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N-(2-mercaptopropionyl) glycine protects against gastric oxidative injury induced by indomethacin in male rats.

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ABSTRACT

Reactive oxygen species (ROS) have been implicated in the etiology of indomethacin-induced gastric mucosal damage. The protective effect of the oral administration of N-(2- Mercaptopropionyl) glycine (MPG) from oxidative gastric mucosal injury induced by the non-steroidal anti-inflammatory drug indomethacin was investigated in the rat. The total areas of the gastric lesions, as well as lipid peroxidation, were significantly increased one hour after oral administration of indomethacin (15 mg/kg) indicating an acute oxidative injury. The activities of superoxide dismutase (SOD), catalase (CAT), glutathione-S-transferase (GST) as well as glutathione (GSH) content were significantly decreased in the gastric mucosa by indomethacin. The administration of indomethacin was associated with a significant decrease in total protein and albumin contents in serum, concomitant with significant increases in glucose concentration and the activities of lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) in serum. Pretreatment with MPG (2.5 mg 1kg body weight, ig) reduced gastric mucosal lesions, decreased the lipid peroxides and undermined the decrease in the activities of SOD, CAT, and GST in gastric mucosa. In addition, the administration of MPG one hour prior to the induction of gastric injury by indomethacin, abolished the indomethacin-induced decrease in total protein and albumin contents, as well as the increase in glucose concentration and the activity of LDH, but did not affect the ALP activity in serum. In conclusion, MPG has a protective potential from indomethacin-induced gastric ulceration and that the gastroprotective properties of MPG might attributed to its positive effects on the antioxidant system in indomethacin-induced gastric ulcers in rats. MPG might serve as a novel co-therapeutic agent when administered in combination with indomethacin to limit its oxidative injury.

Keywords: N-(2-mercaptopropionyl) glycine-gastric, indomethacin, antioxidants, gastric ulcer.

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INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAID) such as aspirin and indomethathin are known to induce gastric mucosal damage including bleeding, ulceration and perforation in humans and experimental animals [1] These adverse effects of NSAID were originally attributed to the inhibition of cyclooxygenase and the deficiency of endogenous prostaglandins, but the role of reactive oxygen species (ROS), lipid peroxidation (LPO) and antioxidizing mechanism in the pathogenesis of indomethacine induced damage has been scarcely studied [2&3]. Several reports suggested that inhibition of prostaglandin synthesis due to inhibition of cycloxygenase, was not the only mechanism responsible for gastric damage induced by NSAID [4&5]

A great attention has been focused on the role of ROS and LPO in mediating the gastric mucosal injury induced by NSAID. Naito *et al.* [4] suggested that LPO, mediated by oxygen radicals, plays an important role in the mechanism of ulcer aggravation induced by indomethacin. In addition, recent data has been reviewed to assess the role of oxygen radicals, LPO and lowered antioxidants levels in the pathogenesis of indomethacininduced gastric mucosal injury in rats and humans [5&6] suggesting that ROS and LPO play a major role in the gastric ulcer induction. In addition, there are reports suggest that NSAID such as indomethacin have pro-oxidant catalytic activity and initiate LPO by generating ROS and thereby interfering with antioxidant system of the mucosal cells [7]. A shift to a more oxidative state may result in uncontrolled LPO, protein oxidation and ultimately cell death.

N-(2-Mercaptopropionyl) glycine (MPG) is a synthetic aminothiol and antioxidant with a wide range of clinical applications [8]. It is an effective radioprotector against radiation- induced oxidative damage [9&10]. It has been used in experimental cardioprotection [11] *in vivo* and *in vitro* because it is effective at very low doses (20 mg/kg), far below the toxic dose of 2100 mg/kg. Therefore, the aim of the present study was focused on the ability of the sulphydryl compound MPG, as oxyradical scavenger; to reduce indomethacin induced gastric injury and oxidative stress.

MATERIALS AND METHODS

Male albino rats weighed about 150 g were used. The animals were deprived of food for 24 h before the experiment but had free access to water. Gastric mucosal haemorrhagic injury was induced by oral administration of indometbaciti (15 mg/kg body weight) in 0.5 ml wtaer. Indomethacin was obtained as meglumine liometacen (Chiesi Farmaceutics S.P.A., Italy), which dissolved in water to give indomethacin. All other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO).

Rats were divided into 4 groups each of six animals. The first group was the control rats did not receive any treatment. In the second group, animals received a single dose of N-2- mercaptopropionyl glycine (2.5 mg/kg body weight, ig) dissolved in distilled water [12]. The animals in the 3rd group received a single ig administration of indomethacin as described above. The animals in group 4 received MPG ig one hour before indomethacin administration at the same doses of groups 2&3. All experimental procedures were carried out in accordance with the guide for care and use of laboratory animals published by the US National institute of Health [NIH publication, 1985].

After one hour of indomethacin treatment, all groups of rats were sacrificed and the stomachs were excised. The stomachs were opened along the greater curvature and examined for gastric lesions. A lesion index (LI) of gross mucosal damage was estimated, using a scoring system based on the number and length of haemorrhagic mucosal erosions [13]. The gastric mucosa was scrapped off using a skin-scrapping spoon, and then homogenized for biochemical assays. Lipid peroxidation products (MDA+HNE), were determined as described by [14]. Total SOD activity was measured initially in mucosal homogenates by the method of [15]. The activity of catalase (CAT) was assayed as described by [16]. Glutathione (GSH) content was estimated as described by [17]. The activity of glutathione-S-transferase GST was determined according to the method of [18]. Total protein, albumin, glucose concentrations and lactate dehydrogenase (LDH), alkaline phosphatase (ALP) activities were determined using kites. Total protein, albumin, glucose, triglyceride concentrations and lactate dehydrogenase (LDH), alkaline phosphatase (ALP) activities were determined using kits from Bio-Merieux, France.

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All data were expressed as mean ± standard error of the mean. For comparison the ANOVA test was used. Differences were considered significant when P<0.05.

RESULTS

The administration of MPG alone did not affect the gastric mucosa and showed insignificant changes in the levels of MDA+HNE and GSH concentrations in serum and gastric mucosa (Table 1), as well as the activities of SOD, GST and CAT (Table 2) as compared with control rats. Gastric haemorrhagic lesions were significantly developed 1 hour after the i.g administration of indomethacin (Table 1). These lesions were accompanied with a significant increase in MDA+HNE concentration, an index of lipid peroxidation in gastric mucosa (Table 1). Oral administration of MPG I hour prior to indomethacin significantly reduced the lesion area and the rise in MDA+HNE concentration in gastric mucosa (Table 1).

Oral administration of indomethacin was associated with a significant decrease in the endogenous antioxidants, as evidenced by the decrease in GSH content in serum and gastric tissue as well as marked inhibition of SOD, CAT and GST activities in gastric mucosa cells. The administration of MPG 1 hour in advance of the induction of gastric injury by indomethacin, ameliorateed the decrease in the antioxidants including activities of SOD, CAT and GST in gastric mucosa cells (Table 2). On the other hand, MPG did not prevent the loss of serum and gastric mucosal GSH contents (Table 1).

Table 1. Effect of 15mg/kg indomethacin (Indo) as well as 2.5 mg/kg MPG or their combination on the lesion index (LI), Lipid peroxidation product (MDA+HNE, nmol/g) and gastric glutathione concentration, (GSH, mol/g) as well as serum GSH (mol/ml).

	Control	MPG	Indo	MPG+Indo
Lesion Index	- -	- -	35 ± 2.3[a]	13 ± 1.7[ab]
MDA+HNE	32.45 ± 2.1	32.72 ± 3.6	45.91 ± 1.9[a]	34.31 ± 1.8[b]
Serum GSH	0.498 ± 0.03	0.511 ± 0.029	0.335 ± 0.25[a]	0.365 ± 0.021[ab]
Gastric GSH	0.196 ± 0.021	0.214 ± 0.032	0.172 ± 0.016[a]	0.176 ± 0.009[a]

Values are expressed as means ± S.E (n=6).
[a] Significant compared to the control group, P<0.05.
[b] Significant compared to indomethacin-treated group, P<0.05.

Table 2. Effect of 15/mg/kg indomethacin (Indo), 2.5 mg/kg MGP or their combination on superoxide dismutase, SOD, (U/mg protein), Catalase, CAT ((umol/H2O2/min/g) and glutathione-S-transferase, GST (U/g) activities in rat gastric mucosa.

	Control	MPG	Indo	MPG+Indo
SOD	3210 ± 55	3426 ± 42	2378[a] ± 46	2935[ab] ± 59
CAT	0.096 ± 0.004	0.089 ± 0.005	0.052[a] ± 0.003	0.075[ab] ± 0.005
GST	18.65 ± 1.6	17.30 ± 1.3	15.97[a] ± 1.6	17.14[b] ± 1.1

Values are expressed as means ± S.E (n=6).
[a] Significant compared to the control group, P<0.05.
[b] Significant compared to indomethacin-treated group, P<0.05.

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Table 3. Effect of 15/mg/kg indomethacin (Indo), 2.5 mg/kg MGP or their combination on serum total protein (g/dl), albumin (g/dl), glucose (mg/dl) concentrations as well as lactic dehydrogenase (LDH), and alkaline phosphatase (ALP) activities (U/L).

	Control	MPG	Indo	MPG+Indo
Total Protein	7.25 ± 0.31	6.7 ± 0.35	6.1 ± 0.41[a]	6.7 ± 0.29
Albumin	4.86 ± 0.21	4.67 ± 0.26	3.66 ± 0.44[a]	4.38 ± 0.43[b]
Glucose	75.4 ± 5.2	80.0 ± 4.9	120.7 ± 6.1[a]	108.0 ± 6.7[ab]
ALP	49.12 ± 3.2	43.03 ± 3.9	65.34 ± 5.2[a]	62.27 ± 4.5[ab]
LDH	310 ± 25	340 ± 21	640 ± 3[a]	510 ± 32[ab]

Values are expressed as means ± S.E (n=6).
[a] Significant compared to the control group, P<0.05.
[b] Significant compared to indomethacin-treated group, P<0.05.

MPG administration did not produce changes in total protein, albumin and glucose concentrations as well as LDH and ALKP activities in serum (Table 3). Oral administration of indomethacin was associated with a significant decrease in total proteins and albumin contents in serum, as well as significant increases in glucose concentration and the activities of LDH and ALP in serum (Table 3).

The administration of MPG one hour prior to the induction of gastric injury by indomethacin diminished the decrease in total proteins and albumin contents, as well as the increase in glucose concentration and the activity of LDH but did not alter serum ALP activity (Table 3).

DISCUSSION

The present study has demonstrated that the process of gastric injury by indomethacin involves free radicals and has shown a considerable protection by MPG. It was observed that indomethacin produced oxidative stress by interfering with the antioxidant system and thereby enhancing lipid peroxidation (LPO) in gastric mucosa. LPO was induced in gastric mucosa soon after i.g administration of indomethacin. It has been reported that the mucosal LPO and vascular permeability were significantly increased 30 mm after subcutaneous treatment with indomethacin [7] and that antioxidants has been proven useful in protecting against indomethacin-induced gastric mucosal injury [19]. These studies have strongly suggested the implication of ROS in the toxicity of indomethacin.

The source of oxygen radicals induced by indomethacin in tissues is still debated. Several studies however, implicated neutrophiles (PMN) in the early pathogenic process [20] in addition to the pro-oxidant catalytic activity of the indomethacin and ethanol [4 &21] .The present study demonstrated a significantly high serum glucose levels in indomethatin-treated rats. It has been shown that hyperglycemia is associated with production of free radicals [22]. Thus, it is suggested that excessive generation of free radicals might be associated with be endomethacin-induced hyperglycemia.

In the present study, indomethacin produced a significant rise in MDA+HNE concentration, which are indicators of increased LPO accompanied with increased activity of LDH and severe ulceration. Similar increases in lipid peroxidation products associated with ulceration were recorded in cases with gastric damage produced by ischaemia-reperfusion and stress [5&6] indomethacin [23] as well as ethanol [24] in rats. Moreover, oxidative damage associated with indomethacin treatment caused dramatic enhancement in gastric epithelial cell apoptosis triggered by increased expression of tumor necrosis factor-alfa (TNF-α) [25]. In addition, the biological oxidation results in the modification of cytoplasmic constituents such as depletion of GSH, a fall in the ATP levels and increased intracellular calcium which leads to loss of plasma membrane integrity and finally to cell damage [26].

The inhibition of SOD, CAT and GST activities in gastric mucosa in rats treated with indomethacin might be responsible for increased LPO and render the gastric mucosa more susceptible to injury. The decreased concentration of GSH might be also another reason for increased LPO and gastric injury. This is in

accordance with the finding that SOD and glutathione peroxidase were reduced by the administration of indomethacin [27]. Thus, the inhibition of the antioxidant mechanisms leads to the accumulation of ROS and the presence of free radical scavenger at the site of ROS production might be a protective factor in the modulation of the pathogenesis of indomethacin induced LPO and gastric injury.

Because indomethacin results in free radical (FR)-mediated damage to cellular organelles and mammalian gastric mucosa, strategies to scavenge FR have shown to be promising. For this reason, identification and assessment of potential FR scavenging agents is of clinical interest. One group of candidate drugs is the thiol containing compounds. N-acetylcysteine, glutathione, S-allyl cysteine and MPG have been found to offer protection in animal models from cardiac and hepatictoxicity [21]. The present study demonstrated that MPG ameliorated the indomethacin-induced perturbations in the levels of total proteins, albumin, glucose, LDH and ALP in serum of rats receiving MPG 1 hour before indomethacin treatment. This was accompanied by a marked protection from LPO as well as amelioration of the indomethacin-induced inhibition of SOD, CAT and GST activities in gastric mucosa in the same treated rats. This is consistent with the finding that MPG strongly inhibited ascorbate induced LPO in rat liver microsomes [28]. Moreover, this implies that thiol compounds may have the ability to decrease LPO by scavenging the initiating and propagating radicals, which might be attributed to the high redox potential of MPG [29]. Because MPG is a small and diffusible molecule, it can easily cross biological membranes and protect polyunsaturated fatty acids from FR mediated LPO by abstracting the hydrogen of the thiol group instead of methylene hydrogen of unsaturated lipids [28].

The up-regulation of the antioxidants recorded after treatment with indomethacin and MPG might lend credence to the proposal that antioxidants are important in gastroprotection and the presence of SOD and CAT is critical to eliminate ROS when it is formed at the site of generation. Thus, the elimination of superoxide anion (O_2^{*-}) by in a reaction catalyzed by SOD is an important factor in the protection process. It has been reported that O_2^{*-} is involved in the pathogenesis of gastric mucosal damage and the pretreatment with SOD reduces ethanol-induced gastric mucosal injury [30]. Moreover, treatment with SOD or SOD plus CAT inhibited the increase in gastric mucosal lesion and lipid peroxides in the gastric mucosa induced by indomethacin [31] and ischemia-reperfusion injury models in rat [32]. The present observation are in accordance with the previous studies and revealed that the administration of MPG in advance to indomethacin treatment decreased the MDA+HNE concentration and maintained the levels of the endogenous antioxidant enzymes close to normal and caused a marked reduction in the gastric lesions.

Since GSH content in serum and gastric mucosa remained almost constant, under the present experimental condition, the mechanism of MPG action remains speculative and needs further investigation. However, the mechanism of MPG action most probably includes SH/S-S interchange reaction and FR scavenging ability [11].

In conclusion, MPG has a protective potential from indomethacin-induced gastric ulceration and that the gastroprotective properties of MPG might attributed to its positive effects on the antioxidant system in indomethacin-induced gastric ulcers in rats. MPG might serve as a novel co-therapeutic agent when administered in combination with indomethacin to limit its oxidative injury.

REFERENCES

[1] RINGIM AH. Review evidence-based insights on nonsteroidal antiinflammatory drugs. J Pharm Cosmet Sci 2015;3(2):8–13

[2] DE LA LASTRA A.C., MOTILVA V., MARTIN M.J., NIETO A., BARRANCO M.D., CABEZA J. AND HERRERIAS J.M. (1999). Protective effect of melatonin on indomethacin-induced gastric injury in rats. J. Pineal Res., 26:101-7.

[3] BRZOZOWSKI T, KWIECIEN S, KONTUREK PC, KONTIJREK SJ, MITIS-MUSIOL M, DUDA A, BIELANSKI W, AND HAHN EG. (2001). Comparison of nitric oxide-releasing NSAID and vitamin C with classic NSAID in healing of chronic gastric ulcers; involvement of reactive oxygen species. Med. Sc Monit., 7:592-9.

[4] NAITO Y., YOSHIKAWA T., MATSUYAMA K., NISHIMURA S., YAGI N. AND KONDO M. (1995). Effects of free radical scavengers on indomethacininduced aggravation of gastric ulcer in rats. Dig. Dis. Sci.; 40: 2019-2 1.

[5] NAITO Y., YOSHIKAWA T., YOSHIDA N. AND KONDO M. (1998). Role of oxygen radical and lipid peroxidation in indomethacin-induced gastric mucosal injury. Dig. Dis. Sd.; 43: 30S-34S.

[6] NAITO Y., YOSHIKAWA T., MATSUYAMA K., YAGI N., ARM M., NAKAMURA Y., KANEKO T., YOSHIDA N. AND KONDON M. (1988). Neutrophils, lipid peroxidation, and nitric oxide in gastric reperfusion injury in rats. Free Radic. Biol. Med.; 24: 494-502.

[7] TAKEUCHI K., UESHIMA K., HIRONAKA Y., FUJIOKA Y., MATSUMOTO J., AND OKABE S. (1991). Oxygen free radicals and lipid peroxidation in the pathogenesis of gastric mucosal lesions induced by indomethacin in rats. Relation to gastric hypermotility. Digestion; 49: 175-84.

[8] KOUICHI TANONAKA, TAKESHI IWAI, KANATAKA MOTEGI, SATOSHI TAKEO. Effects of N-(2-mercaptopropionyl)-glycine on mitochondrial function in ischemic–reperfused heart Cardiovascular Research 57 (2003) 416–425.

[9] Joseph F. Weiss J F. Pharmacologic Approaches to Protection against Radiation-induced Lethality and Other Damage Environmental Health Perspectives 105 (1997) 1173-1178.

[10] AYENE S., KALE R. AND SRI VASTAVA P. (1988). Radioprotective effect of 2- mercaptopropionyl glycine on radiation induced lipid peroxidation and enzyme release in erythrocytes. I. J. Radia. BioL, 55, 627-639.

[11] ZIMMER G. AND EVERS J. (1998). 2-mercaptopropionyl glycine improves aortic flow after reoxygenation in working rat hearts. Basic Research in Cardiology, 83, 445-451.

[12] TANAKA M., FUJIWARA H., YAMASAKI K. AND SASAYAMA S. (1994). Superoxide dismutase and N-2-mercaptopropionil glycine attenuate infarct size limitation effect of ischemic reconditioning in rabbit. Cardiovascular Resarch, 28(7): 980-986.

[13] FRANCO L. AND VELO G.P. (1996). A copper complex reduced gastric damage caused by acetylsalicyclic acid and ethanol. Prostaglandins; 51: 331-118.

[14] OHKAWA H., OHISHI N. AND YAGI K. (1979). Assay for lipid peroxidation in animal tissues by thiobarbituric acid reaction. Anal. Biochem.; 95: 35 1-358.

[15] NISHIKIMI, M., RAO, N. AND YAI K. (1972). The occurrence of superoxide anion in the reaction of reduced phenazine methoxulfate and molecular oxygen. Biochem. Biophys. Res. Comm.; 46: 849-853.

[16] BOCK P.P., KRAMER R., PRAVELKA M.A. (1980): Simple assay for catalase determination. Cell Biol Monogr, 7: 44-74.

[17] BEUTLER, E. (1982). Red cell metabolism a mammal of biochemical methods, 2 edition, Grune and Stratton, New York, pp. 66-72.

[18] HABIG W.H., PABST M.J. AND JAKOBY W.B. (1974). Glutathione S-transferase the first enzyme step in mercapturic acid formation. J. Biol. Chem.; 249: 7130-7 139.

[19] MAGDA M. EL-KOMY, AND FOUKIA E. MOUAFI . Mitigating effect of Avicenna marina on indomethacin induced gastric ulcer in male albino rats. egyptian journal of basic and applied sciences 3 (2016) 155–163.

[20] YOSHIKAWA T., NAITO Y., KISHI A., TOMII T., KANEKO Y., [INTJMA S., ICHIKAWA H., AND YASUDA M. (1993). Takahashi S, Kondo M. Role of active oxygen, lipid peroixdation, and antioxidants in the pathogenesis of gastric mucosal injury induced by indomethacin in rats. Gut; 34: 732-737.

[21] EL-MISSIRY M.A., EL-SAYED I.H., AND OTHMAN A.!. (2001). Protection by metal complexes with SOD-mernetic activity against oxidative gastric injury induced by indomethacin and ehanol in rats. Ann. Clin. Biochem. 38:694-700.

[22] 22-GRAIER, W.F., SIMECEK, S., KUKOVETZ, W.R., AND KOSTNER, G.M. (1996). High Dglucose-induced changes in endothelial Ca2/ EDRF signaling are due to generation of superoxide anion. Diabetes. 45, 1386-1395.

[23] OTHMAN Al., EL-MISSIRY M.A. AND AMER M. (2001). The protective action of melatonin on indomethacin-induced gastric and testicular oxidative stress in rats. Redox Report, 6(3):173-177.

[24] MELCHIORRI D., SEWERYNEK E., REITER R.J., ORTIZ G.G., PROEGGELER B. AND NISTICO G. (1997) Suppressive effect of melatonin administration on ethanol-induced gastroduodenal injury in rats in vivo. Br. J. Pharmacol.; 12: 264-70.

[25] SOLMIANY B.L., PIOTROWSK1 J. AND SLOMIANY' A. (1997). Induction of tumor necrosis factoralfa and apoptosis in gastric mucosal injury by indomethacin: effect of omeprazol and ebrotidine. Scand. J. Gastroentrol.; 32: 638-642.

[26] WILSON J.X. (1997). Antioxidant defense of the brain: a.role for astrocytes. Can. J. Physiol. PharmcoL; 75: 1149-1163.

[27] TANAKA J., AND YUDA Y. (1996). Lipid peroxidation in gastric mucosal lesions induced by indomethacin in rat. BIoL Pharm. Bull.; 19: 716-20.

[28] CHOPRA M, M.BES WICK, CLAPPERTON H., DARGIE J., SMITH W.E. AND MCMURRAY. J. (1992). Antioxidant effects of angiotensin converting enzyme inhilitors (ACE): Free radical and oxidant scavenging are sulfhydryl dependant, but lipid peroxidation is inhibited by both suithydryl and nonsulfhydryl containing ACE inhibitors. J. Cardiovascular Pharmaco., 19, 330-340.

[29] MITA I. (1981). Chemistry, pharmacology and current clinical application of tiopronin. In: Recent Advances in 2-MPG Treatment of Liver Diseases (Eds. R. Williams, G. Gasbarrini and M. Davis) Santen Pharmaceuticla Col, Ltd., Osaka, Japan, pp. 3-10.

[30] PIHAN G., REGILLO C. AND SZABO S. (1987). Free radicals and lipid peroxidation in ethanol or aspirin-induced gastric mucosal injury. Dig. Dis. Sd.; 32: 1395- 140 1.

[31] YOSHIKAWA T., MTNAMIYAMA Y., ICHIKAWA H., TAKAHASHI S.,NAITO Y. AND KONDO M. (1997). Role of lipid peroxidation and antioxidants in gastric mucosal injury induced by the hypoxanthine-xanthine oxidase system in rats. Free Radic. Biol. Med.; 23: 243-50.

[32] YOSHIKAWA T., NAITO Y., KISHI A., TOMII T., KANEKO Y., [INTJMA S., ICHIKAWA H., AND YASUDA M. (1993). Takahashi S, Kondo M. Role of active oxygen, lipid peroixdation, and antioxidants in the pathogenesis of gastric mucosal injury induced by indomethacin in rats. Gut; 34: 732-737.