Physiol. Res. 49: 441-446, 2000

Role of Mucus in Ischemia/Reperfusion-Induced Gastric Mucosal Injury in Rats

J. MOJŽIŠ, R. HEGEDÜŠOVÁ, L. MIROSSAY

Department of Pharmacology, Medical Faculty, P.J. Šafarik University, Košice, Slovak Republic

Received June 10, 1999
Accepted March 10, 2000

Summary

Gastric mucus plays an important role in gastric mucosal protection. Apart from its „barrier" function, it has been demonstrated that mucus protects gastric epithelial cells against toxic oxygen metabolites derived from the xanthine/xanthine oxidase system. In this study, we investigated the effect of malotilate and sucralfate (mucus production stimulators) and N-acetylcysteine (mucolytic agent) on ischemia/reperfusion-induced gastric mucosal injury. Gastric ischemia was induced by 30 min clamping of the coeliac artery followed by 30 min of reperfusion. The mucus content was determined by the Alcian blue method. Sucralfate (100 mg/kg), malotilate (100 mg/kg), and N-acetylcysteine (100 mg/kg) were given orally 30 min before surgery. Both sucralfate and malotilate increased the mucus production in control rats. On the other hand, N-acetylcysteine significantly decreased mucus content in control (sham) group. A significant decrease of mucus content was found in the control and the N-acetylcysteine pretreated group during the period of ischemia. On the other hand, sucralfate and malotilate prevented the decrease the content of mucus during ischemia. A similar result can be seen after ischemia/reperfusion. In the control group and N-acetylcysteine pretreated group a significant decrease of adherent mucus content was found. However, sucralfate and malotilate increased mucus production (sucralfate significantly). Sucralfate and malotilate also significantly protected the gastric mucosa against ischemia/reperfusion-induced injury. However, N-acetylcysteine significantly increased gastric mucosal injury after ischemia/reperfusion. These results suggest that gastric mucus may be involved in the protection of gastric mucosa after ischemia/reperfusion.

Key words

Ischemia/reperfusion • Gastric mucus • Sucralfate • Malotilate • N-acetylcysteine

Introduction

The gastric epithelium is covered by a continuous layer of secreted mucus and bicarbonate which have been widely implicated as an important pre-epithelial protective factor against autodigestion of the gastric mucosa by acid and pepsin (so-called barrier function of mucus) (Copeman *et al.* 1994). The gastric mucus occurs in three forms: a soluble mucin present in gastric juice, insoluble (adherent) mucus covering mucosal cells and mucus present in muciparous cells. The adherent mucus is considered to be the main factor protecting the gastric mucosa (Azzumi *et al.* 1993). It has been reported that a decrease in gastric mucus renders the

Fax+4202 24920590
E-mail: physres@biomed.cas.cz http://www.lf2.cuni.cz/physiolres/physiolres.htm

mucosa more susceptible to injury induced by various aggressive factors (Nosáľová *et al.* 1991, Leonard *et al.* 1994, Farre *et al.* 1995).

It has been suggested that gastric mucus may possess antioxidant properties (Cross *et al.* 1984, Grisham *et al.* 1987). Hiraishi *et al.* (1993) described a protective effect of mucus glycoproteins against oxygen free radicals derived from the xanthine/xanthine oxidase system.

Sucralfate has been shown to be effective in the prevention and treatment of gastric and duodenal ulcers in humans (Lam *et al.* 1985) and also in animals (Tarnawski *et al.* 1985, Višňovský *et. al.* 1990). Several mechanisms of action have been proposed for sucralfate, including the enhancement of gastric mucus secretion (Szabo and Hollander 1989). Malotilate, a synthetic substance with properties comparable with those of natural flavonoids, possesses a gastroprotective effect in different models of gastric injury (Mirossay *et al.* 1996a,b, 1999).

In the present study, we investigated the role of mucus in ischemia/reperfusion-induced gastric mucosal injury by using sucralfate and malotilate (stimulators of mucus production), and N-acetylcysteine (NAC, a mucolytic agent).

Material and Methods

Male Wistar rats weighing 180-200 g were used in this study. All animals were housed in a wire-mash floor cages to prevent the ingestion of hair and feces. They were fasted for 24 h before the experiment but were allowed free access to water. On the day of the experiment, the animals were randomly divided into three groups (28 rats per group). Group I – control (sham operation); group II – only ischemia was performed; group III – animals were subjected to ischemia/reperfusion. Each group was subdivided into four subgroups (7 rats per subgroup) 1: pretreatment with 0.5 % methylcellulose (control); 2: pretreatment with malotilate (100 $mg.kg^{-1}$) (Slovakofarma a.s); 3: pretreatment with sucralfate (100 $mg.kg^{-1}$) (Slovakofarma a.s); 4: pretreatment with NAC (100 $mg.kg^{-1}$) (Sigma). Malotilate, sucralfate and NAC were dissolved in methylcellulose. All these substances were administered orally, 30 min before surgery. The rats were then anesthetized by an intraperitoneal injection of pentobarbital sodium in the dose of 50 $mg.kg^{-1}$. The animals were subjected to 30 min ischemia induced by occlusion of the coeliac artery without reperfusion (group II) or 30 min ischemia induced by occlusion of the coeliac artery and followed by 30 min reperfusion (group III). The coeliac artery was clamped using an atraumatic microvascular clamp (Nakamoto *et al.* 1998).

Adherent gastric mucus was determined by the method of Corne *et al.* (1974). Briefly, the stomach was removed, opened along the great curvature and rinsed in cold saline. The glandular part of the stomach was excised, weighed and immersed for 2 h in 10 ml of 0.1 % w/v Alcian blue (Sigma) in 0.16 $mol.l^{-1}$ sucrose solution. The excess dye was removed by rinsing twice in 0.25 $mol.l^{-1}$ sucrose solution (15 min each). The mucus-bound dye was extracted by immersing the gastric tissue in 0.5 $mol.l^{-1}$ $MgCl_2$ solution which was intermittently shaken for 1 min at 30 min intervals during a 2 h period. The blue extract was shaken with diethylether. The emulsion was then centrifuged at 3600 rpm for 10 min and the optical density of the aqueous phase was measured spectrophotometrically at 600 nm. The results are expressed as absorbance per gram of tissue (A/g of tissue).

At the end of the period of ischemia or ischemia/reperfusion, the extent of gastric lesions was measured, and the length of the lesions was expressed in mm.

The statistical significance of the difference between the means was estimated by Student`s t-test. The value of $p<0.05$ was considered as the level of minimal statistical significance.

Results

The effect of tested drugs on adherent mucus production is shown in Table 1. In sham operated animals (group I) NAC significantly decreased the mucus content in comparison with all other groups ($p<0.05$). During ischemic conditions (group II) the content of mucus in the control subgroup was significantly decreased ($p<0.01$). No significant changes in adherent mucus content were found in animals pretreated with malotilate and sucralfate in comparison with group I. However, NAC significantly decreased the gastric mucus content during ischemic conditions ($p<0.001$). A similar result was found in group III. After ischemia/reperfusion, adherent mucus was significantly lower in the control subgroup ($p<0.05$). However, there was a significantly higher amount of adherent mucus in group III ($p<0.05$) in comparison with group II. Sucralfate significantly increased the content of

adherent mucus in comparison with both group I and II ($p<0.001$). On the other hand, no significant changes in mucus content occurred in all the groups after malotilate administration. NAC significantly decreased the mucus content in group III as compared to the NAC effect in group I ($p<0.001$), but the mucus content was significantly increased in comparison with group II ($p<0.05$).

Table 1. Effect of methylcellulose, sucralfate, malotilate and N-acetylcysteine on gastric mucus content expressed as A/g of tissue and as percentage of control values (Group I)

Agent	Group I		Group II		Group III	
	A/g of tissue	%	A/g of tissue	%	A/g of tissue	%
Methylcellulose (control)	0.18	100	0.13	72**	0.154	86^{*+}
Sucralfate	0.19	100	0.174	91	0.264	139^{***+++}
Malotilate	0.19	100	0.87	98	0.205	108
N-acetylcysteine	$0.6^{\S}$	100	0.079	49***	0.105	65^{***+}

*Group I – sham operation, Group II – ischemia, Group III – ischemia/reperfusion, * $p<0.05$, ** $p<0.01$, *** $p<0.001$, compared to group I, $^{+}$ $p<0.05$; $^{+++}$ $p<0.001$; compared to group II, § $p<0.05$ compared to methylcellulose, sucralfate, and malotilate in Group I*

Figure 1 illustrates the effects of the tested drugs on gastric lesions in all the experimental groups. No gastric lesions were found in group I and II. However, a significant increase in gastric mucosal injury was observed after reperfusion of the ischemic gastric mucosa (group III). The average length of lesions in methylcellulose-pretreated animals was more than 20 mm/rat. Sucralfate and malotilate significantly decreased the length of gross lesions ($p<0.001$). On the other hand, the administration of NAC significantly increased the mean values of gastric lesions ($p<0.001$).

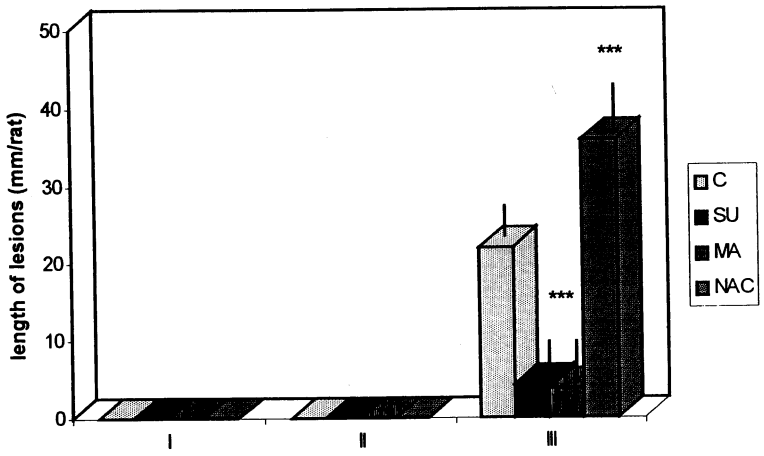

Fig. 1. *Effect of sucralfate (SU), malotilate (MA), and N-acetylcysteine (NAC) on the length of gastric lesions in rats in comparison with methylcellulose-treated animals (C). Results are expressed as means ± S.D. I – sham operation, II – ischemia, III – ischemia/reperfusion, *** $p<0.001$*

Discussion

An imbalance between mucosal defensive and aggressive factors may result in acute gastric injury. It is known that gastric mucosal perfusion is an essential factor in the ability of the mucosa to protect itself against injury (Cheung 1984) and that the gastric mucosal defense is impaired during ischemic conditions mainly because of an energy deficit (Mengury 1981). Although the early restitution of blood flow is essential for preventing further hypoxic injury, it has been found that mucosal injury may be greater when the ischemia is followed by a period of reperfusion. The main factors believed to be responsible for ischemia/reperfusion-

induced injury are oxygen free radicals (Zimmerman and Granger 1994).

As has been mentioned above, gastric mucus plays an important role in the protection of gastric mucosa. It is known that many sugars (e.g. manitol, glucose) are potent scavengers of oxygen free radicals. On the basis of this fact, Cross *et al.* (1984) hypothesized that gastric mucus possesses antioxidant properties because of its rich glycoproteins content. These authors tested the antioxidative effect of mucus glycoproteins and found that a 12 mg/ml solution of these proteins scavenged the hydroxyl radicals as effectively as 10-15 mmol/l solutions of manitol or glucose. It had previously been reported that gastric mucus contains glycoproteins at concentrations as high as 50 mg/ml (Allen 1981). Later, Hiraishi *et al.* (1993) confirmed the protective role of mucus glycoproteins against oxygen free radicals in a cultured gastric epithelial cell system. Gong *et al.* (1990) reported that not only mucus glycoproteins possess antiradical properties but also lipids bound to gastric mucin protect the mucin against oxygen radical attack. Our results and the above mentioned *in vitro* studies indicate that gastric mucus may be involved in the protection of gastric mucosa against oxygen free radicals generated during ischemia/reperfusion.

Additionally to its antioxidant effect, the "barrier function" of mucin may play an important role in protecting the gastric mucosa during ischemia/reperfusion stress (IRS).

It was reported that endogenous luminal acid plays little or no role in the gastric mucosal damage after postischemic reperfusion and no measurable gross lesions were observed in this model of gastric mucosal injury (Kawai *et al.* 1994). On the basis of these results, Seno *et al.* (1995) suggest that it is unlikely that gastric mucus protects the gastric mucosa against ischemia/reperfusion by blocking acid back-diffusion.

Contrary to Kawai's results, we found significantly damaged gastric mucosa after ischemia/reperfusion without exogenous acid. We suggest that endogenous acid may contribute to the mucosal damage after IRS. Our suggestion is supported by the recent reports of Wada *et al.* (1996), Kitano *et al.* (1997) and Nakamoto *et al.* (1998) who have found that inhibitors of gastric acid secretion (omeprazol or cimetidine) significantly decreased the total area of ulcers during ischemia/reperfusion.

Sucralfate increases gastric mucus production *in vivo* (Slomiany *et al.* 1991) and *in vitro* (Takahashi and Okabe 1996) by stimulating phospholipase C activity. We previously found that sucralfate decreased vascular permeability and protected the gastric mucosa during IRS (Mojžiš *et al.* 1995). Furthermore, malotilate has been shown to exert a strong gastroprotective effect in different models of gastric mucosal damage (Mirossay *et al.* 1996a,b), but the mechanism of its protective effects is still not clear. Malotilate is known as a stimulator of protein synthesis, but this effect requires its long-term administration (Imaizumi *et al.* 1982). We may thus hypothesize on the basis of our results that protection against mucus degradation in ischemia or ischemia/reperfusion by malotilate may be a factor responsible for mucosa protection during IRS.

We therefore suggest that gastric mucus is an important factor in gastric mucosal defense and provides certain protection against ischemia/reperfusion-induced gastric mucosal injury, and that stimulation of mucus secretion may be involved in the gastroprotective effect of both malotilate and sucralfate.

References

ALLEN A: Structure and function of gastrointestinal mucus. In: *Physiology of the Gastrointestinal Tract.* LR JOHNSON (ed), New York, Raven Press, 1981, pp 617-639.

AZZUMI Y, ICHIKAWA T, ISHIHARA K, HOTTA K: The validity of the ethanol precipitation method for the measurement of mucin content in human gastric juices and its relationship to gastroduodenal disease. *Clin Chim Acta* **221:** 219-225, 1993.

CHEUNG LY: Gastric mucosal blood flow: its measurement and importance in mucosal defense mechanisms. *J Surg Res* **36:** 282-288, 1984.

COPEMAN M, MATUZ J, LEONARD AJ, PEARSON JP, DETTMAR PW, ALLEN A: The gastroduodenal mucus barrier and its role in protection against luminal pepsins: the effect of 16,16 dimethyl prostaglandin E_2, carbol-polyacrylate, sucralfate and bismuth salicylate. *J Gastroenterol Hepatol* **9** (Suppl 1): S55-S59, 1994.

CORNE SJ, MORRISSEY SM, WOODS RJ: A method for the quantitative estimation of gastric barrier mucus. *J Physiol Lond* **242:** 116P-117P, 1974.

CROSS CE, HALLIWELL B, ALLEN A: Antioxidant protection: a function of tracheobronchial and gastrointestinal mucus. *Lancet* **1:** 328-330, 1984.

FARRE AJ, COLOMBO M, ALVAREZ I, GLAVIN GB: Some novel 5-hydroxytryptamine 1A (5-HT_{1A}) receptor agonists reduce gastric acid and pepsin secretion, reduce experimental gastric mucosal injury and enhance gastric mucus in rats. *J Pharmacol Exp Ther* **272:** 832-837, 1995.

GONG DH, TURNER B, BHASKAR KR, LAMONT JT: Lipid binding to gastric mucin: protective effect against oxygen radicals. *Am J Physiol* **259:** G681-G686, 1990.

GRISHAM MB, VON RITTER C, SMITH BF, LAMMONT JT, GRANGER DN: Interaction between oxygen radicals and gastric mucin. *Am J Physiol* **253:** G93-G96, 1987.

HIRAISHI H, TERANO A, OTA S, MUTOH S, SUGIMOTO T, HARADA T, RAZANDI M, IVEY KJ: Role for mucous glycoprotein in protecting cultured rat gastric mucosal cells against toxic oxygen metabolites. *J Lab Clin Med* **121:** 570-578, 1993.

IMAIZUMI Y, KATOH M, SUGIMOTO T, KASAI T: Effects of malotilate (diisopropyl-1,3-dithiol-2ylidenemalonate) on the protein synthesis in rat liver. *Jpn J Pharmacol* **32:** 369-375, 1982.

KAWAI T, JOH T, IWATA F, ITOH M: Gastric epithelial damage induced by local ischemia-reperfusion with or without exogenous acid. *Am J Physiol* **266:** G263-G270, 1994.

KITANO M, WADA K, KAMISAK Y, NAKAMOTO K, KISHIMOTO Y, KAWASAKI H, ITOH T: Effects of cimetidine on acute gastric mucosal injury induced by ischemia-reperfusion in rats. *Pharmacology* **55:** 154-164, 1997.

LAM SK, LAU W, LAI CL: Efficacy of sucralfate in corpus, prepyloric and duodenal ulcer-associated gastric ulcers. *Am J Med* **79** (Suppl 2C): 24-31, 1985.

LEONARD A., DROY-LEFAIX MT, ALLEN A: Pepsin hydrolysis of the adherent mucus barrier and subsequent gastric mucosal damage in the rat: effect of diosmectite and 16,16-dimethyl prostaglandin E_2. *Gastroenterol Clin Biol* **18:** 609-616, 1994.

MENGURY R: Role of gastric mucosal energy metabolism in the etiology of stress ulceration. *World J Surg* **5:** 175-180, 1981.

MIROSSAY L., MOJŽIŠ J, POMFY M, BODNÁR J, SOKOL L: Two models of gastric mucosal injury and their influence by malotilate. *Slovakofarma Rev* **6:** 89-94, 1996a.

MIROSSAY L, MOJŽIŠ J, ŠALLINGOVÁ Z, BODNÁR J, BENICKÝ M, BÖÖR A, KOHÚT A: Gastroprotective effect of malotilate in indomethacin- and ethanol-induced gastric mucosal damage. *Physiol Res* **45:** 405-411, 1996b.

MIROSSAY L, KOHÚT A, MOJŽIŠ J: Effect of malotilate on ethanol-induced gastric mucosal damage in capsaicin-pretreated rats. *Physiol Res* **48:** 375-381, 1999.

MOJŽIŠ J, KOHÚT A., MIROSSAY L, NICÁK A, POMFY M, BENICKÝ M, BODNÁR J: Effect of sucralfate on ischemia/reperfusion-induced gastric mucosal injury. *Slovakofarma Rev* **5:** 53-57, 1995.

NAKAMOTO K, WADA K, KITANO M, KISHIMOTO Y, ASHIDA K, KAMISAKI Y., KAWASAKI H, ITOH T: The role of endogenous acid in the development of acute gastric ulcer induced by ischemia-reperfusion in the rat. *Life Sci* **62:** 63-69, 1998.

NOSÁĽOVÁ V, JURÁNEK I, BABULOVÁ A: Effect of pentacaine and ranitidine on gastric mucus changes induced by cold-restraint stress in rats. *Agents Actions* **33**: 164-166, 1991.

SENO K, JOH T, YOKOYAMA Y, ITOH M: Role of mucus in gastric mucosal injury induced by local ischemia/reperfusion. *J Lab Clin Med* **126:** 287-293 1995.

SLOMIANY BL, PIOTROWSKI J, TAMURA S, SLOMIANY A: Enhancement of the protective qualities of gastric mucus by sucralfate: role of phosphoinositides. *Am J Med* **91:** S30-S36, 1991.

SZABO S, HOLLANDER D: Pathways of gastrointestinal protection and repair: mechanism of action of sucralfate. *Am J Med* **86** (Suppl. 6A): 23-31, 1989.

TAKAHASHI S, OKABE S: Stimulatory effects of sucralfate on secretion and synthesis of mucus by rabit gastric mucosal cells. Involmemt of phospholipase C. *Dig Dis Sci* **41**: 498-504, 1996.

TARNAWSKI A, HOLLANDER D, GERGELY H, STACHURA J: Comparison of antacid, sucralfate, cimetidine and ranitidine in protection of gastric mucosa against ethanol injury. *Am J Med* **79**: 19-23, 1985.

VIŠŇOVSKÝ P, BÁNOVČIN P, KOCIÁN O.: Effects of H^+/K^+-ATPase inhibitors on rat gastric mucosa experimental injury (in Slovak), *Čs fysiol* **39:** 174, 1990.

WADA K, KAMISAKI Y, KITANO M, KISHIMOTO Y, NAKAMOTO K, ITOH T: A new gastric ulcer model induced by ischemia-reperfusion in the rat: role of leukocytes on ulceration in rat stomach. *Life Sci* **59:** 295-301, 1996.

ZIMMERMAN BJ, GRANGER DN: Oxygen free radicals and the gastrointestinal tract: role in ischemia-reperfusion injury. *Hepatogastroenterology* **41:** 337-342, 1994.

Reprint requests

Assoc. Prof. Ján Mojžiš, Ph.D., Department of Pharmacology, Medical Faculty, P.J. Šafarik University, Tr. SNP 1, 040 66 Košice, Slovak Republic. E-mail: mojzis@central.medic.upjs.sk