J. Pharm. Pharmacol. 2000, 52: 1519–1522
Received May 8, 2000
Accepted July 28, 2000

Effect of Dimethylglycine on Gastric Ulcers in Rats

K. HARIGANESH AND J. PRATHIBA

Department of Pharmacology, Kasturba Medical College, Manipal – 576119, Karnataka, India

Abstract

Dimethylglycine is an anti-stress nutrient with antioxidant properties. Recently, studies have implicated the generation of oxygen-derived free radicals and lipid peroxidation as one of the mechanisms in the pathogenesis of gastric ulcer. Hence, we evaluated the anti-ulcer activity of dimethylglycine in various rat models of ulcer and also investigated the probable antioxidant mechanism of the anti-ulcer effect.

Dimethylglycine at a dose of 25 and 35 mg kg^{-1} significantly reduced ulcer number, ulcer size and ulcer index in pyloric-ligation-, ibuprofen- and stress-induced ulcers. The 35 mg kg^{-1} dose was more effective than 25 mg kg^{-1} and was comparable to famotidine. Dimethylglycine did not produce any significant change in acid secretion, unlike famotidine. There was a significant increase in plasma and tissue malondialdehyde levels in pyloric-ligated rats but these levels fell following dimethylglycine treatment. Also, there was a significant reduction in glutathione levels after dimethylglycine treatment.

The results suggest that the gastroprotective effect of dimethylglycine could be mediated by its free radical scavenging activity and cytoprotection of gastric mucosa.

Peptic ulcer is one of the common diseases affecting man. Severe stress, *Helicobacter pylori* infection and ingestion of alcohol, aspirin and other non-steroidal anti-inflammatory agents (NSAIDs) are predisposing factors. It is well known that peptic ulcer develops when there is an imbalance between protective mechanisms and aggressive factors. An increase in acid and pepsin is found only in a minority of peptic ulcer patients. Nevertheless, drugs used in peptic ulcer have traditionally been directed mainly against a single luminal damaging agent, hydrochloric acid, and a plethora of drugs like antacids, anticholinergics, histamine H_2-antagonists, have flooded the market.

Recently, studies have implicated the generation of oxygen derived free radicals and lipid peroxidation as one of the mechanisms involved in the pathogenesis of gastric ulcers (Naito et al 1995; Desai et al 1997). Antioxidants are known to inhibit lipid peroxidation and scavenge free radicals. Hence, there is a need to develop drugs that are directed towards scavenging of these free radicals and which produce beneficial effects against gastric ulcers.

Dimethylglycine is an anti-stress nutrient and clinically useful in various stress-related disorders, including neurologic disorders, diabetes and cardiovascular diseases (Passwater 1987). It aids detoxification and acts as an antioxidant, protecting the body cells from unwanted reactions caused by free radicals (Passwater 1987). It is therefore possible that dimethylglycine could scavenge free radicals and produce beneficial effects against gastric ulcer. Hence, this study evaluates the anti-ulcer activity of dimethylglycine in different rat models of ulcer and also investigates probable antioxidant mechanisms of the anti-ulcer effect.

Correspondence: J. Prathiba, Department of Pharmacology, Kasturba Medical College, Manipal – 576119, Karnataka, India.

Materials and Methods

Animals and treatment

Healthy inbred male albino Wistar rats, 150–200 g, were used for the study. The rats were individually housed and allowed free access to normal diet and water. The dose of dimethylglycine was 25 or 35 mg kg^{-1} and that of famotidine was 3·6 mg kg^{-1}. The doses were decided upon by computing the human dose to rat. Dimethylglycine was prepared in water and famotidine suspended in gum acacia. Both drugs were administered orally.

For each model of gastric ulcer (pylorus-ligation method, ibuprofen- and stress-induced gastric ulcers), four groups with eight rats in each group were used.

Ulcer production

Pylorus-ligation method. One hour after drug or saline administration, the pylorus of the rats was ligated under light ether anaesthesia as described by Shay et al (1945). Twenty-two hours later rats were killed and their stomachs dissected out after ligating the cardiac end. Each stomach was cut open along the inner curvature and the contents were collected, then the mucosa was washed and the extent of ulceration was scored as per the method of Rao et al (1990). The gastric juice collected from each stomach was centrifuged and its volume was measured. Free and total acid was estimated with 0·01 N NaOH using Toepfer's reagent and phenolphthalein as indicators. The acid present was expressed as mEq h^{-1} per 100 g body weight.

Ibuprofen-induced gastric ulcers. Ibuprofen 300 mg kg^{-1} was given orally at 15-h intervals to rats to produce gastric ulcers. The rats were killed, 6 h after the second dose of ibuprofen, with excess of ether and ulcer scored as described earlier. Test drugs and saline were administered 60 min before each dose of ibuprofen.

Stress-induced ulcers. Rats were deprived of food for 12 h and then immobilised in a stress cage and forced to remain in a cold room (4–6°C) for 3 h. The rats were then killed and ulceration was scored. Saline and test drugs were administered 30 min before immobilisation.

Biochemical studies

Rats were divided into three groups with eight rats in each group. Group I served as the control while rats in groups II and III underwent pyloric ligation. Rats in group III were administered dimethylglycine (35 mg kg^{-1}) one hour before ligation.

Blood (1 mL) was withdrawn by cardiac puncture and the rats were anaesthetised with ether and killed by decapitation. The stomach was removed, washed with ice-cold saline and a 10% (w/v) homogenate was prepared in ice-cold 0·05 M potassium phosphate buffer (pH 7·4). It was then centrifuged at 7000 rev min^{-1} for 10 min at 4°C. The supernatant was collected for estimation.

Lipid peroxidation. The quantitative measurement of lipid peroxidation in plasma was performed according to the method of Satoh (1978) and that in gastric tissue by the method of Ohkawa et al (1979). The amount of malondialdehyde formed was quantified by reaction with thiobarbituric acid and used as an index of lipid peroxidation. The results are expressed as nmol malondialdehyde/mL of plasma and nmol malondialdehyde/g of wet tissue, respectively.

Glutathione estimation. Glutathione was estimated by the method of Beutler et al (1963) and the results are expressed as mg glutathione/g of wet tissue.

Statistical analysis

The results were analysed using one-way analysis of variance followed by studentized range procedure.

Results

Dimethylglycine, at both doses, and famotidine produced significant reduction in ulcer number, ulcer size and ulcer index compared with control rats in pyloric-ligation-, ibuprofen- and stress-

Table 1. Effect of dimethylglycine on pyloric-ligation-, ibuprofen- and stress-induced ulcers in rats.

		Control	Dimethylglycine		Famotidine
			25 mg kg^{-1}	35 mg kg^{-1}	3·6 mg kg^{-1}
Pyloric ligation	Ulcer no.	5·25 ± 0·85	2·25 ± 0·14*	0·25 ± 0·25*†	0·05 ± 0·25*
	Ulcer size (mm)	9·63 ± 2·37	4·25 ± 0·59*	0·25 ± 0·25*†	0·25 ± 0·25*
	Ulcer index	63·75 ± 24·95	10·00 ± 1·96*	0·50 ± 0·50*†	0·50 ± 0·50*
Ibuprofen	Ulcer no.	14·63 ± 1·91	4·50 ± 0·18*	0·75 ± 0·31*†	0*
	Ulcer size (mm)	27·75 ± 4·49	14·38 ± 0·82*	1·50 ± 0·60*†	0*
	Ulcer index	435·25 ± 104·10	65·88 ± 5·02*	2·25 ± 1·03*†	0*
Stress	Ulcer no.	11·75 ± 0·72	1·37 ± 0·73*	0·63 ± 0·26*†	0·63 ± 0·37*
	Ulcer size (mm)	20·00 ± 3·42	2·75 ± 1·69*	0·63 ± 0·26*†	1·38 ± 0·98*
	Ulcer index	251·75 ± 34·77	11·38 ± 7·20*	0·88 ± 0·48*†	3·37 ± 2·96*

Values represent means ± s.e.m., n = 8. *$P < 0·05$ vs control; †$P < 0·05$ vs dimethylglycine 25 mg kg^{-1}.

Table 2. Effect of dimethylglycine on gastric secretion produced by pyloric ligation in rats.

Drug	pH	Vol. of gastric juice (mL (100 mg body weight)$^{-1}$)	Free acidity (mEq L^{-1} h^{-1} (100 g body weight)$^{-1}$)	Total acidity (mEq L^{-1} h^{-1} (100 g body weight)$^{-1}$)
Control	2·71 ± 0·46	4·22 ± 0·45	3·14 ± 0·22	7·42 ± 0·46
Dimethylglycine 25 mg kg^{-1}	2·97 ± 0·36	3·81 ± 0·28	3·09 ± 0·36	6·18 ± 0·45
Dimethylglycine 35 mgkg^{-1}	3·97 ± 0·49	3·18 ± 0·40	2·95 ± 0·24	6·96 ± 0·48
Famotidine 3·6 mg kg^{-1}	4·03 ± 0·39	2·07 ± 0·26*	1·34 ± 0·15*	3·43 ± 0·33*

Values represent means ± s.e.m., n = 8. *$P < 0·05$ vs control.

induced ulcers. There was a significant reduction in ulcer number, ulcer size and ulcer index in rats treated with 35 mg kg^{-1} of dimethylglycine as compared with the lower dose of 25 mg kg^{-1} in all the three rat ulcer models (Table 1).

Famotidine significantly reduced the volume, free acidity and total acidity of gastric juice, while dimethylglycine treatment did not produce any change in these parameters at either of the doses used (Table 2).

There was a significant increase in plasma and tissue malondialdehyde levels in pyloric-ligated rats as compared with control rats. Following dimethylglycine treatment, there was a significant reduction in plasma, as well as tissue, malondialdehyde levels as compared with pyloric-ligated rats (Table 3).

Glutathione levels in pyloric-ligated rats were similar to those in control rats. However, in rats treated with dimethylglycine there was a significant reduction in the glutathione levels as compared with control and pyloric-ligated rats (Table 3).

Discussion

This study was undertaken to evaluate the anti-ulcer activity of dimethylglycine. At both the doses used, it showed significant anti-ulcer effect in pyloric-ligation-, ibuprofen- and stress-induced ulcer models. The higher dose of dimethylglycine was more effective than the lower dose and comparable to the standard drug, famotidine. Increased acid secretion has long been linked to gastric ulcer production and treatment is aimed at reducing acid secretion (Desai et al 1997). Dimethylglycine did not, however, significantly change acid secretion, unlike famotidine which reduced the gastric volume and the free and total acid, consistent with its H_2 receptor antagonist activity. This indicates that dimethylglycine produced its anti-ulcer effect by a mechanism other than alteration of gastric secretion.

It is well known that ibuprofen induces gastric ulcer by inhibiting prostaglandins, which are cytoprotective to gastric mucosa (Desai et al 1997). Dimethylglycine effectively reduced the gastric ulcer produced by ibuprofen, indicating a possible cytoprotective action on gastric mucosa. Several recent lines of evidence implicate the role of free radicals and lipid peroxidation in gastric mucosal damage (Naito et al 1995; Desai et al 1997). Stress is known to cause ischaemic conditions in the gastric mucosa by reducing blood flow following activation of parasympathetic and sympathetic nervous system. This causes generation of reactive oxygen species, especially the hydroxy radical (·OH), by the Haber-Weiss reaction causing lipid peroxidation and oxidative damage of gastric mucosa. Stress is also found to inactivate mucosal prostaglandin synthetase by accumulated H_2O_2 and inhibit the synthesis of prostaglandin which also favours the generation of reactive oxygen species (Bandyopadhyay et al 1999). *H. pylori* is known to

Table 3. Effect of dimethylglycine on plasma and tissue malondialdehyde and tissue glutathione levels in pylorus-ligated rats.

Group	Malondialdehyde concn		Tissue glutathione concn
	Plasma (nmol L^{-1})	Tissue (nmol (g wet tissue)$^{-1}$)	(mg (g wet tissue)$^{-1}$)
Control (group I)	2·84 ± 0·40	17·27 ± 1·67	3·07 ± 0·37
Pyloric ligation (group II)	8·31 ± 1·00*	142·38 ± 5·17*	3·47 ± 0·13
Dimethylglycine + pyloric ligation (group III)	3·23 ± 0·60†	22·12 ± 3·19†	0·94 ± 0·12*†

Values represent means ± s.e.m., n = 8. *$P < 0·05$ vs control; †$P < 0·05$ vs pyloric ligation.

attract neutrophils and on activation produces reactive oxygen species to cause oxidative damage (Davies & Simmonds 1994). Similarly, NSAIDs are also reported to cause lipid peroxidation (Desai et al 1997). Lipid peroxidation leads to loss of membrane fluidity, ion transport and membrane integrity of the surface epithelial cells and helps to generate gastric lesions (Bandyopadhyay et al 1999).

The detection and measurement of lipid peroxidation is the evidence cited to support the involvement of free radicals (Naito et al 1995). The thiobarbituric acid assay is the most popular method to estimate the malondialdehyde level, which is an indication of lipid peroxidation and free radical activity (Holley & Cheeseman 1993). In this study, there was a significant increase in plasma and tissue malondialdehyde levels in pyloric-ligated rats. This is consistent with studies (Oner et al 1994; Naito et al 1995) that associate increased lipid peroxidation with gastric ulcers. Moreover, the tissue malondialdehyde levels, higher than those in plasma, were indicative of localised gastric damage. Szabo et al (1981) demonstrated that exogenous sulfhydryl compounds induce gastric cytoprotection. Conversely, it is reported that depletion of endogenous glutathione (gastric mucosal sulfhydryl) can also induce mucosal protection, possibly by increasing prostaglandin release (Robert et al 1984).

Dimethylglycine reduced malondialdehyde levels in plasma as well as tissue, which correlates with its anti-ulcer effect. This is consistent with its reported antioxidant effect (Passwater 1987). Further, there was depletion of glutathione levels, consistent with previous studies (Robert et al 1984) and similar to that reported with verapamil (Al-Bekairi et al 1994). Glycine is an important substrate necessary for the synthesis of glutathione. It is possible that dimethylglycine, with structural similarity to glycine, could compete for glutathione synthetase, thereby leading to the deprivation of glycine in the biosynthesis of glutathione, and hence the latter's depletion. However, further investigation is required to fully explain glutathione depletion.

This study indicates that the anti-ulcer activity of dimethylglycine may be due to free radical scavenging activity and cytoprotection of gastric mucosa. Stress ulceration of the stomach is associated with clinical conditions like trauma, head injury, burns, shock, anxiety and neurological disorders. Studies have shown dimethylglycine to be a safe anti-stress nutrient and thus it could be specifically useful in the above conditions and also an important supplement against NSAID- and H. pylori-induced ulcers. Hence, dimethylglycine is a promising anti-ulcer drug in peptic ulcer therapy.

Further work could elucidate the other mechanisms involved in the anti-ulcer effect of dimethylglycine.

Acknowledgements

We thank Dr Malini for her assistance in the work, Dr Tom Ommen for providing the sample of dimethylglycine and Dr Gopalan Kutty for revision of the manuscript.

References

Al-Bekairi, A. M., Al Rajhi, A. M., Tariq, M. (1994) Effect of verapamil and hydralazine on stress and chemically-induced gastric ulcers in rats. Pharmacol. Res. 29: 225–236

Bandyopadhyay, U., Das, D., Bandyopadhyay, D., Bhattacharjee, M., Banerjee, R. K. (1999) Role of reactive oxygen species in mercaptomethylimidazole-induced gastric acid secretion and stress-induced gastric ulceration. Curr. Sci. 76: 55–63

Beutler, E., Duron, O., Kelly, B. M. (1963) Improved method for the determination of blood glutathione. J. Lab. Clin. Med. 61: 882–888

Davies, G. R., Simmonds, N. J. (1994) Helicobacter pylori stimulates antral mucosal reactive oxygen metabolite production in vivo. Gut 35: 179–185

Desai, J. K., Goyal, R. K., Parmar, N. S. (1997) Pathogenesis of peptic ulcer disease and current trends in therapy. Ind. J. Physiol. Pharmacol. 41: 3–15

Holley, A. E., Cheeseman, K. H. (1993) Measuring free radical reaction in vivo. Br. Med. Bull. 49: 407–498

Naito, Y., Yoshikawa, T., Matsuyama, K., Yagi, N., Aral, M., Nakamura, S., Yoshida, N., Konda, M. (1995) Effect of oxygen radical scavengers on the quality of gastric ulcer healing in rats. J. Clin. Gastroenterol. 21: S82–S86

Ohkawa, H., Ohishi, N., Yagi, K. (1979) Assay for lipid peroxides in animal tissues by the thiobarbituric acid reaction. Anal. Biochem. 95: 351–358

Oner, G., Izgut-Uysal, V. N., Senturk, U. K. (1994) Role of lipid peroxidation in calcium-induced impairment of the gastric mucosal barrier. Food. Chem. Toxicol. 32: 799–804

Passwater, R. A. (1987) Dimethylglycine (DMG) update: new studies confirm DMG improves health. Let's Live 2: 45–47

Rao, C. M., Ramesh, K. V., Bairy, K. L., Kulkarni, D. R. (1990) Zinc complexes of NSAIDs abolish gastric ulceration propensity of parent drugs. Indian Drugs 28: 64–67

Robert, A., Eberle, D., Kaplowitz, N. (1984) Role of glutathione in gastric mucosal cytoprotection. Am. J. Physiol. 247: G296–G304

Satoh, K. (1978) Serum lipid peroxidation in cerebrovascular disorders determined by a new calorimetric method. Clinica Chimica Acta 90: 37–43

Shay, H., Komarov, S. A., 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: 43–45

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