

Experimental Study of Hypovolaemic Shock-Induced Gastric Mucosal Lesions in the Rat

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Abstract

This study was designed to determine whether oxygen-derived free radicals play a role in the pathogenesis of gastric mucosal lesions produced by haemorrhagic shock and reperfusion experimental model in the rat. Ranitidine (H₂-receptor blocker) in different doses, allopurinol, an inhibitor of xanthine oxidase and SOD (superoxide dysmutase) pre-treatment were used against haemorrhagic shock and reperfusion induced gastric mucosal lesions. Altogether 67 rats were divided into seven different groups. The area of gastric mucosal lesions was measured, the activity of endogenous peroxidase was examined histochemically and histological grading was made. Evans blue was used to demonstrate the improved permeability of gastric mucosal membranes.

Ranitidine, in high dose, allopurinol and superoxide dysmutase significantly protected against haemorrhagic shock-induced gastric mucosal lesions, against improved membrane permeability and peroxidation.

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Key words: Antioxidants, Lipid peroxidation, Membrane permeability, Oxygen free radicals

Introduction

Hypoxic tissue injury is not only caused by lack of oxygen as was previously believed. Now it is clear that tissue injury associated with hypoxia occurs to a large extent in the post-hypoxic reoxygenation period. Several experimental studies have demonstrated that oxygen-derived free radicals are responsible for a large proportion of the gastric mucosal damage observed after ischaemia.¹⁻⁵ The cytotoxic effects of these radicals result from the increased peroxidation of the lipid components of cellular and mitochondrial membranes.^{2,6} On the other hand, activation of neutrophils is accompanied by the release of oxygen-derived free radicals.^{7,8} Ischaemia-reperfusion injury to the intestine and stomach can be reduced by various antioxidants.^{3,9-11} Many have found that treatment with a free radicals-scavenging enzyme or an agent inhibiting free radical synthesis significantly protected against gastric damage induced by haemorrhagic shock. Parks and Granger⁶ demonstrated that the increased intestinal vascular permeability produced by ischaemia was largely prevented by pre-treatment with either allopurinol (xanthine oxidase inhibitor) or dimethyl sulphoxide (a hydroxyl radical scavenger). Treatment with allopurinol or superoxide dysmutase provides beneficial results in hypotension of different organs.^{1,3,6,11,12} Allopurinol as well as superoxide

dysmutase were effective in preventing both the increase in vascular permeability produced by 1 hour of ischaemia and the mucosal lesions resulting from 3 hours of ischaemia.¹³⁻¹⁷ A synthetic antioxidant, MTDQ-DA (6,6'-methylene-bis 2,2-dimethyl-4-methane sulfonic acid sodium-1,2 dihydroquinoline; trade name: Kontrad) has been found to inhibit lipid peroxidation and to exert radicals scavenger action in liver and heart¹⁸⁻²⁰ and it was effective against the gastric mucosal lesions produced by hypovolaemic shock.¹¹ MTDQ-DA having three functional groups also helps to scavenge the superoxide radicals and to block the Haber-Weiss reaction.

In this study, a haemorrhagic shock-reperfusion model was used to produce gastric mucosal lesions. It was decided to examine the increased activity of the non-specific endogenous peroxidase enzyme histochemically and to compare the protective effect of allopurinol (Zilorix, Wellcome Comp, England), superoxide dysmutase (SOD, from bovine liver, S4761, Sigma Chem Comp) and a H₂-receptor antagonist, ranitidine (Zantac, Glaxo Comp).

Materials and Methods

Animal Preparation

Male Wistar rats (300 to 400 g) kept on normal laboratory diet were used. The animals were anaesthetised

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intraperitoneally with 40 mg/kg of pentobarbital sodium (Nembutal). A catheter was inserted into the left femoral artery for monitoring the blood pressure. Another catheter was placed into the right carotid artery to withdraw and reinfuse blood. The abdomen was opened and the cardia was ligated. Through the duodenum a tube was inserted into the stomach to do a proper lavage with physiological saline. 1 ml of 0.1 N HCl per 100 g body weight was instilled into the stomach via the gastric tube. Subsequently the tube was removed and the pylorus was ligated.

Experimental Procedure

In five minutes after the intragastric instillation of HCl blood was withdrawn from the right carotid artery over a 2 min period of time into a syringe containing 0.4 ml heparin. The mean systemic blood pressure was reduced to 20 to 30 mmHg and it was maintained at the same level for 20 min by additional withdrawal of appropriate volumes of blood as required. Then the shed blood was reinfused. Twenty minutes later the rat was sacrificed by thoracotomy and the stomach was removed. One minute before sacrifice 1 ml of 1% Evans blue was injected via the right carotid artery to prove the increased membrane permeability and to enhance the contrast of gastric lesions. The stomach was opened along the greater curvature, pinned out on a cardboard and fixed in 6% formalin or Zamboni²¹ fixative.

Drug Studies

Sixty-seven rats were divided into seven groups. The different drugs were given intra-peritoneally at the same time when the animals received the pentobarbital sodium.

Control group	: no pre-treatment
Ranitidine groups	: 1 mg/kg 2 mg/kg 4 mg/kg 8 mg/kg pre-treatment
Allopurinol group	: 50 mg/kg pre-treatment
Superoxide dysmutase group	: 20,000 U/kg pre-treatment

Measurement of Gastric Lesions

Photographs of the stomachs were taken after the formalin fixation. After magnifying 3 times an independent person measured the areas of gastric lesions and the total gastric mucosal surface with grid (in square-mm). Statistical analysis (multiple variance analysis + Scheffe test) was made by the Computing Centre of Medical University of Szeged.

Enzyme Study

For demonstration of endogenous peroxidase activity,

3 to 4 micron sections were used. After application of normal rabbit serum in a dilution of 1:100 the sections were incubated with peroxidase labelled sheep antirabbit IgG (H+L) serum (Institute Pasteur, Paris). Peroxidase activity was demonstrated with 3-3'-diaminobenzidine tetrahydrochloride (DAB) (Serva, Germany).

Histological Grading

Formalin-fixed specimens stained with haematoxylin-eosin were used for grading of the lesions according to Arvidsson.²² The mucosal lesions were graded from 0 to 4.

Grade 0 = normal mucosa

Grade I = oedema just beneath the superficial epithelium

Grade II = disappearance of the surface epithelial cells

Grade III = damage to the upper half of the glandular cells of gastric crypts

Grade IV = disappearance of the glands

Gastric mucosal damage index was calculated as the sum of the maximum damage in the three fundic area.

Results

In this experiment Evans blue was used to enhance the gastric mucosal lesions. Evans blue was used intraarterially, and it could work because of increased permeability of membranes. Figure 1 shows the stomach of a rat in the control group. The dark areas represent the gastric mucosal lesions.

Figure 2 shows the effect of different drugs on areas of gastric lesions induced by haemorrhagic shock-reperfusion experiment. The ranitidine (4 mg/kg and 8 mg/kg), allopurinol and SOD pre-treatment were effective against these lesions and the damaged areas were significantly smaller in these groups compared to the control group. The ranitidine pre-treatment with smaller concentration of drug, with 1 mg/kg and 2 mg/kg were not proven to be an effective pre-treatment.

Figure 3 shows similar curve to Figure 2. It shows the effect of different pre-treatment on percentage rate of gastric mucosal damage in this experiment.

Figure 4 shows the disappearance of the surface epithelial cells (Grade II).

Figure 5 shows the damage to the upper half of the glandular cells of gastric crypts (Grade III).

Figure 6 shows the changes of gastric mucosal damage index in the different groups. The median values of mucosal damage index were as follows: control group 6; SOD group 2; allopurinol group 2; ranitidine (1 mg/kg) 4; ranitidine (2 mg/kg) 4; ranitidine (4 mg/kg) 2 and ranitidine (8 mg/kg) 2. These values also proved the protective effect of allopurinol and SOD and the high dose ranitidine in this shock model.

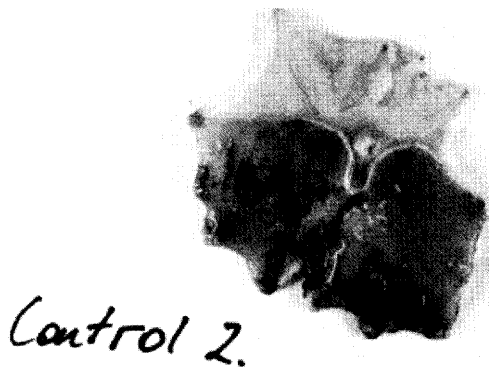


Fig. 1 Stomach of a control rat (untreated). Dark areas represent the gastric mucosal lesions

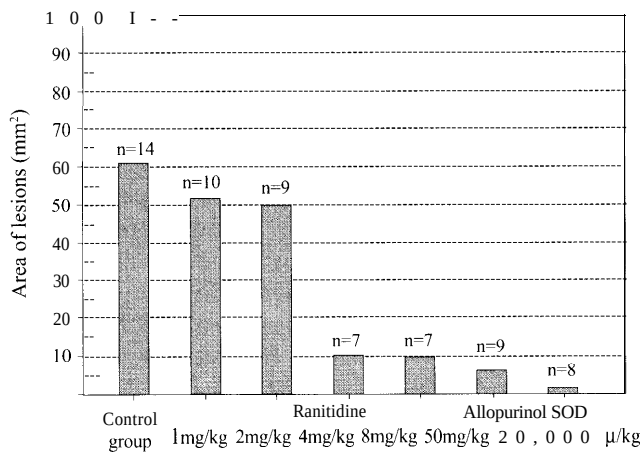


Fig. 2. Effect of ranitidine, allopurinol and superoxide dismutase on areas of gastric mucosal lesions induced by haemorrhagic shock. The vertical column and bar represent mean value and standard error respectively. The values of ranitidine (4 mg/kg), ranitidine (8 mg/kg), allopurinol and superoxide dismutase groups had significant difference from the control (untreated group) ($P < 0.01$).

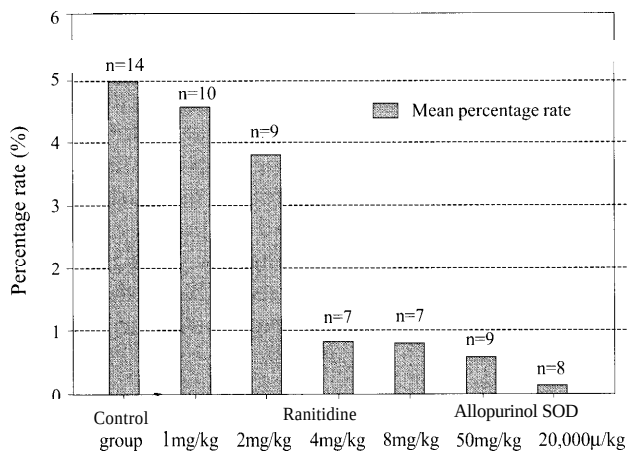


Fig. 3. Effect of ranitidine, allopurinol and superoxide dismutase on the percentage rate of gastric mucosal lesions induced by haemorrhagic shock. The vertical columns represent the mean percentage rates of gastric mucosal lesions. The values of ranitidine (4 mg/kg), ranitidine (8 mg/kg), allopurinol and superoxide dismutase groups had significant difference compared to the control (untreated group) ($P < 0.01$).

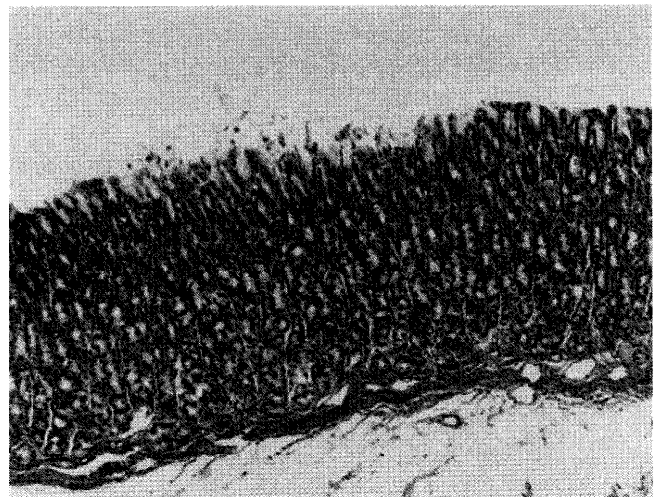


Fig. 4. Light microscopic appearance of histological section of the stomach. Grade II shows the disappearance of the surface epithelial cells (haematoxylin-eosin)(x200).

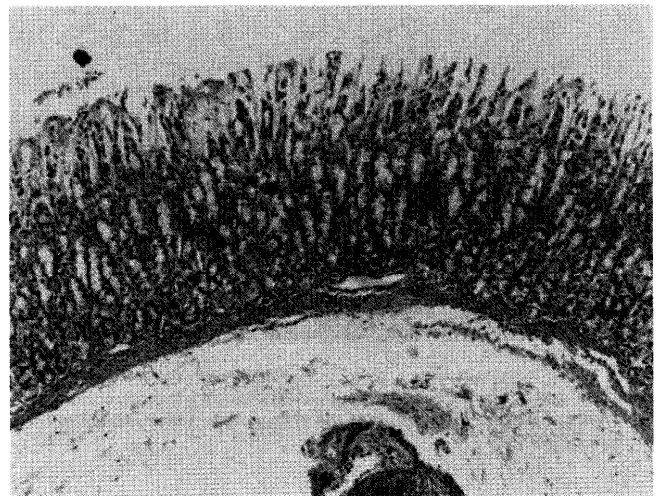


Fig. 5. Light microscopic appearance of histological section of the stomach. Grade III shows the damage to the upper half of the glandular cells of gastric crypts (haematoxylin-eosin) (x200).

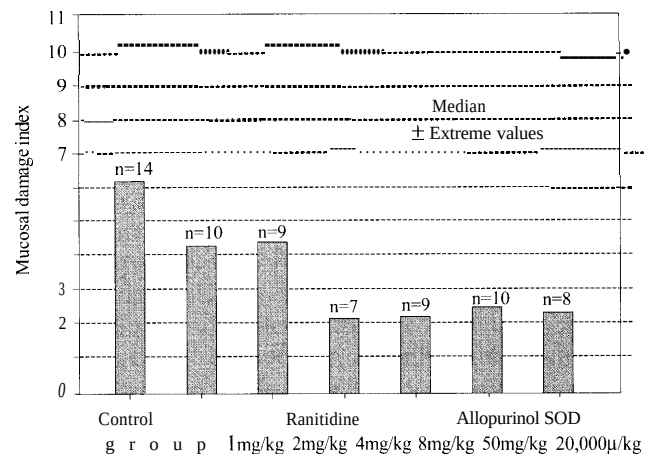


Fig. 6 Changes of gastric mucosal damage index. It was calculated as the sum of the maximum damage in three fundic area (histological grading of gastric mucosal lesions).

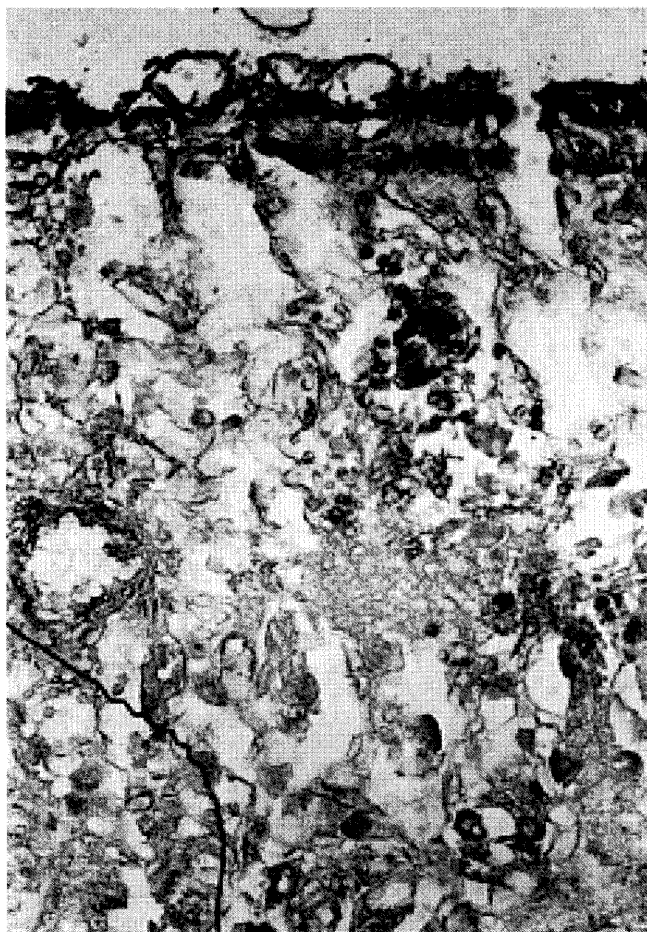


Fig. 7. Endogenous peroxidase activity in the rat stomach. Increased endogenous peroxidase activity can be seen in the superficial layer of lamina propria and in the red blood cells within a dilated capillary. Epithelial cells are completely absent. Indirect PAP technique, $\times 300$.¹¹

Figure 7 shows the results of enzyme study in the control (no pre-treatment) group. An increased peroxidase activity can be seen in the superficial layer of lamina propria and in the red blood cells within a dilated capillary. The epithelial cells disappeared during the experimental time. In the effectively pre-treated animals the structure is well preserved, and a normal aspecific activity of endogenous peroxidase can be observed only in the endothelial cells of vessels.

Discussion

It is a well-known fact that there is an increased intestinal vascular permeability during a hypovolaemic shock. In our study Evans blue went through the wall of gastric mucosal vessels during the hypovolaemic-reperfusion experiment. A protective effect of allopurinol (a specific inhibitor of xanthine oxidase) has been demonstrated previously in haemorrhagic shock or local ischaemia in the stomach^{1,3,6,23} against this increased vascular permeability. Allopurinol prevents oxygen-derived free radicals formation, which are responsible

for a large proportion of the gastric mucosal lesions observed after ischaemia. Superoxide dismutase scavenges the oxygen-derived free radicals, so it has protective effect against these gastric mucosal lesions.¹³⁻¹⁶

An increased lipid peroxidation was proved by indirect method, by the determination malondialdehyde which pointed out the breakdown of polyunsaturated fatty acids in the membranes. An increased glutathione peroxidase activity was measured biochemically^{12,24} in the small intestine during and after the period of regional ischaemia. In our study an increased endogenous peroxidase activity was proved in the damaged tissue histochemically in the control (no pre-treatment) group. Probably, this high peroxidase activity is in connection with the development of oxygen-derived free radicals. Superoxide dismutase, allopurinol and the high dose ranitidine pre-treatment significantly reduced the area of gastric lesions. The depth and severity of these lesions were also reduced. These facts were proved by histological grading. SOD and allopurinol were found effective against the increased activity of endogenous peroxidation. These results suggest that oxygen-derived free radicals have an important role in the pathogenesis of gastric lesions produced by ischaemia-reperfusion. Allopurinol pre-treatment reduces the formation of oxygen-derived free radicals. Superoxide dismutase scavenges the generated superoxide radicals, in that way the pre-treatment with SOD was effective.

Das et al² found that hydroxyl radical is the major causative factor in stress-induced gastric ulceration, and they proved that desferrioxamine, a nontoxic transition metal ion chelator, protected the mucosa against the stress-ulceration in dose dependent.

Stress causes both the sympathetic and parasympathetic stimulation of the stomach, which induces an increased gastric motility and muscular contraction leading to vascular compression and mucosal ischaemia. Although vagal stimulation during stress is expected to increase gastric acid secretion, the role of acidity in stress-ulcer formation is insignificant, as stress itself reduces gastric secretion and acidity. Interestingly, pre-treatment with H₂-receptor blocker ranitidine was effective in high dose,²⁵ but not in small dose in this hypovolaemic-reperfusion study in the rat against gastric mucosal lesions. Ranitidine does not have direct influence on oxygen-derived free radicals, therefore the pathophysiology of this phenomenon has to have other explanation.

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