N-ethylmaleimide antagonizes stress-induced gastric ulcers in rats

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Summary. N-ethylmaleimide (NEM) 10 or 25 mg/kg b.wt, given s.c. 20 min beforehand, dose-dependently and significantly antagonizes the severity of gastric glandular ulcers produced by restraint at 4 °C (stress) for 2 h. These findings suggest that reduced activity of endogenous nonprotein sulfhydryl substances in gastric tissue does not worsen stress-induced ulceration in rat stomachs, unlike the deleterious effect its depletion is claimed to have on ethanol-evoked gastric mucosal damage. Thus, decreased SH activity appears not to play a role in the aetiology of mucosal ulcers due to stress.

Key words. N-ethylmaleimide; cold-restraint gastric ulcers; sulfhydryl substances.

The role of glutathione (GSH) in gastric mucosal cytoprotection is still unclear. Ethanol-induced gastric mucosal damage has been shown to be associated with a decrease in the stomach wall endogenous sulfhydryl (SH) pool [1-3]. Synthetic SH administration indeed antagonizes these ethanol-induced gastric lesions [1,4], and SH alkylation by N-ethylmaleimide (NEM) abolishes the lesion-protecting action of alkyl SH drugs [1,5-7]. However, depletion of GSH, a major endogenous non-protein SH, using diethylmaleate (DEM), paradoxically protects rats against ethanol-produced gastric lesions [8,9]; on the other hand, cysteamine, an exogenous SH agent, can prevent mucosal damage by ethanol [9]. Thus, evidence for a direct relationship between gastric GSH levels and mucosal susceptability to ethanol-induced stomach lesions is equivocal [1,10-12]. It has been suggested that the ability of NEM to worsen ethanol-evoked gastric damage may be due to enhancement of mucosal vascular permeability and inhibition of stomach motility [12]. The role of mucosal SH levels, if any, in the aetiology of stomach ulcers produced by cold-restraint stress is not known. The present study, therefore, examines the influence of NEM on the gastric effects of restraint immobilisation and exposure to a low temperature in rats.

Materials and methods

Female Sprague-Dawley rats (160–190 g) were housed in an air-conditioned room (temperature 22 ± 1 °C and 65–70% humidity). They were fed a normal pellet diet (Ralston Purina Co., U.S.A.) and drank tap water. Solid food was withdrawn 48 h before the animals were used; during this period, the rats were kept in starvation cages with raised wide mesh wire floors and given a drinking solution of sucrose 8% w/v in NaCl 0.2% w/v which was removed 1 h before starting experiments [13]. N-ethylmaleimide (NEM) (Sigma) was freshly dissolved in a solution of NaCl 0.9% w/v (saline) and injected s.c. 20 min before the animals were placed individually into close-fitting tubular wire cages and exposed to 4 °C (stress) for 2 h [14]; rats acting as controls were given a similar volume (1 ml/kg) of saline by the same route. Nonstressed animals were left in their starvation cages, for the same period of time, in the room where they were normally housed. All rats were killed by a sharp blow on the head at the end of 2 h and their stomachs removed. Stomachs, opened along their greater curvatures, were gently rinsed under running tap water before examination under an illuminated magnifier (× 3). Mucosal ulcer size was determined by measuring (mm) each lesion along its greatest diameter. In the case of petechiae, five of these were considered to be equivalent to 1 mm. The total of the lesion lengths in each group was divided by its number of animals and expressed as the mean ulcer index [15]. Differences between the means were examined for significance by the unpaired two-tailed Student t-test [16], and those between groups exposed to the same experimental conditions were further analysed (the values are shown in parentheses) by the one-way ANOVA [16].

Results

Nonstressed control rats pretreated s.c. with saline 1 ml/kg showed a low ulcer index; the lesions presented as

Effects of graded doses of N-ethylmaleimide (NEM) given s.c. 20 min before exposure to cold-restraint stress

	Pretreatment	Dose	Number of rats	Glandular mucosal ulcer index (mm)
A)	No stress (unrestrained at room temperature for 2 h)			
	Saline	1 ml/kg	7	0.06 ± 0.04
	NEM	2.5 mg/kg	5	0.12 ± 0.08
	NEM	5 mg/kg	5	0.18 ± 0.11
	NEM	10 mg/kg	6	0.07 ± 0.07
	NEM	25 mg/kg	6	0.23 ± 0.20
B)	Stress (restrained at 4 °C for 2 h)			
	Saline	1 ml/kg	15	5.13 ± 0.52**
	NEM	2.5 mg/kg	8	4.75 ± 1.07*
	NEM	5 mg/kg	8	5.94 ± 1.37*
	NEM	10 mg/kg	14	2.30 ± 0.43 +*
	NEM	25 mg/kg	11	1.31 ± 0.48 +

The values are the means ± SEM. *$p < 0.02$; **$p < 0.001$, compared with its corresponding control in A. +$p < 0.001$, compared with its own saline-pretreated control.

occasional petechiae in the mucosa of the glandular segment of the stomach (table, A).

Restraint at 4 °C for 2 h produced intense haemorrhagic glandular mucosal ulceration (table, B) in the saline-pretreated animals. Pretreatment s.c. with NEM 10 or 25 mg/kg, in the upper dose range employed in this study, significantly decreased stress ulcer formation by 55 or 74%, respectively (ANOVA: $F = 7.6495$, $p < 0.001$).

Discussion

Evidence suggests that the protective effect of sulphasalazine against ethanol-induced gastric mucosal lesions in rats could be mediated, possibly in part, through a mechanism involving gastric SH substances[2], but not through its ability to inhibit prostaglandin 15-hydroxy dehydrogenase[17]. Past investigations have shown that sulphasalazine also antagonizes cold-restraint stress-induced stomach glandular ulcers in rats[18,19]. In a recent pilot study, pretreatment s.c. with NEM 10 mg/kg failed to antagonize the antiulcer action of sulphasalazine given in doses which provided 80% protection (unpublished data); however, NEM itself appeared to exert a protective effect against these stress-induced lesions (unpublished findings). This novel observation prompted the authors to examine further the action of NEM on stress-induced gastric glandular ulcer formation.

The present results indeed indicate that treatment with NEM 20 min before exposure to 2-h stress effectively antagonizes gastric glandular ulcer formation, when given in doses of 10 mg/kg and above. It is interesting to note that others have found that pretreatment with NEM 10 mg/kg s.c. antagonizes the antiulcer action of sulphasalazine in the ethanol-induced ulcer model, but that this dose of NEM is unable to influence the ulcerogenic properties of ethanol[2].

Various pretreatment doses of NEM, given s.c., have been shown by us (10 mg/kg) and by others (10–50 mg/kg) to worsen ethanol-induced gastric mucosal damage and these findings have been the basis for conclusions regarding involvement of SH activity[1,3,5–7]. Thus, assuming that the higher dose range of NEM employed in the present investigation was able to inactivate SH activity significantly, it would appear that reduced SH levels do not aggravate ulcer formation but, on the contrary, are accompanied by an antiulcer action. This is in contrast to the claimed protective property of nonprotein SH activity in ethanol-evoked mucosal damage. The results are, however, in accord with the conflicting reports of others who have found that reduced nonprotein SH levels, through depletion of GSH by DEM, lessens ethanol-induced gastric damage in rats[8,9]. As NEM has been postulated to inhibit stomach motility[12], it is possible that this action could have contributed to its antiulcer property in the present experiments because increased motility is directly related to ulcer formation[15]. It is not possible to speculate further on the mechanism of the inhibitory action of NEM on stress-induced ulcers because the aetiologies of mucosal lesion formation due to stress and to ethanol are largely dissimilar; further studies are needed. Nevertheless, the findings indeed do suggest that decreased SH activity does not play a role in the mechanism of glandular mucosal ulceration produced by stress.

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