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Gastroprotective Effect of Malotilate in Indomethacin- and Ethanol-Induced Gastric Mucosal Damage

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Summary

Malotilate as a synthetic substance shares comparable hepatoprotective properties with various flavonoids. The gastroprotective effect of some flavonoids prompted us to ascertain the similar effectiveness of malotilate. The possible gastroprotectivity was examined in gastric mucosal damage in rats induced by indomethacin (20 mg.kg^{-1}) or ethanol (96 %). Oral pretreatment with malotilate (25, 50, 100, 200 and 400 mg.kg^{-1}) reduced the extent of lesions induced by both indomethacin and ethanol. Histological analyses also revealed a mitigating effect on the severity of gastric mucosal lesions. Similar results were obtained in the group of rats pretreated with 5 mg.kg^{-1} indomethacin followed by oral administration of 96 % ethanol. This finding suggests that the effect of malotilate on rat gastric mucosa is independent of endogenous prostaglandin production.

Key words
Damage – Ethanol – Gastric – Indomethacin – Malotilate – Gastroprotection

Introduction

Natural flavonoids possess essential antioxidant, antilipoperoxidant, and membrane stabilizing properties. The clinical utilization of these characteristics is centered at present mainly on their hepatoprotective actions. However, some of them exhibit significant gastroprotective effects against gastric lesions induced by cold-restraint stress, pylorus ligation or various necrotizing agents (De la Lastra *et al.* 1992, 1993).

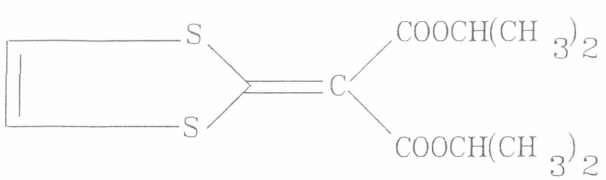

Fig. 1
The chemical structure of malotilate.

Malotilate (Fig. 1) is a synthetic substance with hepatoprotective actions comparable to those of natural flavonoids (Ala-Kokko 1987, Faberová *et al.* 1994).

Besides the existence of some other common properties with certain flavonoids, malotilate exerts anticholinergic, antihistaminergic and antiserotoninergic effects (Matsuda 1982). Furthermore, malotilate causes more than a two-fold increase of cellular regeneration and protein synthesis (Igarashi 1980, Imiazumi 1982). Finally, malotilate-induced augmentation of liver blood flow (Nakayama 1978) and the currently accepted observation that the cytoprotective effect of several agents is not tissue specific, prompted us to study the ability of malotilate to prevent gastric mucosal injury. The study was designed to demonstrate the gastroprotective effect of malotilate against indomethacin- and ethanol-induced gastric injury and to determine whether this cytoprotective effect is mediated by endogenous prostaglandins.

Material and Methods

Male Wistar rats (200–250 g) were deprived of food but allowed free access to water for 24 h before the experiments. They were kept in mesh-floor cages to prevent coprophagy. Malotilate (Drug Research Institute, Modra, Slovakia) was suspended in 0.5 % methylcellulose and indomethacin (Sigma) was dissolved in 2 % $NaHCO_3$ solution (Assouline and Danon 1985). The rats were treated with 25, 50, 100, 200 and 400 mg.kg^{-1} of malotilate, 5 and 20 mg.kg^{-1} of indomethacin and 0.5 ml.100 g^{-1} of 96 % ethanol according to the following schema.

Indomethacin/ethanol control groups: Methylcellulose (p.o.) in a volume of 0.5 ml.kg^{-1} was administered to the animals 60 min before i.p. injection of indomethacin (20 mg.kg^{-1}) or p.o. application of 96 % ethanol.

Malotilate-indomethacin or malotilate-ethanol-treated groups: Malotilate in 5 different doses was given orally 60 min before indomethacin or ethanol treatment.

Endogenous prostaglandin suppression group: The pretreatment with 5 mg.kg^{-1} indomethacin i.p. was followed after 30 min by the administration of malotilate in doses 25, 50, 100, 200 and 400 mg.kg^{-1} p.o. Ethanol was given orally 60 min after malotilate. To compare the effect of malotilate, another group of rats was pretreated with sucralfate in the same manner as with malotilate. Sucralfate was chosen because of its direct gastroprotective effect independent of the inhibition of gastric acid secretion. Sucralfate was given as a suspension in 0.5 % methylcellulose in a single dose of 100 mg.kg^{-1} *via* an oral intragastric tube.

The animals were killed either 4 h or 60 min after administration of indomethacin (20 mg.kg^{-1}) or ethanol, respectively. The stomachs were removed immediately and all macroscopically visible lesions of the mucosa in the glandular part were recorded. The extent of lesions was measured, summed per stomach and expressed in mm. After the gross defects had been scored, the stomachs were immersed in 10 % buffered formaldehyde for 24 h. A section of the glandular portion was embedded in paraffin, sectioned and stained with haematoxylin and eosin.

Statistical analysis: Values are given as means ± S.E.M. The significance of differences between means was evaluated by the ANOVA test. Significant differences were assumed to be real when the test gave probability levels of less than 0.05.

Results

Figure 2 shows that the orally administered malotilate significantly reduced the length of lesions induced by intraperitoneal application of 20 mg.kg^{-1} indomethacin. There were no significant differences between the individual doses of malotilate. The smallest dose of 25 mg.kg^{-1} was as effective as the highest dose of 400 mg.kg^{-1} (Fig. 2).

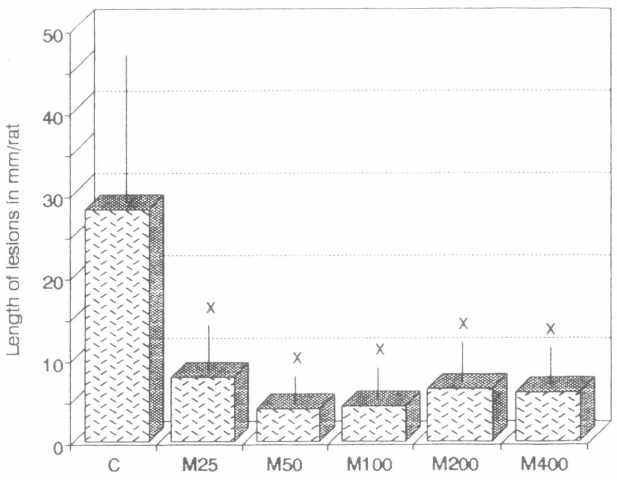

Fig. 2
Effect of malotilate 25, 50, 100, 200, and 400 mg.kg^{-1} (M25, M50, M100, M200, M400) on the average length of indomethacin-induced (20 mg.kg^{-1}) gastric mucosal lesions in mm/rat. Results are means ± S.E.M. X – significantly less than the vehicle group (C).

On the other hand, the gastroprotective effect of malotilate in ethanol-treated rats was expressed in dose-dependent manner (Fig. 3). Similarly, malotilate reduced the length of ethanol-induced mucosal defects in rats pretreated with 5 mg.kg^{-1} indomethacin (Fig. 4).

The effect of sucralfate on indomethacin- or ethanol-induced gastric mucosal damage is shown in Fig. 5. The statistical analysis of results with an analogous dose of malotilate (100 mg.kg^{-1}) indicated the higher efficiency of malotilate in gastric mucosal protection.

The macroscopic appearance of gastric mucosa after ethanol treatment is given in Fig. 6A. The effect of malotilate pretreatment (50 and 100 mg.kg^{-1}), however, completely eliminated the noxious effect of ethanol on rat gastric mucosa (Fig. 6B,C). The effectiveness of malotilate (100 mg.kg^{-1}) was also found in indomethacin-pretreated (5 mg.kg^{-1}) animals with subsequent application of ethanol (not shown).

The histological examinations revealed that the application of indomethacin (20 mg.kg^{-1} i.p.) caused well-defined gastric mucosal lesions in the oxyntic mucosa (not shown). The oral administration of ethanol produced severe damage of the gastric mucosa. The lesions consisted of partial or total detachment of segments of the mucosa and intramural haemorrhages throughout the parietal cell area (Fig. 7A). Malotilate in a dose of 100 mg.kg^{-1} significantly reversed the damaging effect of ethanol (Fig. 7B).

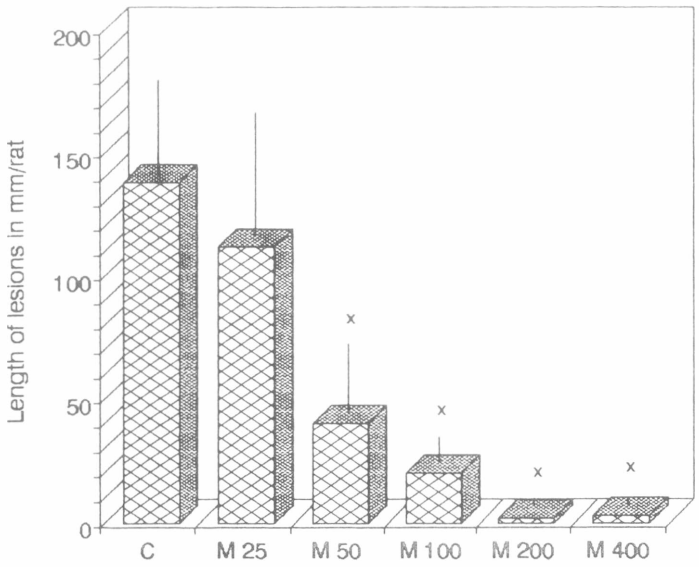

Fig. 3
Effect of malotilate 25, 50, 100, 200, and 400 mg.kg^{-1} (M25, M50, M100, M200, M400) on the average length of ethanol-induced (96 %, 0.5 ml.100 g^{-1}) gastric mucosal lesions in mm/rat. Results are means ± S.E.M.. X – significantly less than the vehicle group (C).

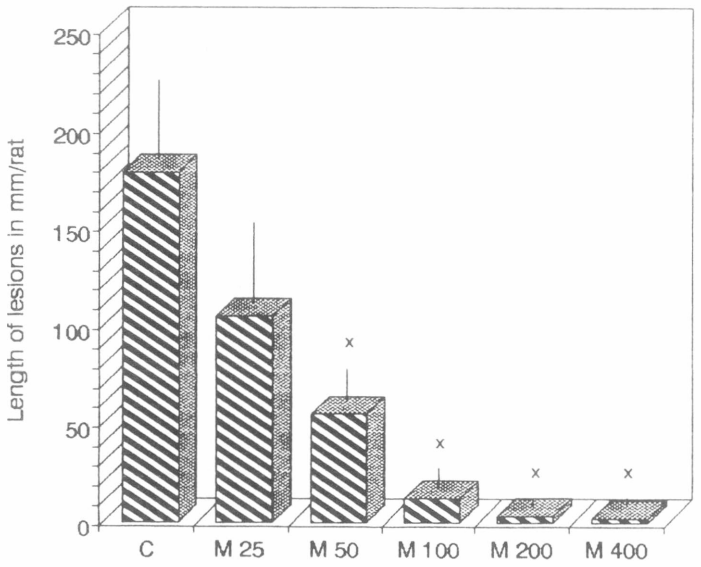

Fig. 4
Effect of malotilate 25, 50, 100, 200, and 400 mg.kg^{-1} (M25, M50, M100, M200, M400) on the average length of indomethacin-pretreated (5 mg.kg^{-1}) and ethanol-induced (96 %, 0.5 ml.100 g^{-1}) gastric mucosal lesions in mm/rat. Results are means ± S.E.M. X – significantly less than the vehicle group (C).

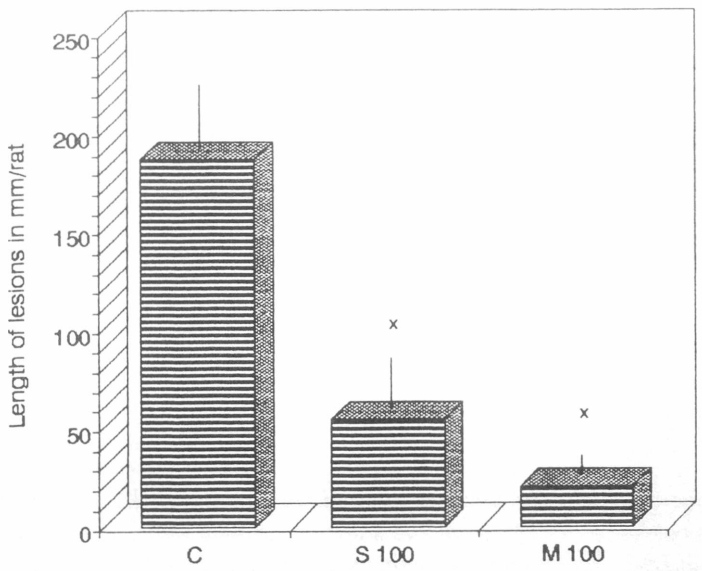

Fig. 5
Effect of sucralfate 100 mg.kg^{-1} (S 100) and malotilate 100 mg.kg^{-1} (M 100) on the average length of ethanol-induced gastric mucosal lesions in mm/rat. Results are means ± S.E.M. X – significantly less than the vehicle group (C).

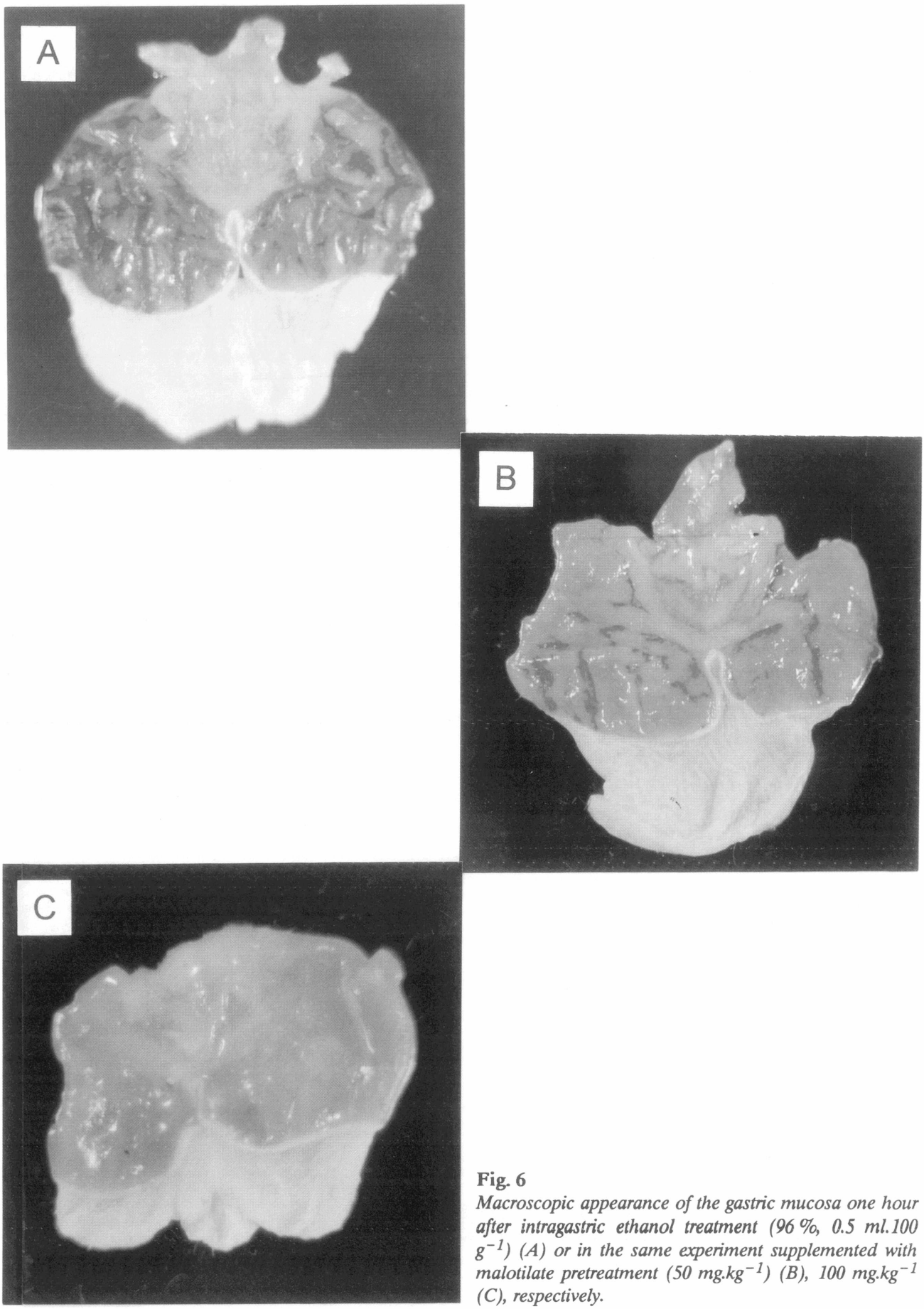

Fig. 6
Macroscopic appearance of the gastric mucosa one hour after intragastric ethanol treatment (96 %, 0.5 ml.100 g^{-1}) (A) or in the same experiment supplemented with malotilate pretreatment (50 $mg.kg^{-1}$) (B), 100 $mg.kg^{-1}$ (C), respectively.

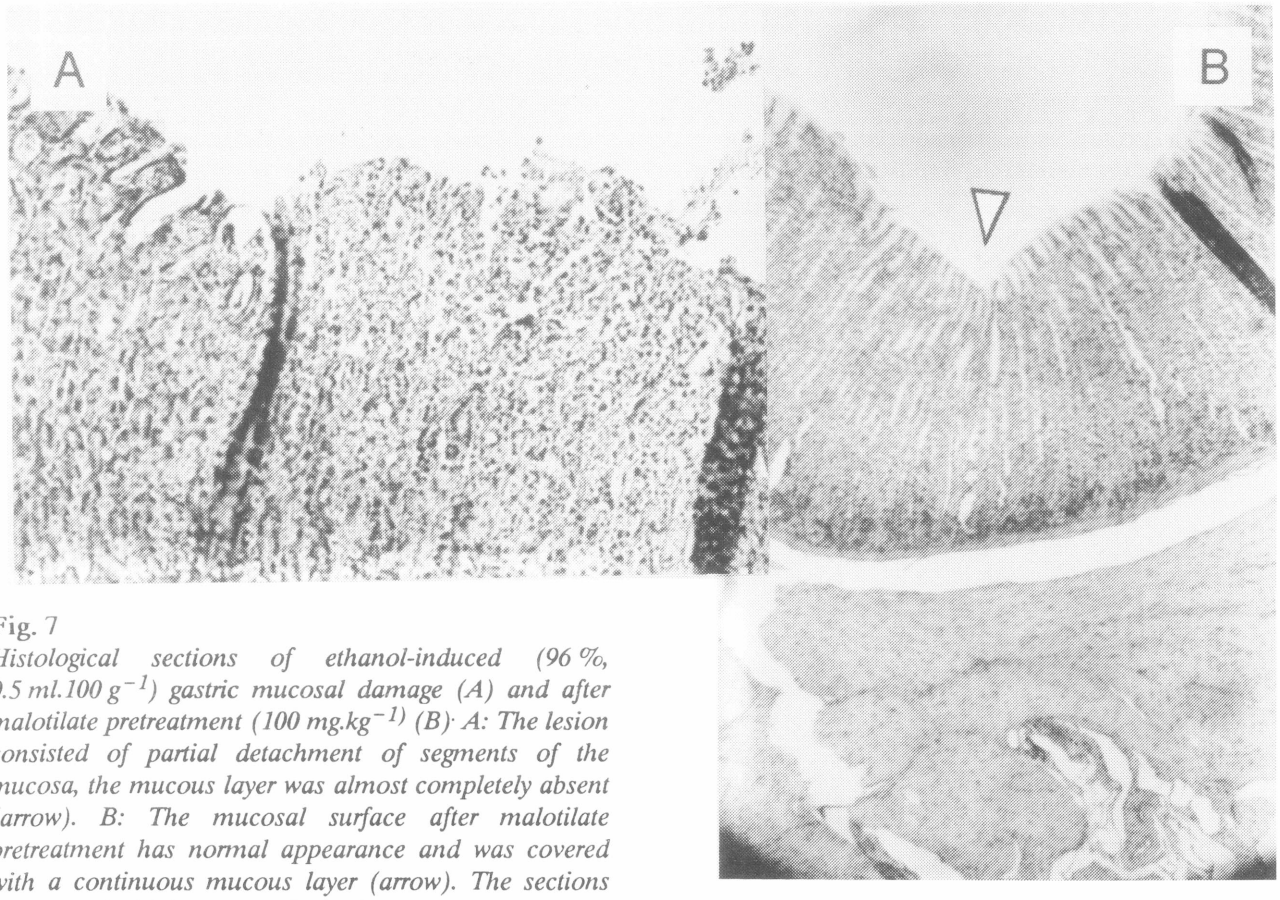

Fig. 7
Histological sections of ethanol-induced (96 %, 0.5 ml.100 g^{-1}) gastric mucosal damage (A) and after malotilate pretreatment (100 mg.kg^{-1}) (B). A: The lesion consisted of partial detachment of segments of the mucosa, the mucous layer was almost completely absent (arrow). B: The mucosal surface after malotilate pretreatment has normal appearance and was covered with a continuous mucous layer (arrow). The sections are stained with haematoxylin-eosin.

Discussion

Gastric mucosal injury due to the administration of nonsteroidal antiinflammatory drugs (NSAID) has been attributed, principally, to the inhibition of prostaglandin production in the gastric mucosa (Whittle 1981). Application of necrotizing agents such as ethanol provokes immediate superficial gastric mucosal damage which is not dependent on mucosal prostaglandin levels (Ito *et al.* 1984). However, prostaglandins protect deeper layers of the gastric mucosa not only against indirectly acting substances (NSAID) but also against macroscopically visible necrotic and haemorrhagic lesions caused by directly irritating agents (ethanol). This beneficial effect of prostaglandins was named cytoprotection and was set apart from the antiulcer effect of antisecretory agents (Robert *et al.* 1984). Although this ability was previously considered as a specific property of prostaglandins, later studies have shown that a variety of compounds with no structural similarity to prostaglandins share this gastric cytoprotective ability.

Several flavonoids, among other pharmacological actions, exert a gastroprotective effect against various noxious stimuli (De la Lastra *et al.* 1994, Izzo *et al.* 1994). Malotilate is the substance with very similar properties as these flavonoids. The results of the present study have shown that malotilate significantly reduced the mucosal lesions in response to indomethacin and ethanol. The attenuation of indomethacin-induced mucosal lesions allowed only the evaluation of malotilate protective activity against indirectly acting ulcerogenic agent. The preventive effect of malotilate on ethanol-induced gastric mucosal injury also confirms its direct gastroprotective activity. The mechanism of malotilate action is not elucidated in the present paper. However, the possible role of prostaglandins was studied in the experiments with indomethacin pretreatment (5 mg.kg^{-1} i.p. 30 min before malotilate administration) in ethanol-induced gastric mucosal injury. The results revealed that indomethacin pretreatment did not attenuate the effect of malotilate. It was therefore assumed that endogenous prostaglandins did not mediate the gastroprotection of malotilate.

It was previously found in our laboratory that intraduodenal application of malotilate in rats with ligated pylorus significantly inhibited gastric acid secretion (Mirossay *et al.* 1995). This effect could help to explain the mucosal protective effect of malotilate. However, the inhibition of gastric acid secretion can account for the biological activity of malotilate only in indirectly-induced (indomethacin-provoked) gastric mucosal damage. This single property of malotilate

should not be considered sufficient for the explanation of its beneficial effect in ethanol-induced mucosal injury.

Sucralfate protects gastric mucosa by forming a mucosal protective layer. The second possibility is the sealing of microlesions by binding with juxtamucosal proteins (Smolow *et al.* 1983, Harrington *et al.* 1981). Our data provide strong evidence that sucralfate acts at this level of defensive mechanisms of the gastroprotective process. The protective effect of sucralfate against ethanol-induced mucosal damage supports this suggestion. In our experiments with malotilate we have demonstrated its higher effectiveness in the same dose as compared to sucralfate. Although it is not possible to determine exactly the mechanism of action of malotilate on the basis of the present experiments, it is reasonable to conclude that the enhancement of defensive components may play an important role.

The gastroprotective effect of malotilate either differs from some flavonoids or is comparable to some others. Silymarin, a lipooxygenase inhibitor, was found to be effective in the prevention of cold-restraint-induced gastric ulceration and rats with pyloric ligation. However, silymarin did not prevent the formation of gastric lesions in absolute ethanol-induced ulcers (De la Lastra *et al.* 1992). Contrary to this, rutin given orally one hour before administration of ethanol significantly reduced the area of macroscopic lesions (Guerrero *et al.* 1994), an effect which is in agreement with that of malotilate.

It was found in the present study that malotilate inhibited the ulcer formation induced by both indomethacin and ethanol. The effects of malotilate were more potent that those of sucralfate under the same conditions. Pretreatment with indomethacin have shown that cytoprotective effect of malotilate against ethanol injury did not appear to be mediated by endogenous prostaglandins.

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