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Protection by Almagate of Ethanol-induced Gastric Mucosal Damage in Rats

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Abstract

The study was designed to analyse the protective effects of almagate on a model of gastric injury, ethanol-induced mucosal damage, in which acid plays little, if any, role.

Pretreatment with almagate dose-dependently reduced the level of gastric damage induced by oral administration of 1 mL 100% ethanol. Administration of 12 μmol kg^{-1} almagate 30 min before ethanol significantly reduced the area of mucosal damage by 65 ± 10%, and the maximum level of inhibition (74 ± 11%) was obtained with 150 μmol kg^{-1} almagate. Administration of higher doses of almagate (200–250 μmol kg^{-1}) did not result in any further increase in the level of protection against ethanol-induced gastric damage. Administration of 1 mL 100% ethanol induces substantial damage to the gastric mucosa, with nearly 40% of the length of the section evaluated exhibiting deep necrotic and haemorrhagic damage. Pretreatment with almagate caused a significant diminution in all parameters of histological damage, whereas damage to the epithelial cell layer was only significantly reduced by pretreatment with the highest doses evaluated (25, 50 and 150 μmol kg^{-1}).

Administration of aluminium hydroxide did not modify ethanol-induced mucosal damage, even at doses containing concentrations of aluminium higher than those present in gastroprotective doses of almagate. Pretreatment with sucralfate, another aluminium containing compound, at doses of 250 μmol kg^{-1} protected the mucosa, although lower doses did not.

The present study has shown that almagate prevents ethanol-induced gastric mucosal damage. This protective effect seems independent of any antacid activity, related to its content in magnesium, and mediated by an increase in gastroprotective prostaglandins in the mucosa of the stomach.

Almagate (hydrated aluminium-magnesium hydroxycarbonate, $Al_2Mg_6[OH]_{14}[CO_3]_2$-$4H_2O$, Almax) is a crystalline aluminium magnesium hydroxide derivative which has shown higher acid neutralizing capacity and greater velocity of neutralization than most amorphous gels and co-gels of aluminium and magnesium hydroxides or hydroxycarbonates (Beneyto et al 1984; Prieto et al 1984). Aluminium-containing antacids are known to protect the gastric mucosa against damage by mechanisms not related to neutralization of acid (Szabo et al 1981; Fitzpatrick 1991). Almagate has been shown to protect against gastric damage induced by the combination of indomethacin and bile salts (Llupiá et al 1984), whereas pretreatment with aluminium hydroxide gel did not influence such damage, thus suggesting that almagate could protect the gastric mucosa by mechanisms independent of its aluminium content. The purpose of the present study was to analyse the protective effects of almagate on a model of gastric injury in which acid plays little, if any, role, such as ethanol-induced mucosal damage. In these experiments, we have compared the effects of almagate with those induced by other aluminium-containing compounds. Furthermore, experiments were also designed to evaluate the mechanisms involved in the protective effects of almagate.

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Materials and Methods

Methods

Wistar rats of either sex (200–250 g) were deprived of food, but not water, for 18–20 h before the experiments. One millilitre of 100% ethanol or vehicle (0·9% NaCl, saline) was administered orally by gavage, and the rats killed by cervical dislocation 5 min later. The stomachs were opened, pinned to a wax block immersed in neutral buffered formalin and photographed on colour transparency film. The extent of damage was calculated via computerized planimetry, and expressed as the percent of the total gastric mucosa showing macroscopically visible damage. In one group of experiments, almagate (1–150 μmol kg^{-1}), aluminium hydroxide (4–250 μmol kg^{-1}), magnesium hydroxide (4–250 μmol kg^{-1}) or sucralfate (25–250 μmol kg^{-1}) was suspended in saline (1 mL) and administered orally 30 min before the administration of 100% ethanol (1 mL, p.o.). Control groups were treated with saline (1 mL, p.o.). In a second group of experiments, rats were treated, 20 min before the administration of almagate, with indomethacin (5 mg kg^{-1}, s.c.) or vehicle (50% saline, 50% ethanol; 1 mL kg^{-1}), and intragastric 100% ethanol administered 30 min later.

Histological assessment of mucosal damage

Two 1·5 × 0·5 cm segments of the corpus stomach were excised from standardized areas of the mucosa, with tissue from both the dorsal and ventral aspects of the midcorpus

region obtained 0·5 cm below the forestomach-limiting ridge. Following processing by routine techniques and embedding in paraffin, the sections (4 μm) were stained with haematoxylin and eosin and examined under a light microscope, according to criteria described previously (Esplugues & Whittle 1990). In brief, the 1-cm length of each histological section was assessed for epithelial cell damage (type I damage); glandular disruption, vasoconges-tion, or oedema in the mid to upper mucosa (type II damage); and haemorrhagic damage and necrosis in the mid to lower mucosa (type III damage). All determinations were performed in a randomized manner with the histo-logical sections coded to eliminate observer bias. The length of each section exhibiting each type of damage was expressed as a percent of the total section length, and the mean value from the two sections of each corpus mucosa was calculated.

Statistical analysis

All data are expressed as mean ± s.e.m. Comparison between groups of parametric data were made by Student's *t*-test for unpaired data. Comparisons between groups of nonparametric data (histological evaluation) were made by the Mann-Whitney U-test. *P* values of less than 0·05 were taken as significant.

Results and Discussion

Pretreatment with almagate dose-dependently reduces the level of gastric damage induced by oral administration of 1 mL 100% ethanol. Administration of 12 μmol kg^{-1} almagate 30 min before ethanol significantly ($P < 0·01$) reduces the area of mucosal damage by 65 ± 10%, and the maximum level of inhibition (74 ± 11%, $P < 0·01$) was obtained with 150 μmol kg^{-1} almagate (Table 1). Adminis-tration of higher doses of almagate (200–250 μmol kg^{-1}) did not result in any further increase in the level of protec-tion against ethanol-induced gastric damage (results not shown). Detailed histological evaluation of the protective effects of almagate is shown in Table 2. Administration of 1 mL 100% ethanol induces substantial damage to the gastric mucosa with nearly 40% of the length of the section evaluated exhibiting deep necrotic and haemorrhagic damage (type III). Control animals receiving only saline did not show significant levels of mucosal damage. Pretreat-ment with almagate caused a significant diminution in all parameters of histological damage, with type II and III being the most reduced, whereas damage to the epithelial cell layer (type I) was only significantly reduced by pretreat-ment with the highest doses evaluated (25, 50 and 150 μmol kg^{-1}).

As shown in Table 3, administration of aluminium hydroxide did not modify ethanol-induced mucosal damage, even at doses containing concentrations of alumin-ium higher than those present in gastroprotective doses of almagate. Pretreatment with sucralfate, another aluminium containing compound, at doses of 250 μmol kg^{-1} protected the mucosa, although lower doses did not. Although differ-ences in the molecular structure of the compounds have to be taken into account, the amount of aluminium in the protective dose of sucralfate is over 150 times higher than that present in equivalent gastroprotective doses of almagate, thus suggesting that the aluminium content of almagate is not the only factor responsible for the prevention of ethanol-induced mucosal injury. Differences in the neutralizing capacity of the compounds seem of little relevance for the understanding of their protective effects,

Table 1. Protective effects of pretreatment with almagate (p.o.) on gastric mucosal damage induced by the intragastric administration of 1 mL 100% ethanol.

	Concn (μmol kg^{-1})	Damaged area (%)	n
Ethanol 100%	0	43 ± 4	19
+ almagate	1	37 ± 9	9
	4	30 ± 8	5
	12	20 ± 4**	5
	25	18 ± 4***	10
	50	18 ± 3**	4
	150	16 ± 5**	5

Results, expressed as the % of the total mucosal area that exhibited macroscopically-assessed damage 5 min after challenge, are shown as mean ± s.e.m. of n experiments. Significant differences from the control (ethanol only) group are given as $^{**}P < 0·01$ and $^{***}P < 0·001$.

Table 2. Histological evaluation of the rat gastric corpus mucosa following a 5 min intragastric challenge with 100% ethanol (1 mL) and the effects of pretreatment with almagate. Data are shown as the length of section exhibiting damage of varying degrees, type I (epithelial cell damage), type II (glandular disruption in the mid to upper mucosa), and type III (deeper haemorrhage and necrosis), expressed as the % of the total section length.

	Concn (μmol kg^{-1})	Damage type (% of total length)			
		I	II	III	n
Ethanol 100%	0	91 ± 6	54 ± 7	39 ± 12	7
+ almagate	4	82 ± 6	56 ± 10	20 ± 5	5
	12	89 ± 4	45 ± 7	16 ± 4	5
	25	68 ± 6*	18 ± 4***	14 ± 6*	6
	50	63 ± 7**	25 ± 7*	15 ± 3**	4
	150	55 ± 9*	16 ± 8**	12 ± 3***	4

Results are given as mean ± s.e.m. of n values, where the statistically significant protection by almagate pretreatment in the extent of damage induced by ethanol 100% is shown as $^{*}P < 0·05$, $^{**}P < 0·01$ and $^{***}P < 0·001$.

Table 3. Effects of pretreatment with various doses (p.o.) of aluminium hydroxide, magnesium hydroxide and sucralfate on gastric mucosal damage induced by the intragastric administration of 1 mL ethanol 100%.

	Concn (μmol kg^{-1})	Damaged area (%)	n
Ethanol 100%	0	38 ± 3	38
+ aluminium hydroxide	4	30 ± 7	5
	12	27 ± 5	5
	25	36 ± 5	7
	250	34 ± 9	6
+ magnesium hydroxide	4	36 ± 12	5
	12	24 ± 4	6
	25	33 ± 7	5
	250	18 ± 4*	6
+ sucralfate	25	44 ± 9	5
	250	7 ± 2**	5

Results, expressed as the % of the total mucosal area that exhibited macroscopically-assessed damage, are shown as mean ± s.e.m. of n experiments. Significant differences from the control (ethanol only) group are given as $^{*}P < 0·05$ and $^{**}P < 0·01$.

Table 4. Pretreatment with indomethacin (5 mg kg^{-1}, s.c.) prevents the protective effects of almagate (25 μmol kg^{-1}) on gastric mucosal damage induced by the intragastric administration of 1 mL ethanol 100%.

	Damaged area (%)	n
Ethanol 100%	42 ± 6	14
+ almagate	20 ± 5*	11
+ indomethacin	41 ± 7	15
+ indomethacin + almagate	43 ± 8	11

Results, expressed as the % of the total mucosal area that exhibited macroscopically-assessed damage, are shown as mean ± s.e.m. of n experiments. Significant differences from the control (ethanol only) group are given as $*P < 0.05$.

since damage induced by ethanol is poorly related to the presence of low levels of endogenous acid (Dupuy & Szabo 1986) and, furthermore, sucralfate has limited antacid properties (McCarthy 1991) but significantly reduced ethanol-induced gastric damage. Pretreatment with magnesium hydroxide (Table 3) does protect at doses that contain levels of magnesium similar to those present in gastroprotective doses of almagate, thus suggesting that it is the magnesium and not the aluminium component that is responsible for the protection elicited by almagate. Divalent cations have been shown to prevent mucosal injury, although the mechanisms responsible are still controversial (Szabo et al 1981; Esplugues et al 1985; Dupuy & Szabo 1986). As shown in Table 4, pretreatment with indomethacin prevents the defensive effects of almagate, thus suggesting that the protective effects of the compound are related to an increase in endogenous gastroprotective prostaglandins (Whittle & Esplugues 1989; Walt 1990; Whittle 1993).

In conclusion, the present study has shown that almagate prevents ethanol-induced gastric mucosal damage. This protective effect seems independent of any antacid activity, related to its content in magnesium, and mediated by an increase in gastroprotective prostaglandins in the mucosa of the stomach.

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References

Beneyto, J. E., Fábregas, J. L., Moragues, J., Spickett, R. G. W. (1984) Evaluation of a new antacid, almagate. Arzneim. Forsch. 34: 1350–1354

Dupuy, D., Szabo, S. (1986) Protection by metals against ethanol-induced gastric mucosal injury in the rat. Comparative biochemical and pharmacological studies implicate protein sulfhydryls. Gastroenterology 91: 966–974

Esplugues, J. V., Whittle, B. J. R. (1990) Morphine potentiation of ethanol-induced gastric mucosal damage in the rat: role of local sensory afferent neurones. Gastroenterology 98: 82–89

Esplugues, J. V., Bulbena, O., Escolar, G., Martí-Bonmatí, E., Esplugues, J. (1985) Effects of zinc acexamate on gastric mucosal resistance factors. Eur. J. Pharmacol. 109: 145–151

Fitzpatrick, L. R. (1991) Protection by acidified maalox against indomethacin-induced gastric antral ulceration in hamsters. Digestion 50: 1–6

Llupiá, J., Lumachi, B., Beckett, P. R., Roberts, D. J. (1984) Protective action of almagate against bile-facilitated gastric ulceration in the pylorous-ligated (Shay) rat. Arzneim. Forsch. 34: 1373–1375

McCarthy, D. M. (1991) Drug therapy—sucralfate. N. Engl. J. Med. 325: 1017–1025

Prieto, R., Martinez-Tobed, A., Fábregas, J. L., Beneyto, J. E. (1984) In vitro comparison of the antacid potencies of almagate in tablets and suspension with those of other commercially available antacid preparations. Arzneim. Forsch. 34: 1360–1364

Szabo, S., Trier, J., Frankel, P. W. (1981) Sulfhydryl compounds may mediate gastric cytoprotection. Science 214: 200–202

Walt, R. P. (1990) Prostaglandins and peptic ulcer therapy. Scand. J. Gastroenterol. 25 (Suppl. 174): 29–36

Whittle, B. J. R. (1993) Neuronal and endothelium-derived mediators in the modulation of the gastric microcirculation: integrity in the balance. Br. J. Pharmacol. 110: 3–17

Whittle, B. J. R., Esplugues, J. V. (1989) Pro-ulcerogenic eicosanoids and related lipid mediators in gastric mucosal damage. In: Garner, A., Whittle, B. J. R. (eds) Advances in Drug Therapy of Gastrointestinal Ulceration. John Wiley & Sons Ltd, Chichester, pp 165–187