PERGAMON

Food and Chemical Toxicology 38 (2000) 577–584

Food and Chemical Toxicology

www.elsevier.com/locate/foodchemtox

Research Section

Inhibition of Gastric Mucosal Damage by Methylglyoxal Pretreatment in Rats

O. A. AL-SHABANAH, S. QURESHI, M. M. AL-HARBI, A. M. AL-BEKAIRI, N. M. AL-GHARABLY and M. RAZA *

Department of Pharmacology, College of Pharmacy, King Saud University, PO Box 2457, Riyadh 11451, Saudi Arabia

(Accepted 15 November 1999)

Abstract—The effect of methylglyoxal pretreatment on gastric mucosal injuries caused by 80% ethanol, 25% NaCl and 0.2 M NaOH, was investigated in rats. The effects caused by pylorous ligation accumulated gastric acid secretions and ethanol-induced changes in gastric mucus secretions, levels of proteins, nucleic acid, malondialdehyde (MDA) and non-protein sulfhydryl groups were also investigated. Methylglyoxal pretreatment at oral doses of 50, 100 and 200 mg/kg body weight was found to provide a dose-dependent protection against the ulcerogenic effects of different necrotizing agents used. With the same dose regimen methylglyoxal offered significant protection against ethanol-induced damage on the parameters evaluated for histopathology. Furthermore, the pretreatment afforded a dose-dependent inhibition of pylorous ligated accumulation of gastric acid secretions and ethanol-induced depletion of stomach wall mucus, proteins, nucleic acids, NP-SH contents and an increase in the MDA levels in gastric tissue. The protective effect of methylglyoxal against ethanol-induced damage to the gastric wall mucosa may be mediated through its effect on mucous production, proteins, nucleic acids, NP-SH groups and its free-radical scavenging property under the influence of polyamines stimulated by ornithine decarboxylase activity (ODC). © ***2000 Elsevier Science Ltd. All rights reserved***

Keywords: methylglyoxal; ulceration; gastric secretions; lipid peroxides; glutathione.

Abbreviations: DTNB = 5,5′-dithiobis (2-nitrobenzoic acid); EtOH = ethanol; MDA = malondialdehyde; MG = methylglyoxal; NP-SH = non-protein sulfhydryls; ODC = ornithine decarboxylase; TCA = trichloroacetic acid.

INTRODUCTION

Methylglyoxal (MG) is present in various frequently consumed beverages and foods, in particular in instant and brewed coffee, black tea, whisky and brandy (Nagao *et al*., 1986), in addition to its existence in mammalian tissues from various endogenous metabolic routes. Because MG is a reactive α-dicarbonyl it is thought to contribute to the physiological states either as a direct toxin or as a precursor for advanced glycation end-products. It is produced primarily from triose phosphates and is detoxified by glyoxalase pathway producing D-lactate. In a recent article, Beisswenger *et al*. (1999) have reported the levels of systemic MG in normal human subjects in the range of 123 ± 37 nmol/litre as measured by HPLC. Furthermore, it is reported to be produced by bacteria present in the human gut (Baskaran *et al*., 1989).

MG is reported to inhibit the proliferative activity of Yoshida ascites hepatoma AH-130 in rats (Tessitore *et al*., 1989), and human osteosarcoma cell lines (Sonoda *et al*., 1995). It is also reported as an antileukemic agent (Regenass *et al*., 1992). It has been shown to injure the cultured rat hepatocytes and cause generation of reactive oxygen species (Kalapos *et al*., 1993) and reduce glutathione in isolated hepatocytes from mice (Kalapos *et al*., 1991, 1992; Vander-Jagt *et al*., 1997). Baskaran and Balasubramanian (1990) found it to inhibit proteins and nucleic acid synthesis in rat enterocytes and colonocytes.

*Corresponding author. Fax: +966 1 467 6383; e-mail: mrsheikh@ksu.edu.sa

PII: S0278-6915(00)00049-1

Nevertheless, MG has been found to react differently to the normal gastric tissue in rats. In the glandular stomach mucosa of rats, an increase in the ornithine decarboxylase (ODC) activity and nucleic acid synthesis has been documented (Furihata and Matsushima, 1987; Furihata *et al.*, 1985). Previous studies have described ODC to be essential for growth of cells and tissues (Coleman *et al.*, 1993; Russell and Durie, 1978). The ODC activity has been found to be more pronounced in the recovery of gastric mucosal tissue after injury (Luk *et al.*, 1980; Wang and Johnson, 1991). Luck and Bass (1994) found it to be related to the elevation of polyamines, the potent antioxidants in different tissues of rat, guinea pig and swine (Matkovics *et al.*, 1993; Pavlovic *et al.*, 1992). Thus, MG appears to possess tissue specific role as an oxidant and/or an antioxidant.

As mentioned earlier, MG has been reported to interfere with the sulfhydryls causing an inactivation of cells. It has also been demonstrated that depletion of sulfhydryls causes an increased toxicity by MG (Dornish and Pettersen, 1989). On the other hand, MG has a tissue-specific role and is shown to increase ODC activity in gastric tissue of the rat, in turn causing an elevation in tissue polyamine contents and modulate lipid peroxidation. The present study on the inhibition of gastric mucosal damage by MG treatment in rats was undertaken in view of: (i) its widespread presence in frequently consumed beverages and foods; (ii) its influence on polyamine biosynthesis, triggered by increased ODC activity; and (iii) the role of polyamines in modulation of lipid peroxidation.

MATERIALS AND METHODS

Animals

Male Wistar albino rats (home bred), approximately of the same age, weighing 180–200 g were randomly assigned to different control and treatment groups. The animals were maintained under standard conditions of temperature, humidity and light (12 hr dark, 12 hr light). They were provided with purina chow and free access to water. Before testing, the animals were fasted for 48 hr with access to water *ad lib*.

Test compound and doses

Methylglyoxal (pyruvaldehyde) 50% in water (Fluka AG, Switzerland) was used as the test compound.

A series of trial doses of MG was administered to see their individual responses at certain dose levels. MG pretreatment was found to produce cytoprotective effects evident by a low number of gastric lesions against ethanol at an oral dose of 100 mg/kg body weight; hence oral doses of 50, 100 and 200 mg/kg body weight were used in the present study. Preliminary experiments revealed that oral gavage of MG at doses of 50–200 mg/kg had no significant effect on any of the parameters studied when compared with untreated control. An aqueous solution of MG (pH 4.7–5.2) or water alone (vehicle) was administered by gavage to the fasted rats.

Gastric lesions induced by necrotizing agents

The animals in the test groups were given 1 ml per rat of different necrotizing agents (80% ethanol, 0.2 M NaOH and 25% NaCl) which are known to produce gastric lesions (Al-Bekairi *et al.*, 1992; Robert *et al.*, 1983). NaCl (25%) and NaOH (0.2 M) were used only in cytoprotection studies. Based on the gastric emptying in fasted rats, MG was given 30 min before the necrotizing agents. Animals were killed under ether anaesthesia 1 hr after treatment with ulcerogenic agents. The stomach was excised and opened along the greater curvature. After washing with normal saline, the gastric lesions were quantified using a binocular magnifier. The ulcers were scored according to the method of Valcavi *et al.* (1982) and assessed on the basis of their dimensions: Deep circular ulcers more than 8 mm = 10; 7–8 mm = 8; 6–7 mm = 7; 5–6 mm = 6; 4–5 mm = 5; 3–4 mm = 4; 2–3 mm = 3; 1–2 mm = 2 and 0–1 mm = 1. The deep linear ulcer more than 10 mm in length = 6 and linear ulcer less than 10 mm in length = 3. The score for each single lesion were then summed up for the determination of ulcer index.

Indomethacin-induced gastric ulcers

Indomethacin was suspended in 1% carboxymethylcellulose in water (6 mg/ml) and administered to the fasted rats in a dose of 30 mg/kg (0.5 ml/100 g). Control rats were treated similarly with an equivalent amount of the vehicle (Bhargava *et al.*, 1973). The animals were killed 5 hr after treatment (Valcavi *et al.*, 1982). The stomachs of the animals were excised off the body, rinsed with normal saline, and studied according to the procedure of Szabo *et al.* (1985).

Histopathological assessment

The gastric tissue samples were fixed in neutral buffered formalin for 24 hr. After fixation each sample was dehydrated, cleared and embedded in paraffin wax. Sections of about 5 μm thickness were cut by employing an American optical rotary microtome. These sections were stained with haemotoxylin and eosin using routine procedures (Culling, 1974). The slides were then examined under a microscope for pathomorphological changes as congestion, haemorrhage, oedema and erosions using an arbitrary scale for the assessment of severity of these changes (Al-Harbi *et al.*, 1995).

Determination of biochemical changes in ethanol-treated rats

Estimation of protein and nucleic acids

The stomachs were rapidly dissected off the animals, frozen in liquid nitrogen, and stored at −20°C

until they were analyzed for total proteins and nucleic acid (RNA, DNA) contents. Total protein was determined by the method of Lowry *et al.* (1951). The method described by Bregman (1983) was used to determine the levels of nucleic acids. Tissues were homogenized and the homogenate was suspended in ice-cold trichloroacetic acid (TCA). After centrifugation, the pellet was leached for fats with ethanol and extracted with hot TCA. DNA was determined by treating the nucleic acid extract with diphenylamine reagent and measuring the intensity of the blue colour at 600 nm. For quantification of RNA, the nucleic acid extract was treated with orcinol and absorbance of green colour was measured at 660 nm. Nucleic acids were quantified using the standard curves.

Estimation of non-protein sulfhydryl groups (NP-SH)

Gastric mucosal NP-SH was measured according to the method of Sedlak and Lindsay (1968). The glandular stomach was removed and homogenized in ice-cold 0.02 M ethylenediaminetetraacetic acid. The homogenate was mixed with distilled water and 50% (w/v) aqueous TCA and centrifuged; the supernatants were mixed with phosphate buffer (pH 8), 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB) was added and the sample was shaken. The absorbance was read within 5 min of addition of DTNB, at 412 nm, against a reagent blank with no homogenate.

Malondialdehyde estimation (MDA)

The method described by Ohkawa *et al.* (1979) was followed. The tissues were homogenized in aqueous KCl, suspended in thiobarbituric acid reagent and incubated. After centrifugation, the optical density of the clear pink supernatant was read at 532 nm. Malondialdehyde bis(dimethyl acetal) was used as standard.

Gastric wall mucus determination

The modified procedure of Corne *et al.* (1974) was used to determine gastric-wall mucus. The glandular segments from the stomachs were removed and weighed. Each segment was transferred immediately to 1% Alcian blue solution (in sucrose solution, buffered with sodium acetate, pH 5), and the excess dye was removed by rinsing with sucrose solution. The dye complexed with the gastric wall mucus was extracted with magnesium chloride solution. A 4-ml aliquot of blue extract was then shaken with an equal volume of diethyl ether. The resulting emulsion was centrifuged and the absorbance of the aqueous layer was recorded at 580 nm. The quantity of Alcian blue extracted/g (net) of glandular tissue was then calculated.

Determination of anti-secretary activity

The method of Shay *et al.* (1945) was used to determine the anti-secretary activity. The animals were fasted for 48 hr with free access to water before ligation of the pylorus under light ether anaesthesia. Care was taken not to bleed or occlude the blood vessels. MG was administered intraduodenally, immediately after pylorus ligation. The animals were sacrificed, 6 hr after the pylorus ligation. The stomachs were removed, contents collected, measured, centrifuged and subjected to analysis for titrable acidity against 0.01 N NaOH to pH 7 and total acid output was calculated.

Statistical analysis

The readings shown are mean ± standard error of means. The mean determination of treatment groups was compared statistically with that of control by using Student's *t*-test.

RESULTS

The results of our present study are summarized in Tables 1–8.

Effect on the gastric lesions induced by different ulcerogenic agents

Pretreatment with MG was found to inhibit the ulcers induced by different ulcerogenic agents (Table 1). Inhibition was statistically significant against 80% ethanol at the higher doses of MG (100 mg/kg, $P < 0.05$ and 200 mg/kg, $P < 0.001$). Ulcers induced by 0.2 M NaOH and 25% NaCl were significantly prevented by all the three doses of MG (50 mg/kg, $P < 0.05$; 100 and 200 mg/kg, $P < 0.001$). Indomethacin-induced ulcers were also inhibited by MG. The inhibition was statistically significant (50 mg/kg, $P < 0.05$; 100 and 200 mg/kg $P < 0.01$) (Table 2).

Effect on histopathological gastric lesions

Treatment of fasted animals with ethanol induced considerable damage in gastric mucosa which was evident by a prominent change in the tissue evaluated for the parameters on congestion, oedema, hemorrhage and erosion formation. Pretreatment of animals with oral MG provided a significant and dose-dependent protection to the destructive action of ethanol evaluated for the same parameters (Table 3, Plates 1–5).

Effect on protein and nucleic acid contents

Treatment with ethanol significantly ($P < 0.001$) reduced the protein and nucleic acid concentrations of gastric mucosa as compared with the control. MG pretreatment was found to significantly protect against the decline in contents of DNA (50 mg/kg, $P < 0.01$; 100 and 200 mg/kg, $P < 0.001$), RNA (100 mg/kg, $P < 0.05$, and 200 mg/kg, $P < 0.001$) and proteins (50 mg/kg, $P < 0.05$, 100 and 200 mg/kg, $P < 0.01$) as compared to the values obtained after treatment with ethanol alone (Table 4).

Effect on gastric mucosal NP-SH contents

Ethanol treatment significantly reduced the NP-SH concentration in the gastric mucosa ($P < 0.001$) as compared with the control. MG pretreatment significantly

Table 1. Effect of methylglyoxal on the induction of gastric ulcers by various necrotizing agents in rats

S1. no.	Treatment and dose (mg/kg)	Ulcer index (mean±SE)			
		Vehicle (1 ml/rat)	80% Ethanol (1 ml/rat)	0.2 M NaOH (1 ml/rat)	25% NaCl (1 ml/rat)
1	Control (distilled water)	—	26.20±3.33	22.80±2.92	24.20±2.31
2	Methylglyoxal (50)	—	16.00±3.96	10.80±2.75*	15.20±1.97*
3	Methylglyoxal (100)	—	11.40±3.44*	6.40±1.50***	6.10±3.00***
4	Methylglyoxal (200)	—	4.00±3.09***	4.60±1.29***	4.80±2.47***

Five rats were used in each group. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$; Student's *t*-test.

Table 2. Effect of methylglyoxal pretreatment on indomethacin-induced gastric mucosal damage in rats

S1. no.	Treatment and dose (mg/kg) gavage	Ulcer index (mean ± SE)	Percent inhibition
1	Indomethacin (30)	37.60±6.23	—
2	Methylglyoxal (50) + indomethacin (30)	20.40±2.42*	45.74
3	Methylglyoxal (100) + indomethacin (30)	10.20±1.79**	72.87
4	Methylglyoxal (200) + indomethacin (30)	7.40±1.80**	80.32

Five rats were used in each group. *$P < 0.05$, **$P < 0.01$; Student's *t*-test.

Table 3. Effect of methylglyoxal pretreatment on the histopathological parameters after ethanol in rat stomach tissue

S1. no.	Treatment and dose (mg/kg) gavage	Oedema	Congestion	Haemorrhage	Erosion
1	Control (Distilled water) (1 ml/rat)	–[a]	–	–	–
2	80% Ethanol (1 ml/rat)	+[b]	+++[d]	+++	+++
3	Methylglyoxal (50) + 80% EtOH (1 ml/rat)	+	++[c]	+	++
4	Methylglyoxal (100) + 80% EtOH (1 ml/rat)	–	++	+	++
5	Methylglyoxal (200) + 80% EtOH (1 ml/rat)	–	–	–	–

[a]–, normal; [b]+, mild; [c]++, severe; [d]+++, intensely severe.

Table 4. Effect of methylglyoxal pretreatment on the nucleic acid and protein concentrations in the stomach wall of rats treated with 80% ethanol

S1. no.	Treatment and dose (mg/kg) gavage	DNA μg/100 mg wet tissue (mean ± SE)	RNA μ/100 mg wet tissue (mean ± SE)	T. protein mg/100 mg wet tissue (mean ± SE)
1	Control (distilled water) (1 ml/rat)	427.05±9.51	525.50±16.69	14.51±0.42
2	80% Ethanol (1 ml/rat)	282.21±8.03***[a]	297.51±25.63***	11.67±0.389***
3	Methylglyoxal (50) + 80% EtOH (1 ml/rat)	325.08±9.86**	342.85±24.90	13.23±0.29*
4	Methylglyoxal (100) + 80% EtOH (1 ml/rat)	369.63±13.71***	401.73±26.05*	14.11±0.56**
5	Methylglyoxal (200) + 80% EtOH (1 ml/rat)	393.19±11.81***	467.28±15.71***	14.72±0.58**

[a]Five rats were used in each group. Group 2 was statistically compared with group 1. Groups 3, 4 and 5 were statistically compared with group 2. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$; Student's *t*-test.

Table 5. Effect of methylglyoxal pretreatment on the concentration of NP-SH in the glandular stomachs of rats treated by gavage with 80% ethanol

S1. no.	Treatment and dose (mg/kg)	No. of rats	NP-SH concentrations (μg/100 mg/ wet tissue, mean ± SE)
1	Control (distilled water) (1 ml/rat)	5	11.12±1.21
2	80% Ethanol (1 ml/rat)	5	3.46±0.26*
3	Methylglyoxal (50) + 80% ethanol (1 ml/rat)	5	7.35±0.43*
4	Methylglyoxal (100) + 80% ethanol (1 ml/rat)	5	9.24±0.86*
5	Methylglyoxal (200) + 80% ethanol (1 ml/rat)	5	11.08±0.18*

prevented the decline in NP-SH concentrations at all the three doses ($P < 0.001$) as compared with the values obtained with ethanol alone (Table 5).

Effect on gastric tissue MDA contents

The administration of ethanol significantly ($P < 0.01$) increased the concentration of MDA in gastric mucosa (Table 6). Pretreatment with MG was found to protect against any rise in MDA levels in a dose-dependent manner. The protection was statistically significant at the higher doses (100 mg/kg, $P < 0.05$ and 200 mg/kg, $P < 0.01$) as compared to the values obtained after treatment with ethanol alone.

Table 6. Effect of methylglyoxal pretreatment on the concentration of lipid peroxides in the glandular stomachs of rats treated with 80% ethanol

Sl. no.	Treatment and dose (mg/kg body weight)	No. of rats	Malondialdehyde concentration (nmol/g wet tissue) Mean±SE
1	Control (distilled water) (1 ml/rat)	5	201.66±13.75
2	80% Ethanol (1 ml/rat)	5	285.86±17.44**
3	Methylglyoxal (50) + 80% ethanol (1 ml/rat)	5	257.00±15.14
4	Methylglyoxal (100) + 80% ethanol (1 ml/rat)	5	231.60±9.03*
5	Methylglyoxal (200) + 80% ethanol (1 ml/rat)	5	218.04±7.73**

Group 2 was statistically compared with group 1. Groups 3, 4 and 5 were statistically compared with group 2. *$P < 0.05$, **$P < 0.01$; Student's t-test.

Table 7. Effect of methylglyoxal on the induction of changes in gastric wall mucus by 80% ethanol

Sl. no.	Treatment and dose (mg/kg body weight)	No. of rats	Gastric wall mucus (μg Alcian blue/g wet glandular tissue)
1	Control (1 ml DW/rat)	5	389.78±16.72
2	80% Ethanol (1 ml/rat)	5	301.24±5.78**[a]
3	Methylglyoxal (50) + 80% ethanol (1 ml/rat)	5	321.74±11.92
4	Methylglyoxal (100) + 80% ethanol (1 ml/rat)	5	348.54±8.74*
5	Methylglyoxal (200) + 80% ethanol (1 ml/rat)	5	372.52±6.78**

[a]Group 2 was statistically compared with group 1. Groups 3, 4 and 5 were statistically compared with group 2. *$P < 0.01$, **$P < 0.001$; Student's *t*-test.

Table 8. Effect of methylglyoxal on the gastric secretions and acidity in pylorus ligated rats

Sl. no.	Treatment and dose (mg/kg body weight)	Volume of gastric secretion (ml) Mean±SE	Titrable acidity mEq/litre Mean±SE
1	Control	12.46±0.61[a]	62.45±9.73
2	Methylglyoxal (50)	10.72±0.39*	54.56±3.97
3	Methylglyoxal (100)	7.87±0.41**	41.32±4.11
4	Methylglyoxal (200)	3.29±0.17**	31.74±2.94*

[a]Five rats were used in each group. *$P < 0.05$, **$P < 0.001$; Student's *t*-test.

Effect on gastric wall mucus

There was a significant ($P < 0.001$) decrease in the gastric wall mucus after treatment with ethanol. Pretreatment with MG was found to protect against any decline in the gastric wall mucus levels of glandular gastric mucosa at the higher doses (100 mg/kg, $P < 0.01$; 200 mg/kg, $P < 0.001$) as compared to ethanol group (Table 7).

Effect on pylorus ligation accumulated gastric acid secretions and acidity

The volume of gastric acid secretions and total titrable acid contents were significantly high in control group after pylorus ligation for 6 hr in comparison of the treatment groups. After ligation, treatment with MG reduced the volume of gastric secretions and the titrable acid contents. The inhibition of gastric secretions were statistically significant at all the three doses (50 mg/kg, $P < 0.05$; 100 and 200 mg/kg, $P < 0.001$). Whereas the reduction in the titrable acidity was statistically significant ($P < 0.05$) only at a dose of 200 mg/kg, body weight, when compared to the control group (Table 8).

DISCUSSION

Results of our present study clearly demonstrate that MG confers a dose-dependent protection against the gross damaging action of ethanol and other destructive agents on gastric mucosa of rats. Results on histopathological assessment revealed that pretreatment with MG prevented erosion formation, congestion, haemorrhage and oedema in the gastric tissue induced by destructive stimuli. It was also found to inhibit the ethanol-induced changes in gastric wall mucus and pylorus ligation accumulated secretions and titrable acid output. These results reveal a relation between the mucosal cytodestruction and the depletion of mucous and gastric acid secretions. Previous reports have also described ulcerogenic process to be caused by an imbalance between mucus and erosive action of acid and gastric mucosal resistance (Salim, 1990; Wallace and Whittle, 1986). An increase in gastric acid output has been considered to be contributing to the development of peptic ulceration and is termed as ''aggressive factor'' (Goa and Monk, 1987).

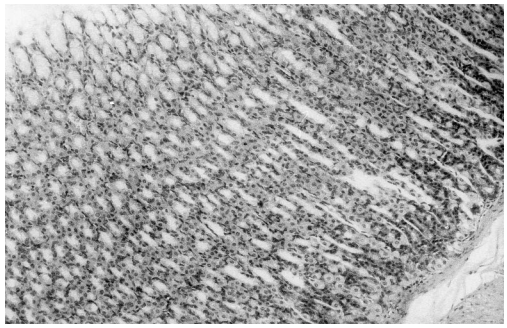

Plate 1. Section through gastric mucosa of control rat showing normal appearance. Haematoxylin and eosin, ×100.

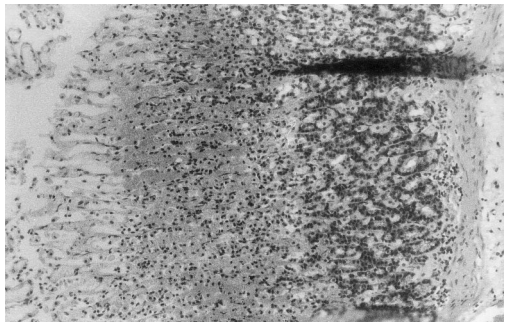

Plate 2. Section through gastric mucosa of rat treated with ethanol (80%, 1 ml) showing congestion, haemorrhage and necrosis. Haematoxylin and eosin, ×100.

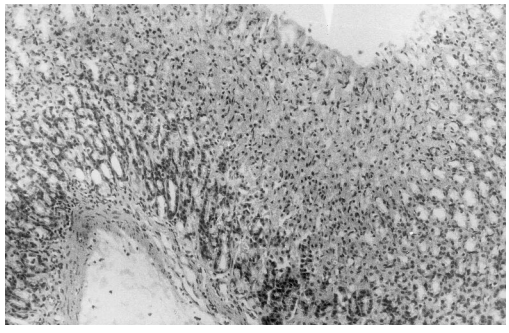

Plate 3. Section through gastric mucosa of rat treated with methylglyoxal (50 mg/kg) and ethanol (80%, 1 ml) showing moderate haemorrhage and erosions. Haematoxylin and eosin, ×100.

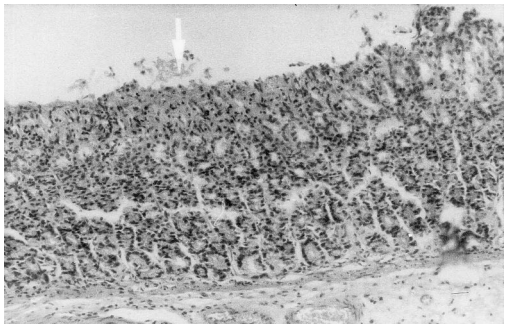

Plate 4. Section through gastric mucosa of rat treated with methylglyoxal (100 mg/kg) and ethanol (80%, 1 ml) showing mild congestion, haemorrhage and necrosis. Haematoxylin and eosin, ×100.

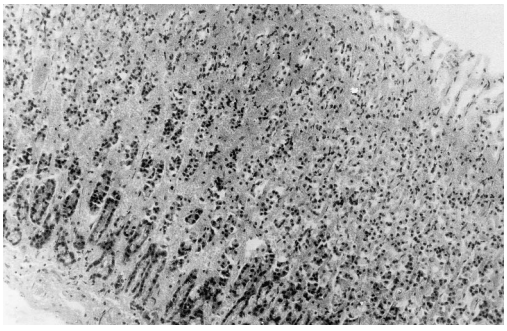

Plate 5. Section through gastric mucosa of rat treated with methylglyoxal (200 mg/kg) and ethanol (80%, 1 ml) showing mild congestion and haemorrhage. Haematoxylin and eosin, ×100.

Our data on the inhibition of indomethacin-induced ulcers observed in the present study suggests the role of prostaglandins in cytoprotection and antisecretary activity of MG. Earlier studies demonstrated that natural prostaglandins and their synthetic analogues inhibit gastric acid secretions in animals and man (Robert *et al*., 1967, 1976; Valcavi *et al*., 1982; Wilson *et al*., 1971) and prevent experimental ulcerations induced by a variety of destructive stimuli (Lee *et al*., 1973; Robert 1976, 1977; Robert *et al*., 1968; Valcavi *et al*., 1982).

Furthermore the results on anti-ulcer and anti-secretary activity of MG pretreatment observed in the present study are supported by our data on its inhibitory effect on ethanol-induced depletion in the contents of proteins, nucleic acids and NP-SH and an increase in the levels of MDA in gastric mucosa. The exact mechanism of MG-induced protection of ulcerogenic and anti-secretary processes is not known. Nevertheless, it appears to be related to the influence of MG on gastric NP-SH levels observed in the present study. Previous studies support that depletion of NP-SH concentrations (Kosower and Kosower, 1978; Szabo *et al*., 1981) and increase in contents of free radicals (Butterfield and McGraw, 1978; Del-Maestro *et al*., 1980; Salim, 1990) mediate tissue injury by stimulating lipid peroxidation and membrane damage by cross-linking proteins, lipids and nucleic acids. Thus, an MG-induced increase in the gastric concentrations of NP-SH might have inhibited the impact of cytodestructive stimuli on the proteins, nucleic acids and malondialdehyde, probably by binding to the reactive moieties (Szabo *et al*., 1981).

Other possible modes of action of protection by MG may be due to its influence on the ODC activity (Furihata *et al*., 1985) which result in the activating biosynthesis of polyamines (Coleman *et al*., 1993;

Hayashi, 1989; Tabor and Tabor, 1984). Earlier studies have reported polyamines to modulate lipid peroxidation (Gilad *et al*., 1993; Matkovics *et al*., 1993; Pavlovic *et al*., 1992) and cause rapid growth of tissues (Russel and Duri, 1978). Luk *et al*. (1980) and Wang and Johnson (1991) have also reported an increase in the concentration of putrescine and ODC enzymes activity in intestinal mucosa during recovery after injury. Thus, it appears that polyamines under the influence of ODC activity may play a major role in the gastroprotection conferred by MG.

In summary, the results of the present investigations clearly demonstrate anti-ulcerogenic and anti-secretary activity of MG against different cytodestructive stimuli used in the present study. Although at this time it is difficult to explain the exact mechanism of action, the effects of MG on gastric mucus production, proteins, nucleic acids, MDA and NP-SH concentrations observed in our present study suggest a multifactorial mechanism, involving its influence on ODC activity and biosynthesis of polyamines. Further studies are warranted to evaluate MG at the pharmacologically effective doses before any consideration for clinical trials.

REFERENCES

Al-Bekairi A. M., Qureshi S., Ahmed M. M., Afzal M. and Shah A. H. (1992) A study of uric acid pretreatment for the protection of rat gastric mucosa against toxic damage. *Food and Chemical Toxicology* **30,** 525–531.

Al-Harbi M. M., Qureshi S., Ahmed M. M., Raza M., Miana G. A., Afzal M. and Shah A. H. (1995) Inhibition of gastric mucosal damage by santonin pretreatment in rats. *Drug Development Research* **34,** 1–8.

Baskaran S. and Balasubramanian K. A. (1990) Toxicity of methylglyoxal towards rat enterocytes and colonocytes. *Biochemistry International* **21,** 165–174.

Baskaran S., Rajan D. P. and Balasubramanian K. A. (1989) Formation of methylglyoxal by bacteria isolated from human faeces. *Journal of Medical Microbiology* **28,** 211–215.

Beisswenger P. J., Howell S. K., Touchette A. D. and Szwergold B. S. (1999) Metformin reduces systemic glyoxal levels in type 2 diabetes. *Diabetes* **48,** 198–202.

Bhargava K. P., Gupta M. B. and Tangri K. K. (1973) Mechanism of ulcerogenic activity of indomethacin and oxyphenbutazone. *European Journal of Pharmacology* **22,** 191–195.

Bregman A.A. (1983) In *Laboratory Investigation and Cell Biology*, pp. 51–60. Wiley, New York.

Butterfield J. D. and McGraw C. P. (1978) Free radical pathology. *Stroke* **9,** 443–445.

Coleman C. S., Stanley B. A. and Pegg A. E. (1993) Effect of mutations at active site residues on the activity of ornithine decarboxylase and its inhibition by active site-directed irreversible inhibitors. *Journal of Biological Chemistry* **268,** 24572–24579.

Corne S. J., Morrisey S. M. and Wood R. J. (1974) A method for quantitative estimation of gastric barrier mucus. *Journal of Physiology* **242,** 116–117.

Culling C.F.A (1974) *Handbook of Histopathological and Histochemical Techniques*, 3rd edn, pp. 73, 126 & 159. Butterworth, London.

Del Maestro R. F., Thaw H. H., Bjork J., Planker M. and Arfors K. E. (1980) Free radicals as mediators of tissue injury. *Acta Physiologica Scandinavica (Suppl.)* **492,** 43–57.

Dornish J. M. and Pettersen E. O. (1989) Potentiation of cis-dichlorodiammineplatinum (II)-induced cytotoxicity by methylglyoxal. *Anticancer Research* **9,** 1137–1142.

Furihata C. and Matsushima T. (1987) Use of *in vivo*/*in vitro* unscheduled DNA synthesis for identification of organ specific carcinogens. *Critical Reviews in Toxicology* **17,** 245–277.

Furihata C., Sato Y., Matsushima T. and Tatematsu M. (1985) Induction of ornithine decarboxylase and DNA synthesis in rat stomach mucosa by methylglyoxal. *Carcinogenesis* **6,** 91–94.

Gilad G. M., Gilad V. H. and Wyatt R. J. (1993) Accumulation of exogenous polyamines in gerbil brain after ischemia. *Molecular Chemical Neuropathology* **18,** 197–210.

Goa K. L. and Monk J. P. (1987) Enprostil: A preliminary review of its pharmaco-dynamics and pharmacokinetic properties and therapeutic efficacy in the treatment of peptic ulcer disease. *Drugs* **3,** 539–559.

Hayashi S. (1989) *Ornithine Decarboxylase: Biology, Enzymology and Molecular Genetics*, pp. 1–147, Pergamon, New York.

Kalapos M. P., Garzo T., Antoni F. and Mandl J. (1992) Accumulation of S.D. lactoyl glutathione and transient decrease of glutathione level caused by methylglyoxal load in isolated hepatocytes. *Biochimica et Biophysica Acta* **1135,** 159–164.

Kalapos M. P., Littauer A. and de-Groot H. (1993) Has reactive oxygen a role in methylglyoxal toxicity? A study on cultured rat hepotocytes. *Archives of Toxicology* **67**, 369–372.

Kalapos M. P., Schaff Z., Garzo T., Antoni F. and Mandl J. (1991) Accumulation of phenols in isolated hepatocytes after pretreatment with methylglyoxal. *Toxicology Letters* **58,** 181–191.

Kosower N. S. and Kosower E. M. (1978) The glutathione status of cells. *International Reviews of Cytology* **54,** 109–160.

Lee Y. H., Cheng W. D., Bianchi R. G., Mollison K. and Hansen J. (1973) Effects of oral administration of PGE_2 on gastric secretion and experimental peptic ulcerations. *Prostaglandins* **3,** 29–45.

Lowry O. H., Rosebrough N. S., Farr A. L. and Randall R. J. (1951) Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry* **193,** 265–275.

Luck M. S. and Bass P. (1994) Inhibition of ornithine decarboxylase does not prevent intestinal smooth muscle hyperplasia in the rat. *American Physiological Society* **0193-1857,** G1021–G1027.

Luk G. D., Marton L. J. and Baylin S. B. (1980) Ornithine decarboxylase is important in intestinal mucosal maturation and recovery from injury in rats. *Science* **210,** 195–198.

Matkovics B., Kecskemeti V., Varga S. I., Novak Z. and Kertesz Z. (1993) Antioxidant properties of di-and polyamines. *Comparative Biochemistry and Physiology B* **104,** 475–479.

Nagao M., Fujita Y., Wakabayashi K., Nukaya H., Kosuge T. and Sugimura T. (1986) Mutagens in coffee and other beverages. *Environmental Health Perspectives* **67,** 89–91.

Ohkawa H., Ohishi N. and Yagi K. (1979) Assay of lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry* **95,** 351–358.

Pavlovic D. D., Uzunova P., Galabova T., Peneva V., Sokolova Z., Bjelakovic G. and Ribarov S. (1992) Polyamines as modulators of lipid peroxidation. *General Physiology and Biophysics* **11,** 203–211.

Regenass U., Caravatti G., Mett H., Stanek J., Schneider P., Muller M., Matter A., Vertino P. and Porter C. W. (1992) New S-adenosylmethionine decarboxylase inhibitors with potent antitumor activity. *Cancer Research* **52,** 4712–4718.

Robert A. (1976) *Advances in Prostaglandin and Thromboxane Research*, ed. B. Samuelsson and R. Paoletti, Vol. 2, pp. 507. Raven Press, New York.

Robert A. (1977) *Prostaglandins and Thromboxanes*, ed. F. Berti, B. Samuelsson and G. P. Velo, pp. 287. Plenum Press, New York.

Robert A., Schultz J. R., Nezamis J. E. and Lancaster C. (1976) Gastric antisecretary and antiulcer prperties of PGE_2, 15-methyl PGE_2 and 16,16-dimethyl PGE_2. Intravenous, oral and intrajejunal administration. *Gastroenterology* **70,** 359–370.

Robert A., Nezamis J. E., Lancaster C., Davis J. P., Field S. O. and Manchar A. J. (1983) Mild irritants prevented gastric necrosis through adaptive cytoprotection mediated by prostaglandins. *American Journal of Physiology* **245,** G113–G121.

Robert A., Nezamis J. E. and Philips J. P. (1967) Inhibition of gastric secretion by prostaglandins. *American Digestive Diseases* **12,** 1073–1076.

Robert A., Nezamis J. E. and Phillips J. P. (1968) Effect of prostaglandin E_1 on gastric secretion and ulcer formation in the cat. *Gastroenterology* **55,** 481–487.

Russell D. H. and Durie B. G. M. (1978) Ornithine decarboxylase: a key enzyme in growth. *Progress in Cancer Research and Therapy* **8,** 43–58.

Salim A. S. (1990) Oxygen-derived free radicals and the prevention of duodenal ulcer relapse: A new approach. *American Journal of Medical Science* **300,** 1–6.

Sedlak J. and Lindsay R. H. (1968) Estimation of total protein-bound and non-protein sulfhydryl groups in tissue with Ellman's reagent. *Analytical Biochemistry* **25,** 192–195.

Shay H., Komarov S. A., Fels S. S., Meranza D., Grunstein M. and Siplet H. (1945) A simple method for uniform production of gastric ulceration in rat. *Gastroenterology* **5,** 43–61.

Sonoda J., Hibasami H., Yamoda H., Fujinami S., Nakashima K. and Ogihara Y. (1995) Methylglyoxal bis(cyclopentyl amidinohydrazone): antitumor effect against human osteosarcoma cells and combined effect with methotrexate, adriamycin and 4-hydroperoxy fosfamide. *Anticancer Research* **15,** 907–909.

Szabo S., Trier J. S., Brown A., Schnoor J., Homan H. D. and Bradford J. C. (1985) A quantification method for assessing the extent of experimental erosions and ulcers. *Journal of Pharmacological Methods* **13,** 59–66.

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

Tabor C. W. and Tabor H. (1984) Polyamines. *Annual Review of Biochemistry* **53,** 749–790.

Tessitore L., Bonelli G., Costelli P., Matera L., Pileri A., Baccino F. M. and Dianzani M. U. (1989) Effect of two aliphatic aldehydes, methylglyoxal and 4-hydroxypentenal, on the growth of Yoshida ascites hepatoma AH-130. *ChemicoBiological Interactions* **70,** 227–240.

Valcavi V., Caponi R., Brambilla A., Palmira F., Monoja F., Bernini F., Mustani R. and Fumagall R. (1982) Gastric antisecretary anti-ulcer and cytoprotective properties of 9-hydroxy-19, 20-bis-nor-prostanic acid in experimental animals. *Arzneimittel Forschung* **32,** 657–663.

Vander-Jagt D. L., Hunsaker L. A., Vander-Jagt T. J., Gomez M. S. and Gonzales D. M. (1997) Inactivation of glutathione reductase by 4-hydroxy-nonenal and other endogenous aldehydes. *Biochemical Pharmacology* **53,** 1133–1140.

Wallace J. L. and Whittle B. J. R. (1986) Role of mucus in the repair of gastric epithelial damage in the rat. *Gastroenterology* **91,** 603–611.

Wang J. Y. and Johnson L. R. (1991) Polyamines and ornithine decarboxylase during repair of duodenal mucosa after stress in rats. *Gastroenterology* **100,** 333–343.

Wilson D. E., Phillips C. and Levine R. A. (1971) Inhibition of gastric secretion in man by prostaglandin A. *Gastroenterology* **61,** 201–206.