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# Synthesis, Gastroprotective, Antisecretory and Anti-helicobacter Effect of *N*-[3-(3-(1-Piperidinylmethyl) phenoxy)propyl]-hydroxyacetamide 2-Hydroxypropane-1,2,3-tricarboxylate Bismuth ($3^+$) Complex ($MX_1$)-$MX_1$

C. IVANOV*, O. PETKOV*, P. PETROV**, M. TASKOV*, R. ATHANASSOVA*, E. TSVETKOVA**, V. KOTSEV**, G. LYUTAKOV*, G. NIKOLOV* AND E. SAVOV**

*Chemical Pharmaceutical Research Institute and **Department of Microbiology, Military Medical Academy, Sofia, Bulgaria

## Abstract

$MX_1$ (*N*-[3-(3-(1-piperidinylmethyl)phenoxy)propyl]-hydroxyacetamide 2-hydroxypropane-1,2,3-tricarboxylate bismuth ($3^+$) complex) is a novel salt of the active metabolite of $H_2$-antagonist roxatidine with a complex of bismuth with citric acid.

In a model of ethanol-induced ulcers in male Wistar rats, both roxatidine and the bismuth salt reduced the number and the total length of lesions. Comparison of roxatidine and $MX_1$ at equimolar doses of 160 $\mu$mol kg$^{-1}$ showed a more potent cytoprotective effect of $MX_1$. The potency of anti-secretory and antiacidic effects of $MX_1$ was more than twice that of roxatidine on histamine-stimulated secretion in female Wistar pylorus-ligated rats. Microbiological tests with the reference bismuth preparation De-Nol showed prominent anti-Helicobacter properties of $MX_1$ in-vitro. Both test compounds had similar range of MICs to *Helicobacter pylori*, from 4 to 64 $\mu$g bismuth mL$^{-1}$.

The cytoprotective, antisecretory, anti-acidic and anti-Helicobacter properties of the new agent $MX_1$ warrant further more extensive pharmacological and clinical trials.

Colloidal bismuth subcitrate (CBS) realizes its gastric mucosal protective effect by forming complexes with mucus, most likely by binding to glycoproteins, and thus increases the barrier function against $H^+$ reverse diffusion (Koo et al 1982; Lee 1982; Hollanders et al 1983). CBS inhibits pepsin activity (Baron et al 1986), stimulates endogenous prostaglandin synthesis (Konturek et al 1986b), and has a potent antibacterial effect on *Helicobacter pylori* (Marshal et al 1987). Furthermore, the combination of CBS with histamine $H_2$-receptor antagonists, such as cimetidine, was found to have better therapeutic effect and shorten healing time in patients with duodenal ulcer disease (Salmon 1987).

The above information prompted us to synthesize and test a novel salt of the active metabolite of the $H_2$-antagonist roxatidine with the complex of bismuth with citric acid: *N*-[3-(3-(1-piperidinylmethyl)phenoxy)propyl]-hydroxyacetamide 2-hydroxypropane-1,2,3-tricarboxylate-bismuth ($3^+$) complex, under the code name $MX_1$. $MX_1$ was selected as the most suitable candidate for testing and development after synthesis and screening of several new bismuth salts. The novel bismuth salt $MX_1$ is expected to provide an effective combination of the gastric antisecretory activity of the metabolite with potentially prominent mucoprotective and antibacterial properties.

This paper presents the method of preparation of $MX_1$ and the results obtained from tests of its mucoprotective effect on ethanol-induced gastric ulcers in rats, its antisecretory effect in a model of histamine-stimulated secretion in rats with pyloric ligation, and its in-vitro anti-helicobacter activity.

Correspondence: C. Ivanov, Chemical Pharmaceutical Research Institute, 3 Kliment Ohridsky Boulevard, 1756 Sofia, Bulgaria.

## Materials and Methods

*Synthesis and analysis of $MX_1$*

The novel bismuth salt $MX_1$ was obtained by treating *N*-[3-(3-(1-piperidinylmethyl) phenoxy)propyl]-hydroxyacetamide free base with bismuth citrate in aqueous medium upon boiling. The crude bismuth salt was purified by subsequent treatment with absolute ethanol and acetone.

The salt $MX_1$ was characterized by proton nuclear magnetic resonance ($^1$H-NMR) and infra-red (IR) spectra and by the data from differential scanning calorimetry (DSC) and elemental analysis (Fig. 1).

IR spectral data clearly show that the compound $MX_1$ is a novel chemical compound, and not a simple physical mixture of bismuth citrate and the basic piperidine derivative. This is also demonstrated by the observed significant IR spectral differences between $MX_1$ and the physical mixture. Thus, a series of absorbance bands at 705, 865, 1280, and 1348 cm$^{-1}$ was found in the IR spectrum of the mixture that was absent in the IR-spectrum of the salt. Another series of absorbance bands absent in the IR spectrum of the mixture was observed in the range 2300–2700 cm$^{-1}$, which is an indication for protonation of the piperidine nitrogen of the salt.

$^1$H-NMR spectral data support these results for the salt where the chemical shifts of the methylene protons at positions 2 and 6 of the piperidine ring appeared in a lower field,

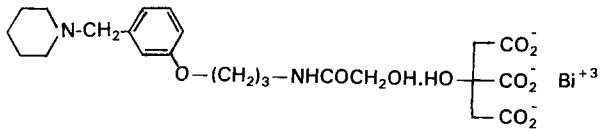

FIG. 1. Structure of $MX_1$.

compared with the chemical shifts of the same protons in the starting base. This difference in the chemical shifts is a proof of the protonation of the piperidine nitrogen in $MX_1$.

Comparison of the data obtained from the DSC-thermograms of $MX_1$ and the physical mixture shows that $MX_1$ is a new compound. Thus, the salt thermogram displayed an endothermal effect at 267°C, whereas the endothermal effect in the DSC-thermograms of the physical mixture was observed at 298 and 310°C.

Substantial differences were found with respect to the solubility of the salt and the starting bismuth citrate in water and methanol. The salt $MX_1$ is very easily soluble in both solvents (100 g $L^{-1}$), whereas bismuth citrate is practically insoluble.

*Preparative chemical work*

$^1$H-NMR spectra were recorded on a Bruker-WN-250 spectrometer in $D_2O$. Chemical shifts are given as s values (ppm): s, singlet; d, doublet; t, triplet; m, multiplet. Infrared (IR) spectra were recorded on a Shimadzu IR 435 infrared spectrophotometer. Differential scanning calorimetry (DSC) was performed at a rate of 10° $min^{-1}$ under nitrogen atmosphere, using a Mettler TA 3000-DSC 20.

Elemental analysis were performed and measured on a Perkin Elmer 240 analyzer. Analytical values were within ± 0·4% of theoretical values.

Starting hydroxyacetamide was obtained as described by Yamakoshi et al (1981). Bismuth citrate was obtained as described by Watson (1989).

*N-[3-(3-1-piperidinylmethyl)phenoxy)propyl]-hydroxyacetamide 2-hydroxypropane-1,2,3-tricarboxylate bismuth (3$^+$) complex (1:1:1) ($MX_1$).* *N*-[3-(3-(1-piperidinylmethyl) phenoxy)propyl]-hydroxyacetamide (3·84 g, 12 mmol) was suspended in water (64 mL). The mixture was heated to 90°C. At that temperature bismuth citrate (5 g, 12 mmol) was added and the reaction mixture was boiled for 4–5 h with vigorous stirring. At the end of the reaction, pH of the mixture was 5·5–6. After cooling to room temperature, unreacted bismuth citrate was removed by filtration. The aqueous filtrate was concentrated in-vacuo, and the obtained residue was dissolved in methanol (40 mL). The solvent was removed in-vacuo and the resulting crystalline residue was resuspended in absolute ethanol (52 mL) and the suspension was stirred for 20 min. Ethanol was decanted, and acetone (50 mL) was added to the crystalline precipitate. After stirring for 1 h, the bismuth salt was filtered and washed with fresh acetone, and dried in-vacuo to give 4·4 g (50% of the theoretical value).

Anal. Calcd for $C_{23}H_{31}N_2O_{10}Bi.H_2O$: C 38·18; H 4·56; N 3·87; Bi 29·66.
Found: C 37·78; H 4·76; N 4·20; Bi 29·20

DSC: endothermic peak at 267°C.

IRv/KBr/:3370 / NH, OH /, 2700-2300 /-$\overset{+}{N}$H /,1642-1540 / series of bands, CO, $CO_2^-$)

$^1$H-NMR /$D_2O$/ δ: 1·52-2·04/ m, 6H, 3,4,5-$CH_2$ of piperidine /2·12/ q, 2H, J = 6·3 Hz, $-OCH_2^-$ $CH_2-CH_2-$ /, 2·65 /d, 2H, J = 11·6 Hz, AB, $CH_2CO$ part of Bi citrate/, 2·73–3·08/ m, 4H, 2-$CH_2$ of piperidine and AB, $CH_2CO$ part of Bi citrate/, 3·52–3·57/ m, 4H, 6-$CH_2$ of piperidine and $-CH_2-CH_2-$ NH /, 4·15/ s, 2H, $COCH_2-O-$/, 4·23/ t, 2H, Ar-O-$CH_2$/, 4·33/ s, 2H, Ar-$CH_2^-$ $N^+$ </, 7·17-7·21/ m, 3H, Ar-H/, 7·54/t, 1H, J = 7·9 Hz, Ar-H/.

*Effect of $MX_1$ on ethanol-induced gastric damage*

Male albino Wistar rats, 200–260 g were used. Test animals were treated with aqueous solutions of $MX_1$ or with roxatidine used as reference (dose volume 0·5 mL/100 g rat). The test compounds were given orally using a metal orogastric tube, 1 h before experimental ulcer induction by ethanol. The animals were divided into groups. Control animals were treated with water by the same treatment schedule and test conditions. All animals were deprived of food for 36 h before the test, but were allowed free access to water. Fundic lesions were induced by oral application of absolute ethanol at a dose level of 0·3 mL/100 g body mass, using a metal gastric tube (Robert 1979; Konturek et al 1987). After 1 h of treatment the animals were killed by intraperitoneal injection of urethane overdose. Stomachs were removed, opened along the greater curvature and kept for 20 min in 5% formaldehyde solution. Isolated stomachs were then inspected visually for destructive mucosal lesions. The extent of damage was estimated by the total length of lesions and by the mean total number of ulcerations.

*Inhibition of histamine-stimulated gastric secretion in rats*

Tests were conducted on a group of female Wistar rats (mean body mass 150 ± 20 g). The animals received no food for 20 h before the test, but had free access to water. The antisecretory effect of the test compounds was evaluated by the method of Shay et al (1945) using pyloric ligation. The ligature was applied under nembutal narcosis (30 mg $kg^{-1}$, i.p.). Test compounds were also applied intraperitoneally, and histamine hydrochloride doses of 25 mg $kg^{-1}$ were injected subcutaneously twice in 1-h intervals (Bickel et al 1986) to stimulate gastric secretion. The animals were killed by urethane overdose 180 min after the last histamine injection. Stomachs were isolated and removed. Gastric content was collected individually for each animal, filtered and centrifuged by Janetzki centrifuge $T_{23}$ at 4000 rev $min^{-1}$ for 10 min, and the volume of gastric juice was measured. Acidity was determined by titration to pH 7 with 0·1 M NaOH on a Seibold pH-meter (Okabe et al 1975). The pH of the samples was measured and acid output was calculated in mmol $H^+$/3 h.

$MX_1$ and roxatidine (dissolved in distilled water) were applied intraperitoneally at 0·1 mL/100 g body mass dose volumes. Control animals were treated with distilled water (0·1 mL/100 g i.p.) after pyloric ligation and histamine stimulation.

Table 1. Effects of $MX_1$ and roxatidine on ethanol-induced ulcers in rats.

| Compound | Dose mg kg$^{-1}$ (p.o.) | Number of rats | Total length of lesions (mm) | Inhibition (%) | Ulcer number | Inhibition (%) |
|---|---|---|---|---|---|---|
| Controls (water) | – | 18 | 83·5 ± 7·4 | – | 14·0 ± 2·4 | – |
| Roxatidine | 62 | 10 | 40·8 ± 15·2** | 54 | 8·6 ± 2·3* | 39 |
| | 100 | 9 | 22·3 ± 9·0** | 73 | 6·1 ± 1·7** | 56 |
| $MX_1$ | 29 | 13 | 39·4 ± 10·1** | 53 | 11·2 ± 2·5 | 20 |
| | 58 | 12 | 48·5 ± 18·3** | 42 | 10·8 ± 3·2 | 23 |
| | 87 | 14 | 7·9 ± 3·7** | 91 | 3·7 ± 0·9** | 74 |
| | 115 | 14 | 14·1 ± 7·9** | 83 | 6·3 ± 2·1** | 55 |

All measured values are presented as means ± s.e.m. *Statistically significant at $P < 0.01$ and **Statistically significant at $P < 0.001$, compared with controls.

*Anti-helicobacter effect of $MX_1$ in-vitro*

Tests were carried out on 32 strains of *H. pylori*, isolated from patients with gastritis and ulcer disease in the Military Medical Academy, Sofia, and on reference strain *H. pylori* 11637. Test strains were isolated from biopsy material by cultivation on Brucella agar (Difco) supplemented with 7% defibrinated sheep blood. Vancomycin (10 $\mu$g mL$^{-1}$), trimethoprim (5 $\mu$g mL$^{-1}$) and nalidixic acid (5 $\mu$g mL$^{-1}$) (Sigma) were added as selective factors.

Test strains and the reference strain were cultivated at 37°C in GasPak (BBL) in a microaerophilic atmosphere provided with CampyPak (BBL). Bacterial growth was evaluated on the 4th–7th day of test. Formed colonies and isolated pure cultures were identified by Gram preparations, urease test, oxidase and catalase test, and susceptibility to nalidixic acid and cephalothin. Isolated strains were stored in trypticase soy broth (Difco) with 15% glycerol at –25°C.

The inhibitory effect of the compound $MX_1$ was compared with that of tripotassium dicitrato bismuthate (De-Nol) which is being widely used in the treatment of peptic ulcer disease. The activity of both compounds was determined by dilution in distilled water until stock solutions of 1280 $\mu$g mL$^{-1}$ were obtained for each compound (bismuth content 384 $\mu$g mL$^{-1}$ for $MX_1$ and 588 $\mu$g mL$^{-1}$ for De-Nol). Serial dilutions to the necessary concentrations were made from the stock solution by the method of Ericsson & Sherris (1971). The minimal inhibitory concentration (MIC) was determined by the serial dilution method on Mueller-Hinton agar (Difco) with 7% defibrinated sheep blood, containing a respective dilution of the tested anti-ulcer agent. Test strains were inoculated with Steers replicator at a density of $6 \times 10^8$ microbial cells mL$^{-1}$. Inoculated petri dishes were incubated at 37°C for 72 h in a micro-aerophilic atmosphere (Campy-Pak Plus-BBL). Two petri dishes, prepared as above without anti-ulcer agents, were incubated in aerobic and micro-aerophilic atmosphere and served as control of strain growth and possible contamination. MIC was determined as the lowest concentration of each agent inhibiting visible growth or allowing single colonies only.

*Statistical analysis*

The obtained results were statistically analysed by analysis of varience with s.e.m. for the pharmacologic tests.

## Results

The obtained data from the ethanol-induced gastric damage test are presented in Table 1. In the control group, ethanol-induced ulcers had mean total length 83·5 ± 7.4 mm and 14·0 ± 2·4 total mean number of ulcerations. These values are similar to those reported by Konturek et al (1986a, 1987) using the same model of gastric ulcer. Both test groups,

Table 2. Effects of $MX_1$ and roxatidine on histamine-stimulated gastric secretion in pylorus-ligated rats.

| Compound | Dose (p.o.) mg kg$^{-1}$ | Dose (p.o.) $\mu$mol kg$^{-1}$ | Number of rats | Gastric juice (mL) | Inhibition (%) | Initial pH | Increase (%) | Acid output (mEq/H$^+$/3h) | Inhibition (%) |
|---|---|---|---|---|---|---|---|---|---|
| Control | – | | 21 | 5·4 ± 0·6 | – | 1·86 ± 0·04 | – | 115·1 ± 3·4 | – |
| Roxatidine | 3·0 | 7·9 | 10 | 3·9 ± 1·0** | 27·8 | 1·81 ± 0·04$^{NS}$ | – | 117·1 ± 4·3$^{NS}$ | – |
| | 10·0 | 26·0 | 11 | 3·0 ± 1·0*** | 44·4 | 2·06 ± 0·15** | 10·8 | 88·6 ± 8·1*** | 23·0 |
| | 30·0 | 78·0 | 12 | 1·3 ± 0·5*** | 76·0 | 2·22 ± 0·18*** | 19·4 | 72·4 ± 12·2*** | 37·1 |
| | 40·0 | 103·0 | 15 | 1·4 ± 0·6*** | 74·1 | 2·40 ± 0·18*** | 29·0 | 70·0 ± 6·6*** | 39·2 |
| | 60·0 | 155·0 | 10 | 1·7 ± 1·0*** | 69·5 | 2·84 ± 0·47*** | 52·7 | 46·2 ± 13·7*** | 59·9 |
| $MX_1$ | 5·5 | 7·9 | 12 | 4·3 ± 0·8* | 20·4 | 1·80 ± 0·05$^{NS}$ | – | 116·2 ± 7·0$^{NS}$ | – |
| | 18·0 | 26·0 | 15 | 2·7 ± 0·9*** | 50·0 | 2·18 ± 0·21** | 17·2 | 73·8 ± 17·7*** | 35·9 |
| | 37·0 | 53·0 | 12 | 1·4 ± 0·5*** | 74·1 | 2·33 ± 0·28*** | 25·3 | 60·0 ± 26·7*** | 47·9 |
| | 55·0 | 78·0 | 12 | 1·6 ± 0·7*** | 70·4 | 2·53 ± 0·30*** | 36·0 | 41·6 ± 16·0 | 63·9 |
| | 73·0 | 103·0 | 11 | 0·6 ± 0·2*** | 88·9 | 3·13 ± 0·30*** | 68·3 | 11·7 ± 4·5*** | 89·8 |

Test compounds were applied intraperitoneally, immediately after pylorus ligation and injection of the first histamine dose for stimulation of gastric secretion. Each value is presented as mean ± s.e.m. *$P < 0.02$, **$P < 0.01$, ***$P < 0.001$ compared with controls.

Table 3. In-vitro activity of $MX_1$ and De-Nol against *H. pylori*.

| Compound | Modal* MIC (range) in μg Bismuth/mL |
|---|---|
| $MX_1$ | 8 (4–32) |
| De-Nol | 16 (8–64) |

*Modal MIC, most commonly occurring minimum inhibitory concentration (MIC) value.

treated with either roxatidine or $MX_1$, showed statistically significant reduction of the total length of lesions ($P < 0.001$). The mean number of ulcer lesions was reduced, compared with the controls. Comparison of roxatidine and $MX_1$ in 160 μmol $kg^{-1}$ equimolar doses (62 mg $kg^{-1}$ and 115 mg $kg^{-1}$, respectively), demonstrated a more potent effect for $MX_1$. The difference (as compared with the controls) was statistically significant ($P < 0.001$) for the effect of each test agent on the length of ulcer lesions. For $MX_1$ the highest degree of inhibition on the two test parameters was found at a dose of 87 mg $kg^{-1}$ (91% reduction of the length and 74% reduction of the number of ulcer lesions). The first of the examined indices decreased for all administered dose volumes of $MX_1$, while the ulcer number decreased significantly only after application of the two highest dose volumes. With respect to gastric secretion (Table 2), all tested doses of roxatidine except the lowest (3 mg $kg^{-1}$) were found to have prominent antisecretory activity. Whereas higher doses (30–60 mg $kg^{-1}$) decreased the volume of gastric juice by an almost equal factor of magnitude, the inhibitory effect on acid output was dose-dependent and the most potent effect was observed at 60 mg $kg^{-1}$ roxatidine. The initial pH of gastric juice increased with dose increase and the highest increase in pH (by 52·7%) was observed at the highest dose of 60 mg $kg^{-1}$.

At equimolar doses with roxatidine, $MX_1$ was found to have higher potency of inhibition on the monitored test parameters. This was particularly prominent at high dose levels. At a dose of 73 mg $kg^{-1}$ equimolar at 40 mg $kg^{-1}$ roxatidine, $MX_1$ decreased the volume of gastric secretion by 90% compared with the control and decreased the acid output by the same order of magnitude. Compared with roxatidine, the changes in pH and acidity values produced by $MX_1$ were twice as large, and the difference was statistically significant.

Comparative microbiological tests with the bismuth preparation De-Nol revealed the anti-helicobacter properties of $MX_1$. The two compounds were found to have similar activity to *H. pylori* with MIC of 4–64 μg bismuth $mL^{-1}$. The reference strain *H. pylori* 11637 had a MIC of 16 μg bismuth $mL^{-1}$ for the two compounds (Table 3).

The results of these tests give us grounds to conclude that $MX_1$ has similar in-vitro anti-helicobacter activity to De-Nol.

## Discussion

The results of our experimental studies show that the new bismuth salt $MX_1$ has expressed gastroprotective activity. Data obtained from the test models employed suggest that the mechanism of the protective effect is complex and goes beyond the $H_2$-receptor blocking properties of roxatidine.

It should be noted that both roxatidine and the bismuth salt $MX_1$ were shown to have protective effects on ethanol-induced gastric mucosal damage. This finding disagrees with views that $H_2$-receptor blockers have no significant protective activity in the ethanol test (Stables et al 1993). The comparatively well-expressed cytoprotective effect of reference roxatidine in this model may be explained by some differences in the test procedure and is, moreover, concordant with data reported by Shiratsuki et al (1988). The stronger inhibition of the effects of ethanol by $MX_1$ suggests that the bismuth compound triggers other mechanisms of protection. The property of bismuth salts to form complexes with mucus is most likely involved, as reported by Goldenberg et al (1975) and Hall & Van den Hoven (1986), providing a protective coating for the gastric mucosal damage. Stimulated endogenous prostaglandin production is also implicated, as well as altered endogenous glutathione production and formation of polyamines, nitrogen oxide, dopamine and leukotrienes (Motilva et al 1994).

The results obtained for the effects of the new bismuth salt $MX_1$ and roxatidine on histamine-stimulated gastric secretion are also interesting. The reference $H_2$-receptor antagonist reduced the volume of gastric secretion and inhibited gastric acid output. Such antisecretory and antiacidic properties of roxatidine were established in other studies and correlate with literature data (Bickel et al 1986). It is worth noting that equimolar doses of $MX_1$ demonstrated more potent effects than the reference compound on the parameters of gastric secretion. This was evident for all three tested parameters, with highly significant differences between the two test groups for pH and acid output. This suggests that mechanisms other than the characteristic effects of $H_2$-blockers on parietal cells are involved in the action of $MX_1$. These probably include inhibition by bismuth of local inflammation due to its mucoprotective and antibacterial activity, with resulting reduction of acidic mucus products and their irritant effect on secretory cells. It should also be recalled that bismuth salts were shown to inhibit the ulcerogenic and irritant properties of pepsin (Pearson et al 1986). Thus, by antagonizing the effects of pepsin, the novel bismuth salt is expected to reduce some of the pathogenic changes in gastric-acid secretion. In our model the anti-helicobacter activity of $MX_1$ is similar in potency to that of De-Nol, one of the most popular bismuth preparations. This finding, combined with its demonstrated cytoprotective and anti-acidic properties, enhances the optimistic expectations from further studies on the novel bismuth compound. These expectations are supported by the established role of *H. pylori* in the etiology and progress of chronic gastritis and peptic duodenal ulcer (Buck et al 1986; Borody et al 1989; DeCross & Marshall 1993), and by reports of more effective anti-ulcer therapy with inclusion of antibacterial agents (Graham et al 1991). Evidence was reported (Rauws 1992) that antibacterial therapy reduces the complications of ulcer disease and ulcer relapse and complications after its surgical treatment.

The possibility for monotherapy of the major aggressive factors of ulcer disease with concomitant beneficial effects

on some defence mechanisms and better convenience for both patient and doctor requires further more extensive studies to evaluate the therapeutic effectiveness and potential of the compound $MX_1$.

## References


Baron, J. H., Barr, J., Batten, J., Sidebotham, R., Spencer, J. (1986) Acid, pepsin and mucus secretion in patients with gastric and duodenal ulcer before and after colloidal bismuth subcitrate (De-Nol). Gut 27: 486–490

Bickel, M., Herling, A. W., Rising, T. J., Wirth, K. (1986) Antisecretory effects of two new histamine $H_2$-receptor antagonists. Arzneim. Forsch. Drug Res. 36: 1358–1363

Borody, T., Noonan, S., Cole, P., Morgan, A., Genne, J. (1989) Recurrence of duodenal ulcer and *Campylobacter pylori* infection after eradication. Med. J. Australia 151: 431–435

Buck, G. E., Gourly, W. K., Lee, W. K., Subramanyam, K., Latimer, J. M. (1986) Relation of *Campylobacter pylori* to gastritis and peptic ulcer. J. Infect. Dis. 153: 664–669

DeCross, A. J., Marshall, B. J. (1993) The role of *Helicobacter pylori* in acid-peptic disease. Am. J. Med. Sci. 306: 381–392

Ericsson, H. M., Sherris, J. C. (1971) Antibiotic sensitivity testing. Report of an international collaborative study. Acta Pathol. Microbiol. Scand. Sect. B. (Suppl.) 217: 1–67

Goldenberg, M. M., Honkomp, L. J., Burrous, S. E., Castellion, A. W. (1975) Protective effect of Pepto-Bismol liquid on the gastric mucosa of rats. Gastroenterology 69: 636–640

Graham, D. Y., Lew, G. M., Evans, D. G., Evans, J., Klein, P. D. (1991) Effect of triple therapy (antibiotics plus bismuth) on duodenal ulcer healing. Ann. Int. Med. 115: 266–269

Hall, D. W. R., Van den Hoven, W. E. (1986) Protective properties of colloidal bismuth subcitrate on gastric mucosa. Scand. J. Gastroenterol. 21 (Suppl. 122): 11–13

Hollanders, D., Morissey, S. M., Metha, J. (1983) Mucus secretion in gastric ulcer patients treated with tripotassium dicitrato bismuthate (De-Nol). Br. J. Clin. Pract. 37: 112–114

Konturek, S. J., Radecki, T., Brzozowski, T., Drozdowicz, D., Piastucki, I., Maramatsu, M., Tanaka, M., Aihara, H. (1986a) Anti-ulcer and gastroprotective effects of solon, a synthetic flavonoid derivative of sophoradin. Role of endogenous prostaglandins. Eur. J. Pharmacol. 125: 185–192

Konturek, S. J., Radecki, T., Piastucki, I., Drozdowicz, D. (1986b) Advances in the understanding of the mechanism of cytoprotective action by colloidal bismuth subcitrate. Scand. J. Gastroenterol. 21 (Suppl. 122): 6–10

Konturek, S. J., Radecki, T., Piastucki, I., Drozdowicz, D. (1987) Studies on the gastroprotective and ulcer-healing effects of colloidal bismuth subcitrate. Digestion 37 (Suppl. 2): 8–15

Koo, J., Ho, J., Lam, S. K., Wong, J., Ong, G. B. (1982) Selective coating of gastric ulcer by tripotassium dicitrato bismuthate in the rat. Gastroenterology 82: 864–870

Lee, S. P. (1982) A potential mechanism of action of colloidal bismuth subcitrate: diffusion barrier to hydrochloric acid. Scand. J. Gastroenterol. 17 (Suppl. 80): 17–21

Marshall, B. J., Armstrong, J. A., Graham, J., Nokes, N. T., Wee, S. H. (1987) Antibacterial action of bismuth in relation to *Campylobacter pyloridis* colonization and gastritis. Digestion 37 (Suppl. 2): 16–30

Motilva, V., Alarcon De La Lastra, C., Martin, M. J., (1994) Ulcer-protecting effects of naringenin on gastric lesions induced by ethanol in the rat: role of endogenous prostaglandins. J. Pharm. Pharmacol. 46: 91–94

Okabe, S., Takeuchi, K., Nakamura, K., Takagi, K. (1975) Influence of the proximal small intestine on the gastric hyper-secretion in pylorus ligated rats. Am. J. Dig. Dis. 20: 138–177

Pearson, J. P., Ward, R., Allen, A., Roberts, N. B., Taylor, W. H. (1986) Mucus degradation by pepsin: comparison of mucolytic activity of human pepsin 1 and pepsin 3: implications in peptic ulceration. Gut 27: 243–248

Rauws, E. A. J. (1992) Role of *Helicobacter pylori* in duodenal ulcer. Drugs 44: 921–927

Robert, A. (1979) Cytoprotection by prostaglandins. Gastroenterology 77: 1086–1094

Salmon, P. R. (1987) Combination treatment by colloidal bismuth subcitrate with $H_2$-antagonists. Digestion 37 (Suppl. 2): 42–46

Shay, H., Komarov, S. H., Fels, S. S., Meranze, D., Gruenstein, M., Siplet, H. (1945) A simple method for the uniform production of gastric ulceration in the rat. Gastroenterology 5: 43–61

Shiratsucki, K., Fuse, H., Hagiwara, M., Mikami, T., Miyasaka, K., Sakuma, H. (1988) Cytoprotective action of Roxatidine acetate HCl. Arch. Int. Pharmacodyn. Ther. 294: 295–304

Stables, R., Campbell, C. J., Clayton, N. M., Clitherow, J. W., Grinham, C. J., McColm, A. A., McLaren, A., Trevethick, M. A. (1993) Gastric anti-secretory, mucosal protective, anti-pepsin and anti-Helicobacter properties of ranitidine bismuth citrate. Aliment. Pharmacol. Ther. 7: 237–246

Watson, J. (1989) G. Br. 2220937 A

Yamakoshi, N., Kurata, S., Koizumi, N., Tarutani, M., Sakuma, H., Konishi, K. (1981) EP 024510