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The role of Gastric Mucosal Sulphydryls in the Ulcer-protecting Effects of Cisapride

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Abstract

The present study was designed to examine the role of endogenous sulphydryls (SHs) in the gastroprotection induced by cisapride (CIS) (10, 25 and 50 mg kg^{-1} i.p.), a potent benzamide stimulating gastrointestinal motility in mucosal injury induced by 50% v/v ethanol. Results were compared with those of 5-hydroxytryptamine (5-HT) (10 mg kg^{-1}).

Ethanol mucosal damage was significantly reduced by treatment with CIS and 5-HT. On the contrary, administration of *n*-ethylmaleimide (NEM) (10 mg kg^{-1}) an SH alkylator, markedly worsened lesion formation and counteracted the protective effect of CIS. Rats pretreated with CIS significantly increased the total sulphydryls as reflected in the non-protein and protein fractions however, 5-HT treatment showed a fall in the non-protein level.

The present results suggest that 5-HT-ergic dependent mechanisms have no relation to the gastroprotection afforded by CIS in this experimental model. It is possible that mucosal SHs could be involved.

It has been reported that properly coordinated motility might be of crucial importance in promoting a damaging agent's clearance from the stomach thereby reducing its duration of contact with the gastric mucosa (Kerrigan et al 1993) and could, therefore, be involved in the mechanism of gastric cytoprotection (Glavin & Szabo 1992). Some prokinetic benzamides like metoclopramide have been effective in experimental models of gastric ulceration (Gupta et al 1989). Preliminary studies have shown that cisapride, a potent benzamide stimulating gastrointestinal motility, was effective in healing ulcers and could prevent ulcer relapse after healing (Simon et al 1990; Kerrigan et al 1993; Testoni et al 1993; Wiseman & Faulds 1994). In addition, previous studies have shown that this drug does not affect gastric acid and pepsin secretion either in animals (Shuurkes & Van Neuten 1988) or man (Muller-Lissner & Fraass 1986). These findings prompted us to study the cytoprotective properties of this drug in mucosal injury produced by 50% v/v ethanol.

Decreased stomach wall sulphydryls (SHs) accompany ethanol-induced damage (Szabo et al 1987; Loguercio et al 1991). Exogenous administration of sulphydryls containing agents has been shown to prevent different forms of injury to the gastric mucosa in experimental animals in-vivo (Gutierrez-Cabano 1989; Garg et al 1991) as well as to gastric mucosal cells in-vitro (Romano et al 1991), thus in the second phase of our studies we investigated the possible role of sulphydryls in the cytoprotection observed after administration of cisapride. In addition, although the mechanisms of action of cisapride are incompletely characterized, a number of studies suggest that it acts via agonism at 5-HT_4 or antagonism at 5-HT_3 receptors (Linnik et al 1991). 5-HT has been shown to be one of the pathological mediators for the mucosal damaging action of ethanol in rat stomach (Wong et al 1990). Ethanol administration releases 5-HT from the enterochromaffin cells of the digestive tract, also 5-HT has been shown to worsen ethanol-induced damage (Wong et al 1990). Thus we examined the possible involvement of 5-HT-ergic dependent mechanisms in the cytoprotection mediated by cisapride.

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Materials and Methods

Animals

Male Wistar rats, 180–200 g, were placed in single cages with wire-net floors in a controlled room (temperature 22–24° C, humidity 70–75%) and were fed a normal laboratory diet. The animals were deprived of food for 24 h before experimentation but allowed free access to tap water throughout. All studies were carried out using 6–8 rats per group.

Drug preparation and treatment

Cisapride (CIS, Janssen Farmacéutica S.A.) (5, 10, 25 and 50 mg kg^{-1}) and 5-HT (Sigma S.A.) (10 mg kg^{-1}) were dissolved in saline (0·9% NaCl), prepared freshly each time and administered intraperitoneally (i.p.). Control rats received saline in a comparable volume (0·2 mL/100 g animal) by the same route. *N*-ethylmaleimide (NEM, Sigma S.A.) (10 mg kg^{-1}) in SH alkylator was given subcutaneously (s.c.)

Protection against 50% (v/v) ethanol: involvement of endogenous SHs

Ulceration was induced as described by Wong et al (1990) by instillation of 1 mL/100 g animal of 50% (v/v) ethanol in distilled water. CIS (10, 25 and 50 mg kg^{-1}) and 5-HT (10 mg kg^{-1}) were injected intraperitoneally 4 h before the peroral administration of the ethanol. One hour after the

experimental period, the animals were sacrificed using an overdose of anaesthetic and their stomachs removed and opened along the greater curvature. Any lesions were examined macroscopically. The number of erosions per stomach was assessed for severity according to our scoring system (Alarcón de la Lastra et al 1993): 0, no lesions; 1, one or more haemorrhagic ulcers length < 5 mm and thin; 2, one haemorrhagic ulcer length > 5 mm and thin; 3, more than one ulcer grade 2; 4, one ulcer length > 5 mm and diameter < 2 mm; 5, two or three ulcers of grade 4; 6, from four to five ulcers of grade 4; 7, more than six ulcers of grade 4; 8, complete lesion of the mucosa. Mean scores for each group were calculated and expressed as ulcer index (UI).

In additional experiments the importance of endogenous SHs in the beneficial effect of cisapride was assessed. Fasted rats thus received a dose of 10 mg kg^{-1} of NEM subcutaneously followed 30 min later by CIS (10 mg kg^{-1}) and after a further 240 min 50% (v/v) ethanol. Rats were autopsied 1 h after ethanol and their stomachs were processed as indicated above.

Determination of mucosal sulphydryls

The amount of mucosal sulphydryls was measured in the gastric mucosa of rats after administration of NEM (10 mg kg^{-1} s.c.), CIS (10, 25 and 50 mg kg^{-1} i.p.) and 5-HT (10 mg kg^{-1} i.p.) according to the method described by Garg et al (1991). The animals were given the above agents and they were sacrificed 4 h later. In other groups of rats 1 mL/100 g animal of 50% (v/v) ethanol was given by gavage 4 h after administration of the drugs. One hour after the experimental period, the animals were sacrificed, their stomachs 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 (Perkin-Elmer, 1310). Sulphydryl concentrations, calculated from freshly prepared standard curves of glutathione (GSH) (Sigma S.A.) were expressed as μmol (g tissue)$^{-1}$.

Statistical analysis

Values are given as arithmetic means ± s.e.m. The significance of observed differences between means was evaluated by the Mann-Whitney U-test and Student's *t*-test for unpaired data.

Results

Oral administration of 50% v/v ethanol induced multiple elongated reddish bands of lesions in the corpus mucosa along the long axis of the stomach. The lesion index was 4·0 ± 0·2 (Table 1). 5-HT injection (10 mg kg^{-1}) at 4 h before ethanol tended to lower the severity of lesions to a significant level ($P < 0·05$).

Table 1. Effects of pretreatment with cisapride (CIS: 10, 25 and 50 mg kg^{-1} i.p.), 5-HT (10 mg kg^{-1} i.p.) and *N*-ethylmaleimide (NEM; 10 mg kg^{-1} s.c.) on susceptibility to 50% ethanol (v/v)-induced gastric damage. Control 1 (vehicle), control 2 (vehicle + 1 mL/100 g animal of 50% (v/v) ethanol in distilled water.

Treatment (mg kg^{-1})	Ulcer index (mean ± s.e.m.)
Control 1	0
Control 2	4·0 ± 0·2
5-HT 10	2·5 ± 0·2*
CIS 10	2·4 ± 0·3*
CIS 25	2·3 ± 0·2*
CIS 50	1·8 ± 0·2**
Control 2 + NEM 10	7·8 ± 0·2**
CIS 10 + NEM 10	6·9 ± 0·5**†

Mann-Whitney *U*-test *$P < 0·05$, **$P < 0·01$ compared with control 2. †$P < 0·01$ compared with CIS 10.

Ethanol mucosal damage was significantly reduced in both the size and the severity by pretreatment of the animals with CIS (10, 25 and 50 mg kg^{-1}) 4 h before the start of ethanol administration ($P < 0·05$, $P < 0·01$), although with the lower (10 and 25 mg kg^{-1}) doses no dose-dependency was observed (Table 1). On the contrary administration of NEM (10 mg kg^{-1}) in the SH alkylating agent significantly worsened the lesions in response to 50% v/v and the lesion index was 7·8 ± 0·2, which was more than double the severity of mucosal lesions after ethanol administration (Table 1). In addition, this dose of NEM counteracted the protective effect of CIS (10 mg kg^{-1}) and restored the haemorrhagic nature of these lesions. In normal rats the levels of non-protein and protein SH in the gastric mucosa were respectively 3·451 ± 0·09 μmol (g tissue)$^{-1}$ and 12·663± 0·22 μmol (g tissue)$^{-1}$. Administration of NEM, 5-HT or CIS did not significantly affect the amount of the mucosal SHs in relation to the control group (control 1) (Table 2). In agreement with the previous observations by other authors (Takeuchi et al 1988), ethanol (p.o.) caused a marked reduction in the mucosal levels of SHs in the stomach. The non-protein fraction was decreased from 3·451± 0·09 μmol (g tissue)$^{-1}$ to 1·570 ± 0·09 μmol (g tissue)$^{-1}$ (Table 3). 5-HT pretreatment also resulted in a marked fall in the non-protein level ($P < 0·05$) although the protein fraction was significantly enhanced ($P < 0·01$). In

Table 2. Effects of cisapride (CIS: 10, 25 and 50 mg kg^{-1} i.p.), 5-HT (10 mg kg^{-1} i.p.) and NEM (10 mg kg^{-1} s.c.) on changes in mucosal sulphydryls in the rat stomach. Control 1 (vehicle).

Treatment (mg kg^{-1})	Mucosal sulphydryls (μmol(g tissue)$^{-1}$)		
	Non-protein	Protein	Total
Control 1	3·451 ± 0·09	12·663 ± 0·22	16·114 ± 0·31
NEM 10	2·751 ± 0·04	12·238 ± 0·54	14·989 ± 0·58
5-HT 10	2·934 ± 0·04	11·573 ± 0·22	14·507 ± 0·22
CIS 10	2·772 ± 0·04	11·735 ± 0·36	14·507 ± 0·45
25	2·988 ± 0·04	11·885 ± 0·22	14·843 ± 0·13
50	3·280 ± 0·09	11·997 ± 0·18	15·277 ± 0·31

Mean ± s.e.m. (n = 6).

Table 3. Effects of cisapride (CIS: 10, 25 and 50 mg kg^{-1} i.p.) and 5-HT (10 mg kg^{-1} i.p.) on changes in mucosal sulphydryls caused by 50% (v/v) ethanol in the rat stomach. Control 2 (vehicle + 1 mL/100 g animal of 50% (v/v) ethanol in distilled water).

Treatment (mg kg^{-1})	Mucosal sulphydryls (μmol (g tissue)$^{-1}$)		
	Non-protein	Protein	Total
Control 2	1·570 ± 0·09	3·391 ± 0·27	5·161 ± 0·27
NEM 10 + ethanol	1·417 ± 0·10	5·040 ± 0·31	6·462 ± 0·45
5-HT 10 + ethanol	1·003 ± 0·13 *(a)	6·421 ± 0·58 **(a)	7·429 ± 0·72 *(a)
CIS 10 + ethanol	2·961 ± 0·13 ***(a, b, c)	8·172 ± 0·13 ***(a, c) *(b)	11·137 ± 0·20 **(a, b, c)
CIS 25 + ethanol	3·117 ± 0·18 ***(a, b, c)	8·379 ± 0·99 ***(a, c) *(b)	11·556 ± 0·40 ***(a, b, c)
CIS 50 + ethanol	3·312 ± 0·20 ***(a, b, c)	8·428 ± 0·99 ***(a, c) *(b)	11·740 ± 0·90 ***(a, b, c)

Mean ± s.e.m. (n = 6). Student's t-test *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, compared with control 2 (a), NEM 10 + ethanol (b), 5-HT 10 + ethanol (c).

contrast, administration of 10, 25 and 50 mg kg^{-1} of CIS could restore completely the reduced mucosal SH content induced by ethanol, as reflected in the non-protein ($P < 0.001$) and protein ($P < 0.001$) fractions (Table 3).

Discussion

Our experiments have shown that CIS (10, 25 or 50 mg kg^{-1}) after 4 h of treatment lowers the severity of alcohol-induced gastric ulceration; the results were statistically significant with the three tested doses. At the same time it was seen that NEM, an SH alkylator, reversed CIS protection; these results suggested that the beneficial effects of CIS could be partially SH-mediated. However this study confirmed that CIS restored the reduced mucosal SHs induced by ethanol.

Ethanol-induced damage to the gastric mucosa is associated with a significant decrease in mucosal SHs especially glutathione gastric tissue levels (GSH) in experimental animals (Szabo et al 1987) and man (Loguercio et al 1991). This reduction may have been contributed to by oxidation of glutathione because of the ethanol-induced generation of toxic metabolites (Shaw et al 1990) or binding of glutathione to acetaldehyde generated through the oxidation of ethanol by the gastric alcohol dehydrogenase activity (Gutierrez-Cabano 1989). In addition, depletion of endogenous SHs is associated with increased susceptibility of gastric mucous cells to oxygen metabolite and acid-induced cell damage in-vitro (Romano et al 1991).

SH-containing compounds and also agents that modify SH groups, oxidize SH groups or bind SH groups prevent the acute haemorrhagic erosions caused by ethanol, non-steroidal anti-inflammatory drugs or stress in animal models (Szabo et al 1987). The common mechanism of gastroprotection by SH-related compounds seems to be similar to that of endogenous prostaglandins, that is vasoprotection because they decrease the ethanol chemically induced vascular damage and maintenance of the gastric mucosal blood flow that allows the energy-dependent rapid substitution to cover initial epithelial surface damage (Szabo & Vattay 1990). In addition, SH compounds scavenge free radicals and consequently prevent membrane damage brought about by lipid peroxidation. Furthermore, biochemical data indicate that protein SH or cysteine levels, i.e. sulphoproteins, modulate also the beneficial effects of SH drugs (Szabo et al 1987).

On the other hand, 5-HT, although present, has been localized in the enterochromaffin cells of the digestive tract and in the enteric nerves, large numbers of which are present in the gastric mucosa of a variety of animal species (Dhasmana et al 1993). The amine has been shown to inhibit gastric secretion (Cho et al 1986) and possess ulcerogenic properties (Wong et al 1990). Cho et al (1992) demonstrated a dual action of 5-HT on ethanol-induced damage. It was demonstrated that 5-HT given 30 min before ethanol administration markedly worsened lesion formation, however this lesion-aggravating action of ethanol was reversed when the amine was given from 120 to 450 min. Our findings showed similar results because a significant protective effect at 240 min was shown, however, they were not accompanied by an increase of GSH levels. The underlying mechanism for this is unknown, perhaps the defense mechanism of the stomach is triggered by 5-HT metabolites e.g. melatonin, which possess the opposite actions to those of 5-HT (Cho et al 1989, 1992). In addition the stimulatory or the depressive action of 5-HT on gastric mucosal blood flow could explain its lesion-protecting or lesion-worsening property respectively.

Since CIS caused a rise in GSH levels and 5-HT caused a fall, it is unlikely that their protective mechanisms are connected.

Accordingly the present results could suggest that 5-HT-ergic-dependent mechanisms are not involved in the gastroprotection afforded by CIS. It is also suggested that mucosal SHs could contribute to the functional mechanisms of protection by CIS in this experimental model.

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