that sulphapyridine elevates the levels of adherent gastric mucus. Although it is unclear whether this increase is related directly to the elevated sulphydryls seen in the present study, it should be noted that thiols, like glutathione, favourably influence the physical properties of mucus in which the subunits are joined by disulphide bridges (Allen 1978). This possible antiulcer mechanism merits further investigation.

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