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The protective effects of sulphasalazine against ethanol-induced gastric damage in rats

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1 The inhibitory action of sulphasalazine on ethanol-induced gastric damage was studied in rats.

2 Sulphasalazine (62.5 or 125 mg kg^{-1}, s.c.) did not affect basal gastric acid secretion but increased pepsin output.

3 Ethanol (40% v/v, 10 ml kg^{-1}, p.o.) produced severe gastric glandular mucosal damage and lessened the stomach emptying rate of resin pellets, but it increased the levels of prostaglandin E_2 (PGE_2)-like activity in the glandular mucosa.

4 Sulphasalazine markedly prevented ethanol-induced damage and significantly elevated gastric wall mucus levels both in basal conditions and in the presence of ethanol.

5 Sulphasalazine caused a small insignificant increase in mucosal PGE_2 levels in both control and ethanol-treated rats. The drug significantly increased mucosal PGE_2 levels in indomethacin-treated animals, but did not prevent indomethacin-induced mucosal damage.

6 Sulphapyridine but not 5-aminosalicylic acid, constituents of sulphasalazine, showed a similar antilesion action to the parent drug, and prevented gastric wall mucus depletion in ethanol-treated animals.

7 This study elucidates the protective effects of sulphasalazine against ethanol-induced gastric lesions. The antagonistic action appears to be mediated, at least partly, through the preservation of gastric wall mucus by sulphapyridine.

Introduction

Sulphasalazine is able to reduce stress-induced gastric mucosal lesion formation in rats, although the precise mechanism of this action is unclear (Ogle & Cho, 1985; Ogle *et al.*, 1985). Exogenous prostaglandins also protect the gastric mucosa from damage by a variety of ulcerogens including stress (Robert, 1981), and it has been proposed that mucosal protection induced by non-prostanoid compounds may be mediated through the mobilization of endogenous prostaglandins (Robert *et al.*, 1983; Konturek *et al.*, 1987). However, it is unlikely that sulphasalazine produced mucosal protection in such a way since this compound did not change the activity of prostaglandin E_2 (PGE_2) in rat gastric mucosa (Ogle *et al.*, 1985). Therefore, we have investigated further the protective actions of sulphasalazine by determining its effect against two additional ulcerogenic agents, ethanol and indomethacin. Also, insight into the mechanism of action of sulphasalazine has been sought by examining the effect of the compound on gastric motility and the secretion of acid, pepsin and mucus.

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Methods

Female Sprague-Dawley rats (175–190 g) were housed in a temperature (22° ± 1°C)- and humidity (60–70%)-controlled room. They were fed a normal pellet diet (Ralston Purina Company) and given tap water to drink. The animals were deprived of food for 48 h before the experiments, but allowed free access to a drinking solution of 8% sucrose in 0.2% NaCl (w/v) which was removed 1 h before experimentation. Sulphasalazine (Sigma) 62.5 or 125 mg kg^{-1} was injected subcutaneously 30 min before oral administration of either ethanol (BDH) 40% v/v, 10 ml kg^{-1}, or indomethacin (Sigma), 20 mg kg^{-1}; the rats were killed

by a sharp blow on the head 1 or 6 h after treatment with ethanol or indomethacin, respectively.

Determination of gastric acid and pepsin secretion

Sulphasalazine 62.5 or 125 mg kg^{-1} s.c. was injected 30 min before pylorus ligation by the method of Shay *et al.* (1945). The rats were lightly anaesthetized with diethylether before opening up the abdominal cavity and occluding the pyloric sphincter with thread; damage to the mesenteric circulation was avoided. The laparotomy incision was closed by suture and the animals allowed to recover from the anaesthesia. They were killed by a sharp blow on the head 1 h later. Stomachs were removed and their gastric contents collected, via an incision in the forestomach, into individual centrifuge tubes. The mucosae of the stomachs were then washed with distilled water which was also collected. The samples were finally centrifuged at 3000 r.p.m. for 10 min. Aliquots (0.1 ml) of the supernatant were used for determining the acid and pepsin output. Acid content of the gastric solutions was determined by titration with 0.01 N NaOH to pH 7.4 with an autotitration system (Radiometer, Model No. TTT 80), and pepsin output by the method of Berstad (1970).

Measurement of gastric emptying of resin pellets and mucus content in the gastric wall

The rate of gastric emptying of resin pellets was measured by use of the method of Brodie & Kundrats (1965). Following a 48 h fast, 15 amberlite pellets (Rohm & Haas ion exchange resin IRC-50; 1 mm diameter) in distilled water or in 40% v/v ethanol, 10 ml kg^{-1}, were given orally through a stainless steel gastric tube. The animals were killed 2 h later by a sharp blow on the head; their stomachs were removed and the number of pellets remaining in the organs counted. The rate of gastric emptying was expressed as the percentage of pellets expelled.

The amount of mucus on the gastric glandular mucosa was measured by the Alcian Blue method (Corne *et al.*, 1974). After the animals had been killed, the glandular portions of the stomachs were removed, weighed, rinsed in 0.9% w/v NaCl solution (saline) and immediately transferred to buffered Alcian Blue (Sigma) solution. The tissues were stained and excess dye removed by two separate rinses with 0.25 M sucrose (Sigma) solution. Dye complexed with gastric wall mucus in each stomach was extracted individually with 15 ml of 0.5 M magnesium chloride (BDH) and finally shaken with an equal volume of diethylether (BDH). The amount of Alcian Blue in the aqueous layer was measured spectrophotometrically (Varian, Cary 219 spectrophotometer) at a wavelength of 540 nm.

Measurement of prostaglandin E_2-like activity and severity of damage in the gastric glandular mucosa

The amount of PGE_2-like activity in the gastric glandular mucosa was measured by a superfusion technique (Konturek *et al.*, 1981). The mucosae from the corpus and antrum were separated from the muscle using the method developed by Bennett *et al.* (1985). After dissecting the mucosa from the muscle, the tissue was weighed and homogenized in a Krebs/ethanol solution. The homogenate was finally diluted with an appropriate amount of Krebs solution. The contractions of the rat gastric fundus strip, produced by PGE_2-like activity present in the samples, were recorded on a physiograph (E & M Instrument Co., Inc., Houston, Texas) via an isotonic myograph transducer (E & M Instrument Co., Inc., Houston, Texas). Stomach mucosal erosion size was determined by measuring the lengths of the lesions along their greatest diameters; in the case of petechiae, five of these were taken as the equivalent of a 1 mm lesion. The sum of the lesion lengths, from each rat, was divided by the number of animals in the group and expressed as the mean ulcer index (Cho & Ogle, 1978).

Drugs and statistical analysis

All drugs were freshly prepared before use. Sulphasalazine, sulphapyridine (Sigma) and 5-aminosalicylic acid (Sigma) were dissolved in 0.1 N NaOH (BDH). A 40% ethanol solution was prepared by appropriate dilution v/v with distilled water, and indomethacin was suspended in 1% methylcellulose (Sigma) w/v. Statistical significance of differences between means was analysed by Student's two-tailed *t* test. *P* levels of <0.05 were considered significant.

Results

Effects of sulphasalazine on gastric secretion and emptying

Pretreatment with sulphasalazine 62.5 or 125 mg kg^{-1} did not significantly increase the gastric secretory volume or acid output during the 1 h experimental period. However, 125 mg kg^{-1} significantly elevated pepsin secretion (Table 1). The same doses of sulphasalazine did not affect the gastric emptying rate of resin pellets (Table 2).

Effects of sulphasalazine on ethanol-induced gastric damage, gastric emptying and mucosal mucus and prostaglandin E_2 content

Sulphasalazine-pretreated rats given distilled H_2O showed a low ulcer index (Tables 2 and 3). Administra-

Table 1 Effects of sulphasalazine on gastric secretion in pylorus-ligated rats

Pretreatment	*No. of rats*	*Gastric secretion* *Volume* ($ml\,h^{-1}\,100\,g^{-1}$)	*Acidity* ($\mu Eq\,HCl\,h^{-1}\,100\,g^{-1}$)	*Pepsin* ($\mu g\,h^{-1}\,100\,g^{-1}$)
NaOH 0.1N ($5\,ml\,kg^{-1}$)	9	0.37 ± 0.07	39.9 ± 10.9	230 ± 29.4
Sulphasalazine ($62.5\,mg\,kg^{-1}$)	8	0.71 ± 0.17	58.8 ± 14.7	330.6 ± 38.3
Sulphasalazine ($125\,mg\,kg^{-1}$)	9	0.47 ± 0.09	51.0 ± 6.1	343.4 ± 42.1*

Sulphasalazine was given s.c. 30 min before pylorus ligation; the rats were killed 1 h after ligation.
Values are means ± s.e.mean.
*$P < 0.05$ when compared with the corresponding value in the NaOH-pretreated group.

tion of 40% ethanol in a volume of $10\,ml\,kg^{-1}$ produced marked damage (Tables 2, 3 and 5), in the form of linear haemorrhagic erosions located in the glandular mucosa. Sulphasalazine pretreatment reduced lesion severity in a dose-related manner. Significant protection was seen after the higher dose (Tables 2 and 3). Ethanol ingestion also markedly delayed the gastric emptying rate of resin pellets, but this was not significantly affected by sulphasalazine pretreatment (Table 2). Gastric wall mucus was reduced by ethanol but not to a statistically significant level; sulphasalazine dose-dependently increased the mucosal mucus content both in the distilled H_2O- and ethanol-treated animals (Table 3). Ethanol treatment significantly raised stomach PGE_2-like activity; pretreatment with sulphasalazine produced a further increase in PGE_2 levels, but this did not reach statistical significance (Table 4). Sulphasalazine itself tended to increase PGE_2-like activity, as seen in the distilled H_2O-treated rats, but the change was statistically insignificant.

Effects of sulphasalazine on indomethacin-induced gastric damage and mucosal prostaglandin E_2 content

Indomethacin produced severe haemorrhagic lesions (Table 4), which presented as dark haemorrhagic areas, usually about 1 mm in length, and were scattered in the glandular mucosa. This dose of indomethacin decreased PGE_2-like activity in the gastric glan-

Table 2 Effects of sulphasalazine on gastric damage and emptying in ethanol-treated rats

Pretreatment	*No. of rats*	*Gastric glandular ulceration* (mm)	*Rate of gastric emptying* (% of pellets expelled)
A *Rats given distilled H_2O ($10\,ml\,kg^{-1}$) p.o.*			
NaOH 0.1 N ($5\,ml\,kg^{-1}$)	9	0.03 ± 0.13	74.1 ± 9.3
Sulphasalazine ($62.5\,mg\,kg^{-1}$)	10	0.02 ± 0.11	48.0 ± 12.9
Sulphasalazine ($125\,mg\,kg^{-1}$)	10	0.02 ± 0.09	62.0 ± 14.7
B *Rats given ethanol (40%, $10\,ml\,kg^{-1}$) p.o.*			
NaOH 0.1 N ($5\,ml\,kg^{-1}$)	9	44.1 ± 8.9†††	15.6 ± 5.6†††
Sulphasalazine ($62.5\,mg\,kg^{-1}$)	10	26.5 ± 6.4†††	21.3 ± 8.9†
Sulphasalazine ($125\,mg\,kg^{-1}$)	10	13.4 ± 3.7*††	12.0 ± 5.4††

Sulphasalazine was given s.c. 30 min before the oral administration of 15 resin pellets in ethanol; the rats were killed 1 h after ethanol administration.
Values are means ± s.e.mean.
*$P < 0.01$ when compared with the corresponding value in the NaOH-pretreated group.
†$P < 0.05$, ††$P < 0.01$, †††$P < 0.001$ when compared with the corresponding value in (A).

Table 3 Effects of sulphasalazine on gastric damage and mucosal mucus content in ethanol-treated rats

Pretreatment	*No. of rats*	*Gastric glandular ulceration* (mm)	*Mucus content* (μg Alcian Blue g^{-1} wet weight)
A *Rats given distilled H_2O (10 mg kg^{-1}) p.o.*			
NaOH 0.1 N (5 ml kg^{-1})	10	0.02 ± 0.01	166.9 ± 14.9
Sulphasalazine (62.5 mg kg^{-1})	9	0.01 ± 0.01	192.3 ± 14.7
Sulphasalazine (125 mg kg^{-1})	10	0.02 ± 0.02	216.3 ± 13.9*
B *Rats given ethanol (40%, 10 ml kg^{-1}) p.o.*			
NaOH 0.1 N (5 ml kg^{-1})	10	53.0 ± 5.7††	139.0 ± 10.2
Sulphasalazine (62.5 mg kg^{-1})	9	44.7 ± 6.4††	152.1 ± 14.2
Sulphasalazine (125 mg kg^{-1})	10	7.4 ± 2.6**†	178.2 ± 5.1*

Sulphasalazine was given s.c. 30 min before the oral administration of ethanol; the rats were killed 1 h after ethanol administration.
Values are means ± s.e.mean.
*$P<0.05$, **$P<0.001$ when compared with the corresponding value in the NaOH-pretreated group.
†$P<0.02$, ††$P<0.001$ when compared with the corresponding value in (A).

dular mucosa by about 36%, when compared with the methylcellulose-treated controls. Sulphasalazine pretreatment did not affect the lesion-producing effect of indomethacin, but significantly prevented depletion of PGE_2-like activity by the latter in the areas of mucosal damage (Table 4).

Effects of sulphapyridine and 5-aminosalicylic acid on ethanol-induced gastric damage, gastric emptying and mucosal mucus content

Sulphapyridine, a component of sulphasalazine, given in a dose (78.3 mg kg^{-1}) equivalent to the amount contained in sulphasalazine 125 mg kg^{-1}, significantly lessened the ethanol-induced lesions and elevated the mucus content (Table 5), the latter was raised to the same level as that of the parent drug (Table 3). It also significantly delayed the gastric emptying rate of resin pellets (Table 5). 5-Aminosalicylic acid, the other component of sulphasalazine, also given in a dose (48 mg kg^{-1}) equivalent to that released by sulphasalazine 125 mg kg^{-1}, did not noticeably influence the parameters measured; its effects were similar to those of the NaOH-injected controls.

Discussion

Sulphasalazine is used to treat ulcerative colitis (Svartz, 1942; Gould, 1975). Its therapeutic application to other types of disease in the gastrointestinal tract has not been investigated. The drug has been shown to prevent stress-induced gastric ulceration in rats (Ogle & Cho, 1985; Ogle *et al.*, 1985), and the findings suggest that sulphasalazine could be useful for treating gastric lesions.

The protective mechanisms of the drug against stress-induced ulceration are thought to be largely due to inhibition of stomach lipoxygenase activity (Sircar *et al.*, 1983; Ogle *et al.*, 1985) and of leukotriene C_4 (LTC_4) synthetase in particular (Bach *et al.*, 1985). Indeed, in the rat gastric mucosa, some of the effects elicited by exogenous LTC_4 resemble those produced by ethanol (Guth *et al.*, 1984; Szabo *et al.*, 1985; Whittle *et al.*, 1985). Recently, it has been reported that ethanol stimulates the formation of LTC_4 resulting in damage to the rat gastric mucosa (Dreyling *et al.*, 1986; Peskar *et al.*, 1986). These findings suggest that suppression of the lipoxygenase pathway may be an important mechanism for some drugs which exert gastric antiulcerogenic actions. Thus, the protective action of sulphasalazine against ethanol-induced lesions may be closely related to its ability to depress lipoxygenase activity.

Histamine has also been shown to be involved in ethanol-evoked gastric damage (Cho *et al.*, 1983; 1985), because blockade of the histamine H_1-receptors strongly protects against these lesions in rats. Sulphasalazine has been reported to prevent IgE-mediated peritoneal mast cell degranulation (Barrett

Table 4 Effects of sulphasalazine on gastric damage and mucosal prostaglandin (PGE_2)-like activity in ethanol- or indomethacin-treated rats

Pretreatment	*Treatment* (p.o.)	*Gastric glandular ulceration* (mm)	*PGE_2-like substance* (ng mg^{-1} wet glandular mucosa)
A NaOH 0.1 N ($5 ml kg^{-1}$)	Distilled H_2O ($10 ml kg^{-1}$)	0.2 ± 0.1	1.09 ± 0.09
B NaOH 0.1 N ($5 ml kg^{-1}$)	1% methylcellulose ($10 ml kg^{-1}$)	0.1 ± 0.1	1.04 ± 0.06
C Sulphasalazine ($125 mg kg^{-1}$)	Distilled H_2O ($10 ml kg^{-1}$)	0.1 ± 0.1	1.23 ± 0.07
D NaOH 0.1 N ($5 ml kg^{-1}$)	40% ethanol ($10 ml kg^{-1}$)	67.0 ± 11.5*	1.43 ± 0.04*
E NaOH 0.1 N ($5 ml kg^{-1}$)	Indomethacin ($20 mg kg^{-1}$)	13.9 ± 3.6**	0.67 ± 0.10**
F Sulphasalazine ($125 mg kg^{-1}$)	40% ethanol ($10 ml kg^{-1}$)	17.2 ± 7.2†	1.67 ± 0.13
G Sulphasalazine ($125 mg kg^{-1}$)	Indomethacin ($20 mg kg^{-1}$)	10.5 ± 2.2	1.02 ± 0.08††

Sulphasalazine was given s.c. 30 min before the oral administration of ethanol or indomethacin; the rats were killed 1 h after ethanol or 6 h after indomethacin administration.
Values are means ± s.e.mean of 7 rats.
*$P < 0.001$ when compared with the corresponding value in (A).
**$P < 0.01$ when compared with the corresponding value in (B).
†$P < 0.01$ when compared with the corresponding value in (D).
††$P < 0.02$ when compared with the corresponding value in (E).

et al., 1985) and stress-induced gastric glandular mucosal mast cell depletion (Ogle & Cho, 1985). Such an action would reduce mast cell histamine release and, therefore, lessen lesion formation by ethanol; past findings (Cho *et al.*, 1985) have indeed indicated that the involvement of mast cell degranulation cannot be excluded. This hypothesis is confirmed by a recent finding that sodium cromoglycate, a mast cell stabilizer, decreases ethanol-induced damage to the gastric epithelium (Beck & Morris, 1986).

The present study suggests that there is an increase, although not statistically significant, in PGE_2-like activity in the gastric mucosa after sulphasalazine administration (Table 4). However, it remains to be seen whether this effect could have partially contributed to its protective property against ethanol-

Table 5 Effects of sulphapyridine or 5-aminosalicylic acid on gastric damage, gastric emptying and mucosal mucus content in ethanol-treated rats

Pretreatment	*Treatment* (p.o.)	*Gastric glandular ulceration* (mm) ($n = 20$)	*Rate of gastric emptying* (% of pellets expelled) ($n = 15$)	*Mucus content* (μg Alcian Blue g^{-1} wet glandular mucosa) ($n = 10$)
A NaOH 0.1 N ($5 ml kg^{-1}$)	40% ethanol ($10 mg kg^{-1}$)	37.8 ± 6.3	15.1 ± 2.5	120.0 ± 12.7
B Sulphapyridine ($78.3 mg kg^{-1}$)	40% ethanol ($10 ml kg^{-1}$)	16.6 ± 3.6**	8.0 ± 1.7*	172.0 ± 9.3**
C 5-Aminosalicylic acid ($48 mg kg^{-1}$)	40% ethanol ($10 ml kg^{-1}$)	26.3 ± 5.7	19.7 ± 5.6	127.3 ± 12.0

Sulphapyridine or 5-aminosalicylic acid was given s.c. 30 min before the oral administration of ethanol; the rats were killed 1 h after ethanol administration.
Values are means ± s.e.mean.
*$P < 0.05$, **$P < 0.01$ when compared with the corresponding value in (A).

evoked lesion formation. Any increase in PGE_2 levels by sulphasalazine occurring before ethanol challenge could exert some antiulcerogenic effect, as Robert *et al.* (1979) have shown that pretreatment with this prostaglandin has this action against noxious agents. It is interesting to note that sulphasalazine pretreatment did not significantly prevent indomethacin-induced mucosal damage, although it raised the gastric PGE_2-like content to near-normal levels, possibly by inhibiting breakdown of the autacoid by prostaglandin 15-hydroxydehydrogenase (Hoult & Moore, 1980). These findings imply that indomethacin-induced ulceration may not be entirely the result of PGE_2 depletion in the stomach mucosa. Thus, it can at present be concluded that the aetiologies of ethanol- and indomethacin-induced gastric damage are different (Cho *et al.*, 1985), and that sulphasalazine is only able to protect against ethanol-induced lesions.

The amounts of sulphasalazine used in this study did not affect gastric acid secretion but the higher dose of 125 mg kg^{-1} significantly increased pepsin output. The mechanism for the elevation of pepsin secretion is unclear, but it is unlikely that increased pepsin production accounted for the lesion-preventing effect. Sulphasalazine also increased the mucosal mucus in ethanol-treated rats. It is indeed widely known that increased mucus in the gastric mucosa significantly protects it from damage (Rainsford & Whitehouse, 1976) and gastric acid back-diffusion (Pfeiffer, 1981). Thus, this effect could be an important factor in the ability of the drug to antagonize lesion formation.

Ethanol administration markedly delayed gastric emptying of resin pellets, indicating that the contractions of the organ were reduced, since the emptying rate of solids from the stomach is controlled by its contraction frequency (Koo *et al.*, 1985; 1986). As sulphasalazine pretreatment did not affect ethanol-induced slowing of gastric movement or the basal gastric emptying rate, this excludes the possibility that the antagonistic action of sulphasalazine against gastric damage was due to acceleration of ethanol-emptying from the stomach, which would consequently decrease the duration of mucosal exposure to ethanol.

Sulphasalazine is broken down to equimolar amounts of sulphapyridine and 5-aminosalicylic acid in the body (Das & Dubin, 1976). The present study shows that only sulphapyridine, given in a dose equivalent to that present in sulphasalazine 125 mg kg^{-1}, significantly prevented ethanol-induced lesions and permitted more mucus to be retained in the gastric wall. These findings were similar to those seen with sulphasalazine itself. Therefore, it is possible that the protective action of sulphasalazine may be partially or even wholly mediated through sulphapyridine rather than by 5-aminosalicylic acid. Further studies are needed to support this hypothesis in view of *in vitro* findings which indicate that 5-aminosalicylic acid is able to inhibit lipoxygenase (Sircar *et al.*, 1983) and prostaglandin-15-hydroxydehydrogenase (Hoult & Moore, 1980) activity.

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