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Gastroprotective Effect of MX_1 (a Novel Salt of the Active Metabolite of Roxatidine with a Complex of Bismuth and Citric Acid) Against Stress Ulcers in Rats

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Abstract

We have studied the effect of the newly synthesized agent MX_1, a salt of the active metabolite of the H_2-blocker roxatidine with a complex of bismuth and citric acid (*N*-[3-(3-(1-piperidinylmethyl)phenoxy)propyl]-hydroxyacetamide-2-hydroxypropane-1,2,3-tricarboxilate-bismuth(3+) complex), against restraint stress ulcers in rats (24 h immobilization).

The effects of MX_1 (12.5, 50, 125, 184 and 250 mg kg^{-1}) were compared with the effects of equimolar doses of roxatidine (6·5, 25, 70, 100 and 140 mg kg^{-1}) and bismuth subcitrate (6·5, 25, 70, 100 and 140 mg kg^{-1}). The results show that MX_1-pre-treatment, at all the doses used, significantly reduces the mean number and size of ulcers. Even at the lowest dose the number of ulcers was reduced by 64·3% and the size of the ulcer by 55·9%. Roxatidine (25, 70, 100 and 140 mg kg^{-1}) dose-dependently reduces ulcer size and number by 24·6, 55·6, 85·3 and 89·0% and by (+7·2), 14·3, 57·1 and 67·9%, respectively. Bismuth subcitrate significantly reduces ulcer size and number only at the highest dose employed (−28·5 and −44·8%, respectively). The morphometric results have been confirmed histomorphologically.

The results suggest that MX_1 has a gastroprotective effect against stress-induced ulcers which is similar to that of the parent compound and more pronounced than that of bismuth subcitrate.

Ulcer disease is a multifactorial condition that can be viewed as imbalance between co-existing factors either aggressive towards or protective of the gastric mucosa. Recently, *Helicobacter pylori* has emerged as a new and important factor that can cause or predispose development of ulceration (Taylor & Blaser 1991; Blaser 1992; Labenz & Borsch 1994). Clinical investigations clearly show that 70% of patients with peptic ulcer disease are infected by *Helicobacter pylori* and although gastric and duodenal ulcers differ one from another, both are associated with *Helicobacter* infection (Tytgat & Rauws 1990; Blaser 1992; Graham et al 1992).

It is well known that ulcer disease can be treated with acid-reducing drugs, such as H_2-histaminergic blockers and proton-pump inhibitors. Clinical data show that long-term maintenance therapy with ranitidine is effective, safe and reduces the risk of recurrent bleeding (Jensen et al 1994). However, when the therapy ceases, the ulcers recur at a rate of 70% per year. In contrast, elimination of *Helicobacter* results in healing of ulcers and low recurrence rate (Blaser 1992). As an alternative to long-term drug-maintenance therapy or elective surgery, use of a combination of anti-*Helicobacter* and acid-reducing drugs is suggested (Hentschel et al 1993). This new family of anti-ulcer drugs is represented by the complex agent ranitidine bismuth citrate. Its anti-ulcer efficacy has been shown both in experimental and in clinical conditions (Hudson et al 1992; Stables et al 1993).

A search for new effective ways of treating ulcer disease has led to the synthesis of a novel substance, a salt of the active metabolite of the H_2-histaminergic antagonist roxatidine with the complex of bismuth and citric acid: *N*-[3-(3-(1-piperidinylmethyl)phenoxy)propyl]-hydroxyacetamide-2-hydroxypropane-1,2,3-tricarboxilate-bismuth(3+) complex, code name MX_1 (Ivanov et al 1996). It is expected that this substance will exert anti-secretory, mucoprotective and anti-*Helicobacter* activity. Literature data show that MX_1 markedly reduces the number and the size of the ulcerative lesions in a model of ethanol-induced gastric mucosal damage, reduces acid secretion in pylorus-ligated rats and has anti-*Helicobacter* activity in-vitro (Ivanov et al 1996).

The purpose of this study was to elucidate the effects of MX_1 on gastric mucosal damage induced by restraint stress in rats and to compare the effects with those of roxatidine and bismuth subcitrate alone.

Materials and Methods

Experiments were performed on male Wistar rats, 240–260 g. All experiments were conducted according to requirements for humane animal experimentation. The animals were fasted 24 h before the experiment and during the stress period, but had free access to water. The test substances were applied intragastrically in 0·2 mL $(100\ g)^{-1}$ by metal orogastric tube 30 min before experimental ulcer induction, in the corresponding equimolar doses MX_1: 12·5, 50, 125, 184 and 250 mg kg^{-1}; roxatidine: 6·5, 25, 70, 100 and 140 mg kg^{-1}; bismuth subcitrate: 6·5, 25, 70, 100 and 140 mg kg^{-1}. Control animals were treated with distilled water in the same test schedule and under the same conditions. Gastric mucosal damage was provoked by restraint stress for 24 h (Takagi et al 1964). The animals were immobilized in 20×10×10 cm perforated plastic cages. After the stress period the animals were killed by rapid decapitation. The stomach was removed

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immediately, opened along the greater curvature, gently washed in physiological solution, spread over a pad and observed macroscopically for the appearance of mucosal lesions. The size of each lesion was measured. In the event of petechia, five were considered as a 1-mm lesion. Mean ulcer number and mean ulcer size were calculated. Pieces of the stomach wall were immersed in 8% formalin solution for 20 min, embedded in paraffin and stained with haematoxylin-eosin, Van Gieson, Gomori or Weigert solutions for histopathological study. The histopathological results were obtained by light microscopy and documented by means of a Jena Lumar micro-photocamera.

Data were analysed statistically by Student's t-test using the IBM-compatible program InStat. A value of $P < 0.05$ was considered indicative of statistical significance.

Results

Macroscopic observation showed that restraint stress was highly harmful to the gastric mucosa. Lesions varying in number, depth and size were observed (Fig. 1). The lesions were filled with brown-coloured haematin material, loosely fixed to the liable tissue. The destruction seemed to penetrate deeply into the gastric wall. Even when the number of the lesions was not very large, the lesions often merged.

The mean number of ulcers was 9.33 ± 0.54 and the mean ulcer length was 9.06 ± 0.79 mm. The results show that pre-treatment with MX_1 at all doses significantly reduced the mean number of ulcers and their size (Table 1). Even for the lowest dose applied, the number of ulcers was found to be reduced by 64·31% and their size by 55·85%. When the dose of MX_1 was increased further inhibition was slightly increased, up to 75·03 and 83·44%, respectively.

Pre-treatment with roxatidine in doses of 6·5 and 25 mg kg^{-1} did not exert a protective effect against stress-induced gastric injury, indeed there was even a trend for mucosal damage to be increased. The number of ulcers increased by 23·25% and by 7·18%, respectively. The size of the ulcers increased by 14·02% when the dose was 6·5 mg kg^{-1}. For the higher doses (70, 100 and 140 mg kg^{-1}) roxatidine dose-dependently reduced the number of ulcers by 14·26, 57·13 and 67·85% and their size by 55·85, 85·32 and 88·96% (Table 2).

Bismuth subcitrate in doses up to 100 mg kg^{-1} did not exert a pronounced ulceroprotective effect (Table 3). Only when the drug was applied at the highest dose tested (140 mg kg^{-1}) were mean ulcer number and size found to have decreased significantly (by 28·51% and by 44·81%, respectively).

The histomorphological results showed that the experimental ulcers were manifested as acute erosive defects, more often superficial or implicating the total thickness of mucosa, including the glandular part of the wall (Fig. 2). There were also instances when the destructive changes reached the muscularis mucosa propria-layer of the stomach wall and even deeper; in these circumstances the lesions were filled with blood coagulates and haematin materials. In the adjacent areas the cytoplasm of the superficial epithelium showed signs of typical mucous dysplasia with the appearance of vacuolization and nuclear heterotopia and the disappearance of mucoid granula. Oxynthic cells were found swollen or wrinkled or completely destroyed. Mucus was absent. Dilation of the micro-vessels was observed. No inflammatory reaction was found. Stress-induced pathohistological changes were altered to different extents by the substances tested. Protection against lesion formation was found in the MX_1-pre-treated group (more pronounced at doses 184 and 250 mg kg^{-1}) and in the roxatidine-pre-treated group (only at doses 100 and

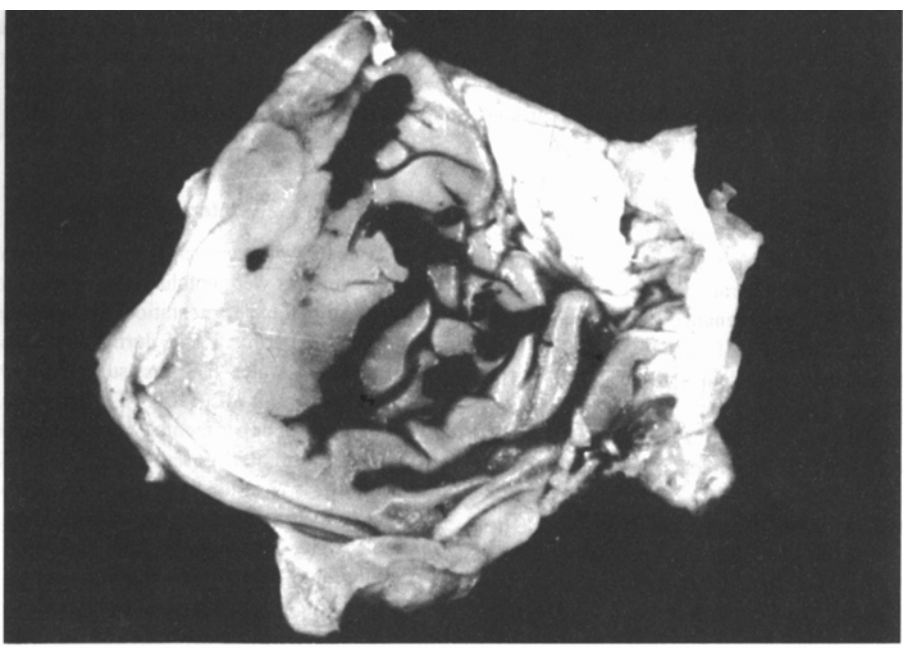

FIG. 1. Macroscopic observation of a stress ulcer (control); magnification × 2.

Table 1. Effect of MX_1 on the number and size of restraint-stress-induced gastric mucosal lesions in rats.

Dose (mg kg^{-1})	Mean number of ulcers	Inhibition (%)	Mean length of ulcer (mm)	Inhibition (%)
Stressed controls	9·33 ± 0·54	–	9·06 ± 0·79	–
12·5	3·33 ± 0·21*	64·31	4·00 ± 0·37*	55·85
50	3·00 ± 0·26*	67·85	4·00 ± 0·36*	55·85
125	2·50 ± 0·62*	73·21	2·33 ± 0·45*	74·28
184	2·83 ± 0·91*	69·67	1·67 ± 0·56*	81·57
250	2·33 ± 0·76*	75·03	1·50 ± 0·50*	83·44

*$P < 0·0001$, significantly different compared with control.

Table 2. Effect of roxatidine on the number and size of restraint-stress-induced gastric mucosal lesions in rats.

Dose (mg kg^{-1})	Mean number of ulcers	Inhibition (%)	Mean length of ulcer (mm)	Inhibition (%)
Stressed controls	9·33 ± 0·54	–	9·06 ± 0·79	–
6·5	11·50 ± 0·43	+23·25	10·33 ± 3·56	+14·02
25	10·00 ± 0·45	+7·18	6·83 ± 0·79*	24·61
70	8·00 ± 0·58	14·26	4·00 ± 0·86†	55·85
100	4·00 ± 0·86‡	57·13	1·33 ± 0·42‡	85·32
140	3·00 ± 1·03‡	67·85	1·00 ± 0·37‡	88·96

‡$P < 0·0001$, †$P < 0·001$, *$P < 0·05$, significantly different compared with control.

Table 3. Effect of bismuth subcitrate on the number and length of restraint-stress-induced gastric mucosal lesions in rats.

Dose (mg kg^{-1})	Mean number of ulcers	Inhibition (%)	Mean length of ulcer (mm)	Inhibition (%)
Stressed controls	9·33 ± 0·54	–	9·06 ± 0·79	
6·5	9·00 ± 0·36	3·54	8·50 ± 1·18	6·18
25	8·33 ± 0·42	10·72	7·83 ± 0·75	13·58
70	8·00 ± 0·68	14·26	7·50 ± 0·62	17·22
100	8·00 ± 0·63	14·26	7·17 ± 0·60*	20·86
140	6·67 ± 0·49†	28·51	5·00 ± 0·36‡	44·81

‡$P < 0·001$ †$P < 0·01$ *$P < 0·05$, significantly different compared with control.

140 mg kg^{-1}). Only slight or no protection was observed in the bismuth subcitrate-treated group.

Discussion

In stress-induced ulceration, in addition to increased acid secretion and pepsin activity, reduced gastric mucosal blood flow and oxygenation and the reduced secretion of mucus and bicarbonate are also pathogenic mechanisms of importance (Miller 1987). Successful therapy against stress ulcers might be achieved either by reducing the aggressive factors (acid and pepsin) or by enhancing the defensive processes (mucus production, micro-circulation) in the gastric mucosa. H_2-histaminergic blockers are widely used to prevent the risk of stress ulcers in patients in intensive care units and to treat stress ulcers associated with active bleeding (Feldman & Burton 1990). By maintaining intraluminal pH above 4·0, H_2-blockers prevent stress ulcers not only by reducing acid secretion but also by reducing pepsin activity, which is quite low under these conditions.

The results of this study show that the complex agent MX_1, which combines the anti-secretory activity of the H_2-blockers with the anti-*Helicobacter* activity of bismuth salts, potently prevents the formation of stress ulcers at all the doses applied. MX_1 inhibits both the incidence and the severity of the stress-induced mucosal lesions. Its effect is more pronounced than that of roxatidine and much more pronounced than that of bismuth subcitrate. These findings are in accord with literature data which show the anti-secretory effects of MX_1 to be two-fold greater than those of roxatidine in pylorus-ligated rats, and also show the cytoprotective effect against ethanol-induced gastric injury to be more potent (Ivanov et al 1996). The results obtained can be explained not only by the H_2-blocker activity of the complex agent tested, but also by the pharmacological properties of the bismuth salt, which has been shown to provide a protective coating of the ulcer crater, to stimulate

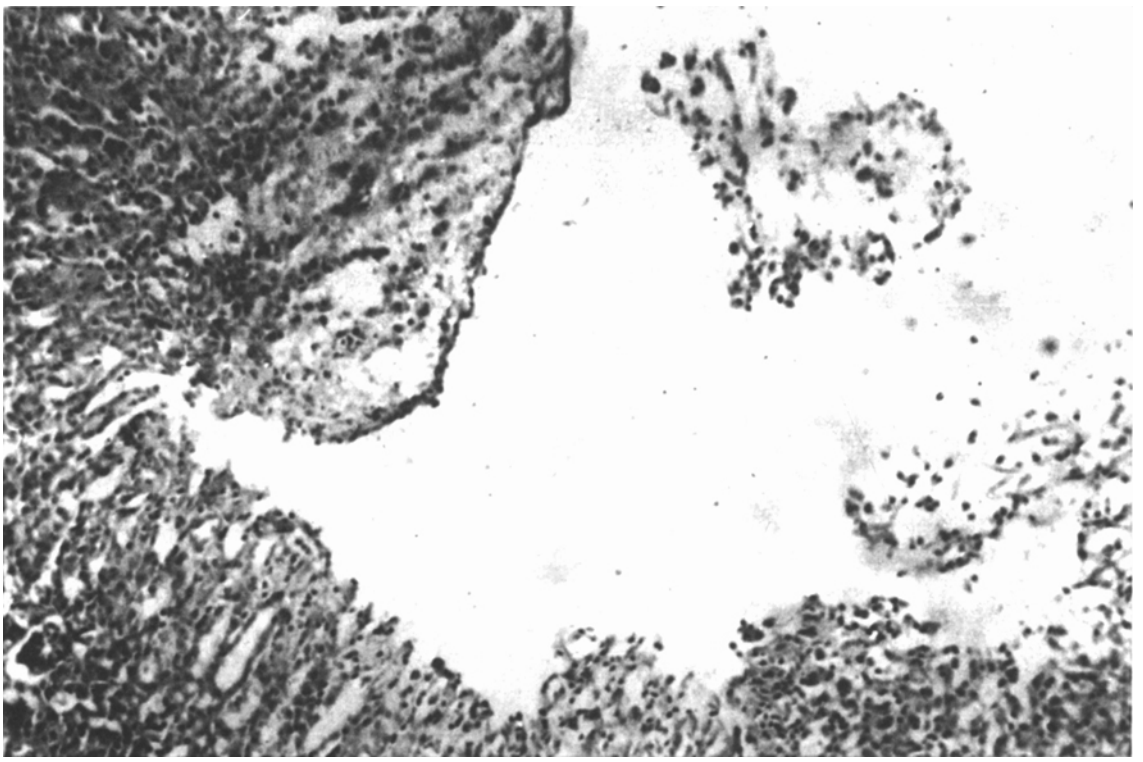

FIG. 2. Stress-induced erosive defect of gastric mucosa; light microscope, Menalumaz; magnification × 80.

endogenous prostaglandin production, to inhibit pepsin activity and to exert anti-*Helicobacter* activity (Wagstaff et al 1988; Stables et al 1993). The complex compound ranitidine bismuth citrate is shown to inhibit gastric acid secretion like ranitidine itself and, in contrast with ranitidine alone, to protect gastric mucosa against ethanol- or indomethacin-induced damage, and to exert inhibitory activity against human pepsin isoenzymes in-vitro and in-vivo (Stables et al 1993). Ranitidine bismuth citrate completely prevents gastric erosion and micro-bleeding caused by aspirin in healthy volunteers (Hudson et al 1992). Recently, bismuth compounds have been widely used in combination with antibiotics to eradicate *Helicobacter* infection, because they act by different mechanisms and have complementary effects (Graham et al 1991; Blaser 1992; Hentschel et al 1993). The addition of anti-secretory components to the complex therapy might be of great importance, because with decreased luminal acidity the anti-*Helicobacter* activity of the pH-sensitive antibiotics such as amoxycillin and erythromycin increases more than tenfold (Walsh & Peterson et al 1995). Clinical trials reveal that combination anti-ulcer therapy produces better results than monotherapy. Regimens consisting of a single or double antimicrobial medication plus an anti-secretory drug result in eradication and ulcer healing in more than 80% of patients (Hentschel et al 1993; Peterson et al 1995). Thus agents, e.g. roxatidine bismuth citrate or the novel compound MX_1, combining potent inhibition of gastric acid secretion with anti-*Helicobacter* activity might be expected to be promising anti-ulcer drugs in peptic ulcer therapy.

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