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# ROLE OF OXYGEN-DERIVED FREE RADICALS IN HEMORRHAGIC SHOCK-INDUCED GASTRIC LESIONS OF RATS

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This study was designed to determine whether oxygen-derived free radicals play a role in the pathogenesis of gastric lesions produced by hemorrhagic shock in the rat. Allopurinol (Zyloric), an inhibitor of xanthine oxidase (responsible for the formation of superoxide radicals) and MTDQ-DA (Kontrad), a synthetic antioxidant of dihydroquinoline type were used. In the anesthetized rat 0.1 N HCl was instilled into the stomach and the rat was bled to reduce the blood pressure to 30 mmHg for 20 min. The blood shed was retransfused. Twenty min later the stomach was removed. The area of gastric mucosal lesions were measured, the activity of endogenous peroxidase was examined histochemically and a histological grading was made.

Both allopurinol and MTDQ-DA significantly protected against hemorrhagic shock-induced gastric lesions and peroxidation. These results suggest that oxygen-derived free radicals play an important role in the formation of gastric lesions produced by ischemia plus 0.1 N HCl.

**Keywords:** oxygen radicals, hemorrhagic shock, gastric lesion, lipid peroxidation, allopurinol, antioxidant.

Several experimental studies have demonstrated the association between hemorrhagic hypotension and the formation of gastric lesions [4, 6, 10, 13]. There is growing evidence that oxygen-derived free radicals play a role in the pathogenesis, but the precise mechanism of tissue injury is not known. The cytotoxic effects of these radicals result from the increased peroxidation of lipid components of cellular and mitochondrial membranes [5]. One of the sources of the superoxide radical in tissue is the xanthine oxidase system. Treatment with allopurinol, a specific inhibitor of xanthine oxidase provides beneficial results in hypotension of different organs [4, 5, 6, 7]. 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 radical scavenger action in liver and heart [3, 9, 11].

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In this study a hemorrhagic shock model was used to produce gastric lesions. We decided to examine the increased activity of the nonspecificic endogenous peroxidase enzyme histochemically and to compare the protective effect of allopurinol (Zyloric, Wellcome Comp. England) and MTDQ-DA (HUMÁN, Budapest, Hungary) against the gastric lesions produced by hemorrhagic shock in the rat.

*Animal preparation*

Male CFY rats (300—400 g) were used which were kept on normal laboratory diet. The animals were anesthetized intraperitoneally with 36 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 wt 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. 20 min later the rat was killed by thoracotomy and the stomach was removed. One minute before killing 1 ml of 1% Evans blue was injected via the right carotid artery 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 Zamboni [8] fixative. In the control group everything was made in the same way except blood was not withdrawn.

*Drug studies*

50 mg/kg of allopurinol or 50 mg/kg of MTDQ-DA were given intraperitoneally at the same time when the animals received the pentobarbital sodium.

*Measurement of gastric lesions*

Photos were made after the formalin fixation from each stomach. After 3x magnification an independent person measured the areas of gastric lesions

and the total gastric mucosal surface with grid (in $mm^2$). Statistical analysis (multiple variance analysis + Scheffe test) was made by the Computing Centre of the Szent-Györgyi Albert University of Medicine.

*Histological grading*

The formalin fixed specimens stained with hematoxylin-eosin were used for grading of the lesions according to Arvidsson [1]. The mucosal lesions were graded 0—4. The gastric mucosal damage index was calculated as the sum of the maximum damage in the three fundic areas.

*Enzyme study*

For the demonstration of endogenous peroxidase activity 3—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).

## Results

Figure 1 shows the effect of allopurinol and MTDQ-DA on area of gastric lesions induced by hemorrhagic shock. The damaged area was significantly smaller in the control group and in the allopurinol study as well as the MTDQ-DA study compared to the "shock" group.

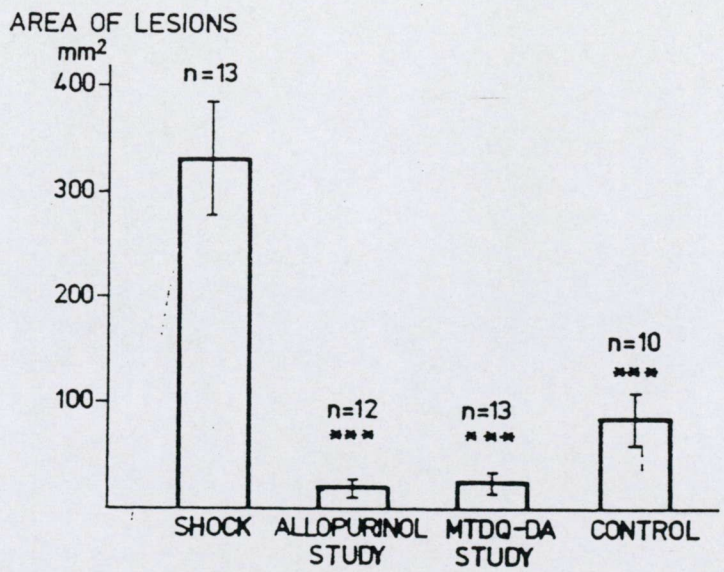


*Fig. 1.* Effect of allopurinol and MTDQ-DA on area of gastric lesions induced by hemorrhagic shock. The vertical column and bar represent mean value and standard error respectively. Asterisks demonstrate significant difference from the "shock" group. Multiple variance analysis + Scheffe test were used. $p < 0.001$


*Fig. 2.* Measurement of endogenous peroxidase activity in the rat stomach. A) Fundic mucosa from a rat of group "shock" Increased endogenous peroxidase activity can be seen in the superficial layer of lamina propria and in the red blood cells within a dilated capillary. Epithel cells are completely absent. Indirect PAP technique, x 100. B) Fundic mucosa from a rat of the MTDQ-DA study A well preserved structure is demonstrated. Normal aspecific activity of endogenous peroxidase can be observed only in the endothelial cells of vessels. Indirect PAP technique, x 100

Gastric mucosal damage index was calculated by histological grading. The median values of these measurements are as follows: "shock" group: 8, control group: 1, allopurinol study: 1.5, MTDQ-DA study: 3. These values proved the protective effect of allopurinol and MTDQ-DA, too.

Figure 2 shows the result of enzyme study. An increased peroxidase activity can be seen in the superficial layer of lamina propria and in the red blood cells in the "shock" group. The epithel cells disappeared during the

experimental time. In the drug studies well preserved structures can be observed. Only the normal aspecific activity of endogenous peroxidase can be seen in the endothel cells of vessels.

## Discussion

A protective effect of allopurinol (a specific inhibitor of xanthine oxidase) has been demonstrated previously in hemorrhagic shock or local ischemia in the stomach [2, 4, 6]. The MTDQ-DA (an antioxidant) pretreatment was effective in ischemic condition of heart [11]. An increased lipid peroxidation was proved by an 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 [7, 12] in the small intestine during and after the period of regional ischemia.

In our study an increased endogenous peroxidase activity was proved in the damaged tissue histochemically in the "shock" group. Probably this high peroxidase activity is in connection with an increased lipid peroxidation and glutathione peroxidation, too.

It was the first occasion when MTDQ-DA was applied in a hemorrhagic shock model to protect the gastric mucosa. Both MTDQ-DA and allopurinol pretreatment significantly reduced the area of gastric lesions produced by hemorrhagic shock and the depth and severity of the lesions were reduced, too. The latter was proved by histological grading. Both allopurinol and MTDQ-DA pretreatment 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 ischemia. Allopurinol inhibits the xanthine oxidase enzyme, so it reduces the formation of superoxide radicals. MTDQ-DA having three functional groups helps to scavenge the superoxide radicals and to block the Haber-Weis reaction.

## REFERENCES

1. Arvidsson, S., Fält, K., Haglund, U.: Acut gastric mucosal ulceration in septic shock. Acta Chir. Scand. **150**, 541—547 (1984).
2. Cunningham, S. K., Keaveny, T. V.: Effect of a xanthine oxidase inhibitor on adenine nucleotide degradation in hemorrhagic shock. Eur. Surg. Res. **10**, 305—313 (1978).
3. Fehér, J., Pollák, Zs., Stréter, L., Toncsev, H., Cornides, Á., Gógl, Á., Vereckei, A.: Experimental models for the study of hepatoprotection. Acta Physiol. Hung. **64**, 401—407 (1984).
4. Itoh, M., Guth, P. H.: Role of oxygen-derived free radicals in hemorrhagic shock-induced gastric lesions in the rat. Gastroenterology **88**, 1162—1167 (1985).
5. Parks, D. A., Granger, D. N.: Ischemia-induced vascular changes: role of xanthine oxidase and hydroxyl radicals. Am. J. Physiol. **245**, G285—289 (1983).

6. Perry, M. A., Wadhwa, S., Parks, D. A., Pickard, W., Granger, D. N.: Role of oxygen radicals in ischemia-induced lesions in the cat stomach. Gastroenterology **90**, 362—367 (1986).
7. Schoenberg, M. H., Muhl, E., Sellin, D., Younes, M., Schildberg, F. W., Haglund, U.: Posthypotensive generation of superoxide free radicals — possible in the pathogenesis of the intestinal mucosal damage. Acta Chir. Schand. **150**, 301—309 (1984).
8. Stefanini, M., De Martino, C., Zamboni, L.: Fixation of ejeculated spermatozoa for electron microscopy. Nature (London) **216**, 173—174 (1967).
9. Sulyok, S., Bär-Pollák, Zs., Fehér, E., Kemenes, I., Kántor, I., Fehér, J.: Liver lipid peroxidation induced by cholesterol and its treatment with a dihydroquinoline type free radical scavenger in rabbits. Acta Physiol. Hung. **64**, 437—442 (1984).
10. Takeuchi, K., Ohno, T., Okabe, S.: Variation of gastric transmucosal potential difference and lesion formation during hemorrhagic shock in the rat. Gastroenterology **91**, 1113—1123 (1986).
11. Török, B., Rőth, E., Bär, V., Pollák, Zs.: Effects of antioxidant therapy in experimentally induced heart infarcts. Basic Res. Cardiol. **81**, 167—179 (1986).
12. Younes, M., Schoenberg, M. H., Jung, H., Fredholm, B. B., Haglund, U., Schildberg, F. W.: Oxidative tissue damage following regional intestinal ischemia and reperfusion in the cat. Res. Exp. Med. **184**, 259—264 (1984).
13. Zavagno, G., Cagol, P. P., Da Pian, P. P., Vallongo, P., Ishaq-Waris, K., Lise, M.: Variations in transmucosal gastric potential difference during hemorrhagic shock in the rat. Eur. Surg. Res. **17**, 38—43 (1985).

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# HISTAMINE RELEASE AND SOD, ALLOPURINOL AND RANITIDINE PRETREATMENT IN HAEMORRHAGIC SHOCK IN THE RAT

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Histamine release have been demonstrated in haemorrhagic shock. There are some observations that oxygen free radicals can cause histamine release. Oxygen free radicals play a role in the pathogenesis of gastric mucosal lesions. The goal of this study was to determine whether ranitinide or SOD and allopurinol pretreatment modify the histamine release during and after the haemorrhagic shock in the rat.

In the anaesthetized rat 0.1 N HCl was instilled into the stomach and the rat was bled to reduce the blood pressure to 30 mmHg for 20 min. The shed blood was reinfused. Twenty min later the stomach was removed. The area of gastric mucosal lesions were measured, histological grading was made. Blood samples taken from the carotid artery were examined by radioimmunoassay (IMMUNOTECH) to determine the plasma histamine level.

Plasma histamine level did not change significantly during the preparative surgery, but there was a significant increase of histamine level by the end of shock period. After the reinfusion of the blood the plasma histamine remained essentially at the same level for five min. Oxygen free radicals did not cause an important histamine release. By the end of the experiment the histamine level decreased dramatically.

Ranitidine, allopurinol and SOD pretreatment provided significant protection against the gastric mucosal lesions. Allopurinol and SOD did not influence significantly the histamine level. Ranitidine caused significant histamine release immediately after the injection and every histamine value was significantly higher in this group except for the final value which was lower than the control one.

The oxygen free radicals were not found as endogenous histamine releasers in this study.

**Keywords:** haemorrhagic shock, oxygen free radicals, histamine release, allopurinol, SOD, ranitidine

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Histamine release has been demonstrated in haemorrhagic shock [10]. Several experimental studies proved the association between haemorrhagic hypotension-reperfusion and the formation of gastric lesions [7, 11, 13, 15]. Oxygen derived free radicals arising from the increased peroxidation of lipid components of cellular and mitochondrial membranes play an important role in the pathogenesis of this phenomenon [8, 15]. The link between oxygen free radicals and histamine release [8, 9] has been established by *in vitro* system. A local histamine release [5] was found *in vivo* after intestinal ischaemia and reperfusion, and antioxidant pretreatment was effective against this histamine release. These data suggest that oxygen free radicals may play an important role of histamine release during and after the shock period.

The goal of this study has been to investigate in our shock model whether there is any histamine release after the reperfusion of the shed blood when the oxygen free radicals damage the membranes.

The next purpose of this study was to investigate whether the increased histamine level in the end of shock period and the possible new histamine release after the reperfusion can be modified or not with allopurinol, xanthine oxidase inhibitor (Ziloric, Wellcome Comp.), and superoxide dysmutase (SOD, from bovine liver, S 4761, Sigma Chem. Comp.) and a H2-receptor antagonist, ranitidine (Zantac, Glaxo).

## Materials and methods

### *Animal preparation*

Male Wistar rats (300–400 g) kept on normal laboratory diet were used. The animals were anaesthetized 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 wt. 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. 20 min later the rat was killed by thoracotomy and the stomach was removed. One minute before killing 1 ml of 1% Evans blue was injected via the right carotid artery to enhance the contrast of gastric lesions. The stomach was opened along the greater curvature, pinned out on a cardboard and fixed in 10% formalin.

Blood samples for histamine measurements were taken from the carotid artery seven times: the first after the cannulation of carotid artery, the second after the preparative surgery, the third when the

systemic blood pressure was reduced to 25 mmHg, the fourth at the final second of shock-period, the fifth, sixth and seventh at the 1st, 5th, 19th min in the reperfusion period.

*Measurement of gastric lesions*

Photos were made after the formalin fixation from each stomach. After 3× magnification an independent person measured the areas of gastric lesions and the total gastric mucosal surface with grid (in $mm^2$). Statistical analysis (multiple variance + Scheffe test) was made by the Computing Centre.

*Histological grading*

The formalin fixed specimens stained with hematoxilin-eosin were used for grading of the lesions according to Arvidsson [3]. The mucosal lesions were graded 0–4.

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

The gastric mucosal damage index was calculated as the sum of the maximum damage in the three fundic areas.

*Drug studies*

The animals were divided into seven groups.
Control group: no pretreatment
Ranitidine groups:
1 mg/kg
2 mg/kg pretreatment
4 mg/kg
8 mg/kg
Allopurinol group: 50 mg/kg pretreatment
SOD group: 20000 U/kg pretreatment

The different drugs were given at the same time when animals received the pentobarbital sodium intraperitoneally.

*Measurements of plasma histamine levels*

Histamine levels were determined in the control group and allopurinol, SOD, Ranitidine (8 mg/kg) pretreated groups. Arterial blood from carotid artery was taken into cooled centrifuge tubes and immediately centrifuged at 4 °C for 10 min with 4000 g (Kokusan H-500 R centrifugal instrument). The plasma was separated and stored below −20 °C a maximum of 3 weeks before analysis. Histamine plasma levels were determined by radioimmunoassay (IMMUNOTECH S.A., France). The detection was carried out by 1282 COMPUGAMMA LKB WALLAC instrument. Wilcoxon's rank sum test was carried out for estimation of stochastic probability of the intergroup comparison.

## Results

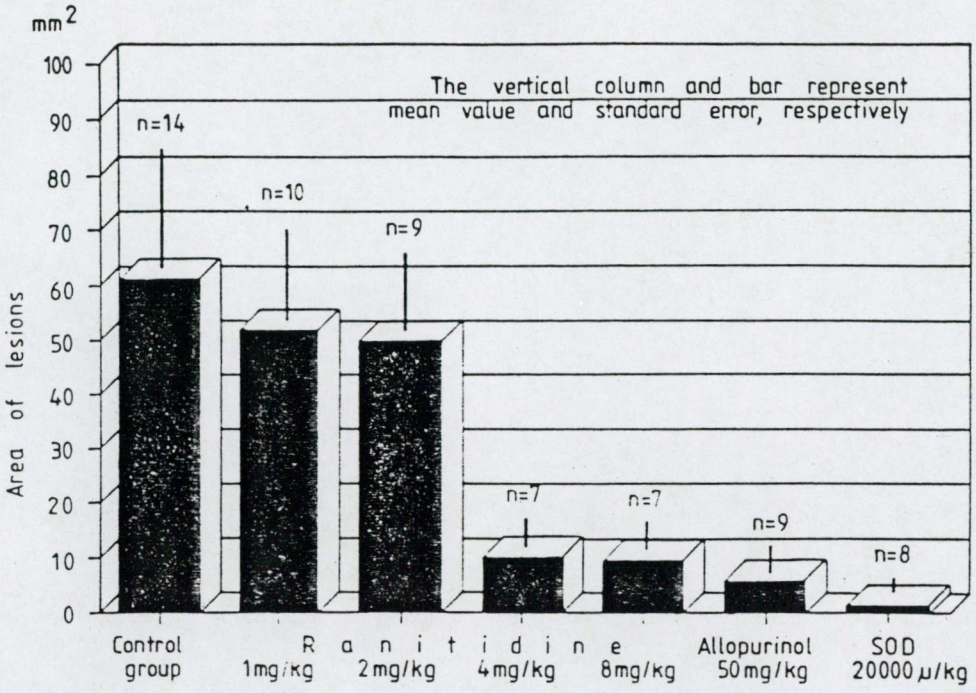


*Fig. 1*

Figure 1 shows the effect of different drugs on area of gastric lesions induced by haemorrhagic shock. The allopurinol, SOD and ranitidine (4 mg/kg and 8 mg/kg) pretreatment were effective against these lesions and the damaged areae were significantly smaller in these groups compared to the control group.

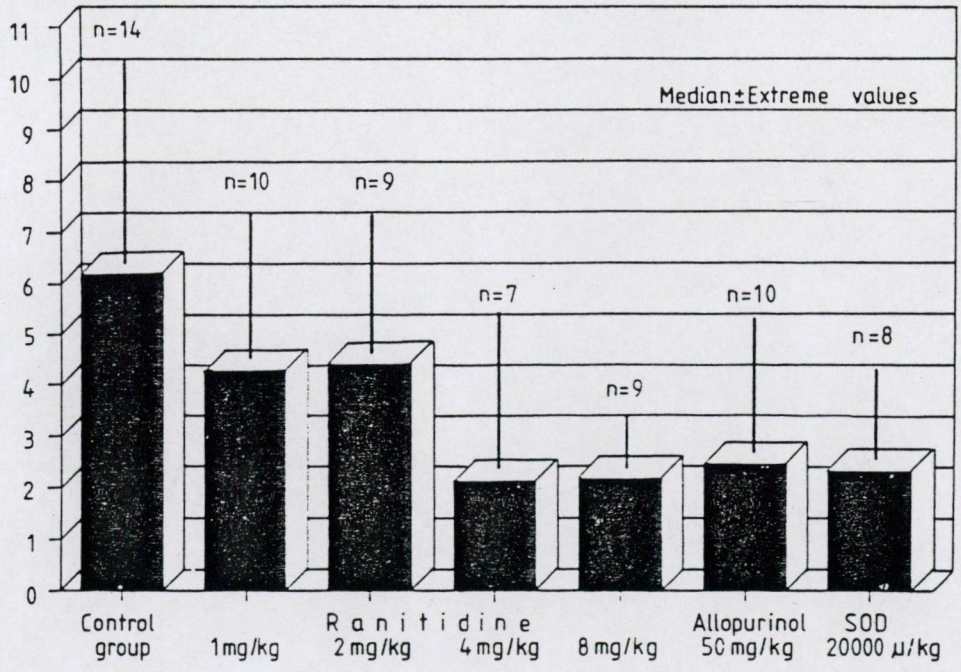


*Fig. 2*

Figure 2 shows the result of histological examination. The median values of mucosal damage index are as follows: control group: 6, ranitidine (1 mg/kg): 4, ranitidine (2 mg/kg): 4, ranitidine (4 mg/kg): 2, ranitidine (8 mg/kg): 2, allopurinol group: 2, SOD group: 2. These values also proved the protective effect of allopurinol, SOD and the high dose ranitidine in this shock model.

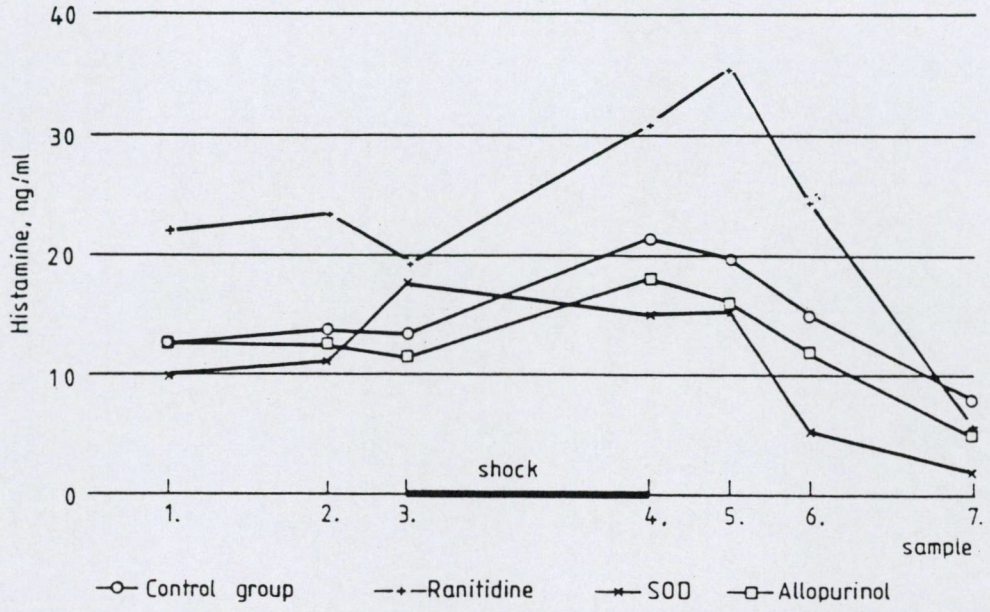


*Fig. 3*

Figure 3 shows the results of histamine measurements. Histamine levels were determined in the control group, and the allopurinol, SOD and ranitidine (8 mg/kg) pretreated groups.

In the control group the histamine level did not change significantly during the preparative surgery, but there was a significant increase of histamine level by the end of shock period. The histamine concentration in the plasma after the reinfusion of the shed blood remained essentially at the same level for five minutes, and later it decreased dramatically.

Allopurinol and SOD pretreatment did not influence significantly the values of histamine. The histamine plasma level in these groups were slightly smaller compared to the control but the difference was not significant. After the reinfusion of shed blood no histamine release was detected in these three groups. In the ranitidine pretreated group the plasma histamine values were different compared to the values of the control group. The ranitidine bolus injection caused significant histamine release immediately and every histamine level was significantly higher in this group compared to the control except for the final value which was lower than the control one. Beside the initial histamine release another release of histamine could be

observed by the end of shock period. After the reinfusion of shed blood a small increase of histamine level was found, but it was not significant.

## Discussion

Oxygen free radicals play an important role in the pathogenesis of gastric mucosal lesions in hypotension-reperfusion model [7, 15]. These oxygen free radicals can cause histamine release in vitro system [8, 9]. Local histamine release [5] was found after intestinal ischaemia and reperfusion, and antioxidant pretreatment was effective against this histamine release in canine. The plasma histamine level of rats is high [1, 4] because of the low activity of histamine-methyltransferase and histamino-diamineoxidase of microvascular endothelial cells. In human normal level of plasma histamine is 0.2–1.4 ng/ml, but the level is 17 ng/ml in rats [1, 4].

The role of histamine and the regulation of histamine release [14] has been studied for many years and further investigation is necessary. It was published that there is histamine cytoprotection against gastric lesions induced by HCl in rats [12]. The mucosal protective action of histamine may be mediated by endogenous prostaglandins through stimulation of H2-receptors.

Histamine release has been demonstrated in haemorrhagic shock [10] in canine. The histamine curve is similar in this experiment. Significant histamine release is proved by the end of shock period. The reinfusion of shed blood was not followed by a further histamine release as it must have been anticipated when the oxygen free radicals damage the cell membranes. The oxygen free radicals were not found as endogenous releasers.

Allopurinol (xanthine oxidase inhibitor) and (superoxide dysmutase) SOD pretreatment were effective against the formation of gastric lesions, but they did not modify significantly the histamine curve.

Ranitidine, H2-receptor blocker was effective against the formation of gastric lesions induced by haemorrhagic shock at the doses 4 mg/kg and 8 mg/kg. The smaller doses, 1 and 2 mg/kg were ineffective. Ranitidine, H2-receptor blocker given intraperitoneally as a bolus (8 mg/kg) caused a histamine release immediately after the injection. This phenomenon was published earlier [6]. A new histamine release was observed by the end of the shock period, but there was no significant histamine release after the reperfusion of the shed blood. It was published [2] that in shock mortality rate of rats in the ranitidine pretreated groups were higher compared to the other groups. We observed a similar fact in the ranitidine group (4 mg/kg). The correct pathomechanism is unclear.

## REFERENCES


1. Almeida, A.P., Flye, W., Deveraux, D., Horakova, Z., Beaven, M.A.: Distribution of histamine and histaminase (diamineoxidase) in lood of various species. Comp. Biochem. Physiol. 67, 187–190 (1980).
2. Altura. B.M.: Reticuloendothelial system function and histamine release in shock and trauma: relationship to microcirculation. Klin. Wochenschr. 60. 882–890 (1982).
3. Arvidsson, S., Falt, K., Haglund, U.: Acute gastric mucosal ulceration in septic shock. Acta Chir. Scand. 150, 541–547 (1984).
4. Beaven, M.A., Robinson-White, A., Roderick, N.B., Kauffmann, G.: The demonstration of histamine release in clinical conditions: A review of past and present assay procedures. Klin. Wochenschr. 60, 873–881 (1982).
5. Boros, M., Kaszaki, J., Nagy, S.: Oxygen free radical induced histamine release during intestinal ischemia and reperfusion. Eur. Surg. Res. 21, 297–304 (1989).
6. Doemicke, A., Lorenz, W.: Histamine release in anaesthesia and surgery. Premedication with H1-, and H2 receptor antagonists. Indications, benefits and possible problems. Klin. Wochenschr. 60, 1039–1045 (1982).
7. Itoh, M., Guth, P.H.: Role of oxygen-derived free radicals in hemorrhagic shock-induced gastric lesions in the rat. Gastroenterology 88. 1162–1167 (1985).
8. Mannaioni, P.F., Gianella, E., Palmerani, B., Pistelli, A., Gambassi, F., Bani-Sacchi, T., Bianchi, S.: Free radicals as endogenous histamine releasers. Agent and Actions 23, 129–142 (1988).
9. Masini, E., Lodovici, M., Fantozzi, R., Brunelleschi, S., Conti, A., Mannaioni, P.F.: Histamine release by free radicals: paracetamol induced histamine release from rat peritoneal mast cells after in vitro activation by monooxygenase. Agents and Actions 18, 85–88 (1986).
10. Nagy, S., Nagy, Á., Szabó, I., Tárnoky, K., Traub, A.: Histamine level changes in the plasma and tissues in haemorrhagic shock. Circulatory Shock 18, 227–239 (1986).
11. Perry, M.A., Wadhwa, S., Parks, D.A., Pickard, W., Granger, D.N.: Role of oxygen radicals in ischemia-induced lesions in the cat stomach. Gastroenterology 90, 362–367 (1986).
12. Takeuchi, K., Nishiwaki, H., Okada, M., Okabe, S.: Mucosal protective action of histamine against gastric lesions induced by HCl in rats: Importance of antigastric motor activity mediated by H2-receptors. J. Pharmacology and Experimental Therapeutics 245/2, 632–638 (1988).
13. Takeuchi, K., Ohno, T., Okabe, S.: Variation of gastric transmucosal potential difference and lesion formation during hemorrhagic shock in the rat. Gastroenterology 91, 1113–1123 (1986).
14. Waldum, H.L., Sandvik, A.K.: Histamine and the stomach. Scand. J. Gastroenterol. 24, 130–139 (1989).
15. Zöllei, I., Karácsony, G., Baltás, B., Nagy, S.: Role of oxygen-derived free radicals in haemorrhagic shock-induced gastric lesions of rats. Acta Physiol. Hung. 73/2–3/, 357–362 (1989).

# 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 Granger[6] demonstrated that the increased intestinal vascular permeability produced by ischaemia was largely prevented by pre-treatment with either allopurinol (xantine 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-dimethyi-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 heart[18-20] 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 peroxydase enzyme histochemically and to compare the protective effect of allopurinol (Ziloric, 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 Zamboni[21] 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 takes 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.

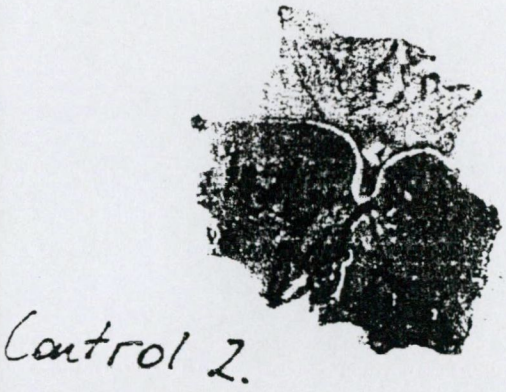


*Fig. 1. Stomach of a control rat (untreated). Dark areas represent the gastric mucosal lesions.*

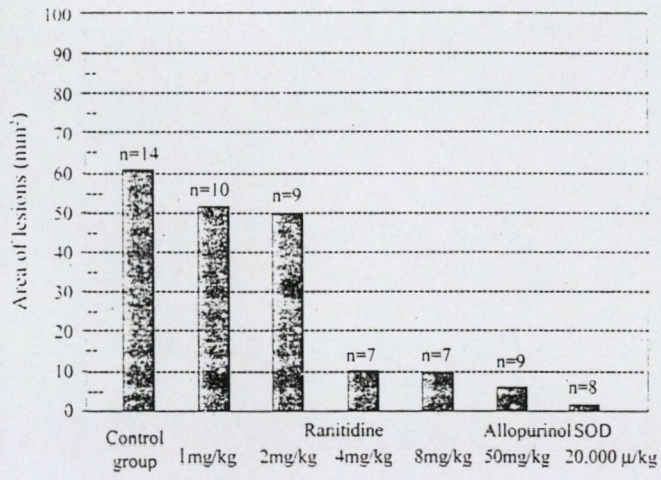


*Fig. 2. Effect of ranitidine, allopurinol and superoxide dysmutase on areas of gastric mucosal lesions induced by haemorrhagic shock. The vertical column and bar represent mean value and standard error respectively. The values of ranitidine (4 mg/kg), ranitidine (8 mg/kg), allopurinol and superoxide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

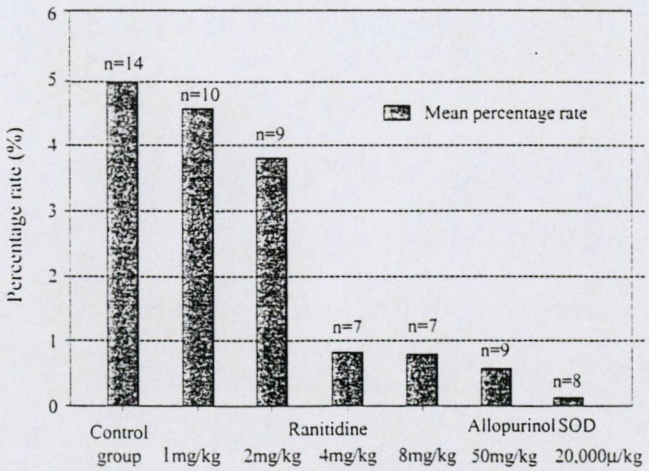


*Fig. 3. Effect of ranitidine, allopurinol and superoxide dysmutase on the percentage rate of gastric mucosal lesions induced by haemorrhagic shock. The vertical columns represent the mean percentage rates of gastric mucosal lesions. The values of ranitidine (4 mg/kg), ranitidine (8 mg/kg), allopurinol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

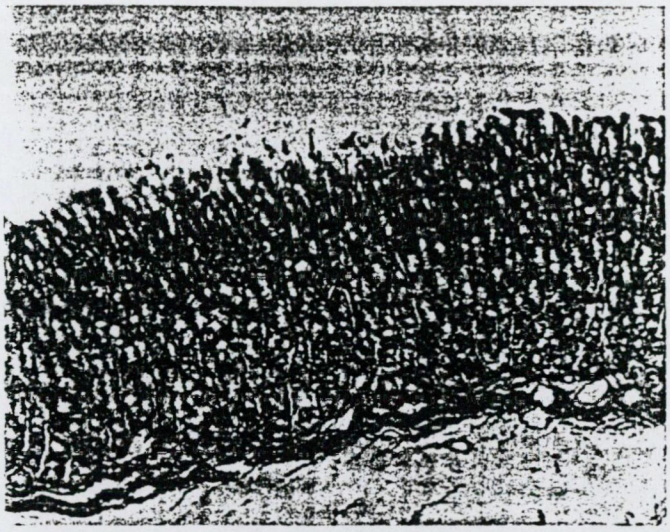

*Fig. 4. Light microscopic appearance of histological section of the stomach. Grade II shows the disappearance of the surface epithelial cells (haematoxylin-eosin) (x200).*

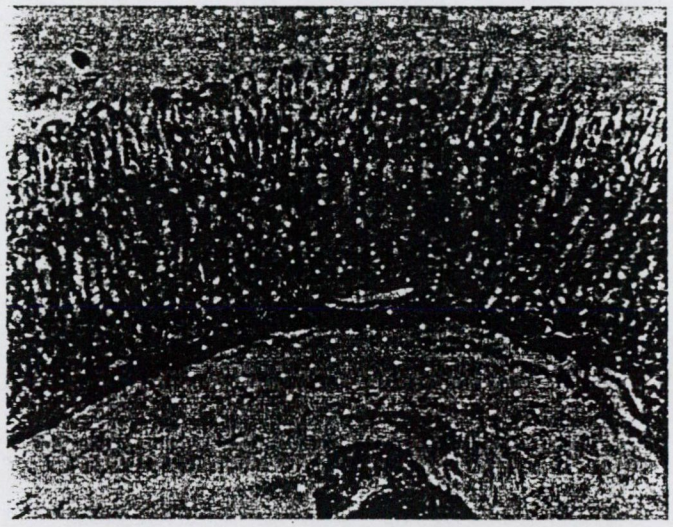

*Fig. 5. Light microscopic appearance of histological section of the stomach. Grade III shows the damage to the upper half of the glandular cells of gastric crypts (haemotoxylin-eosin) (x200).*

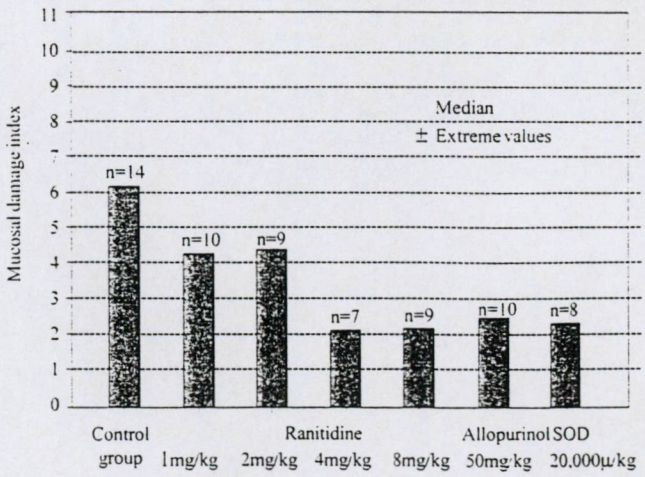


*Fig. 6 Changes of gastric mucosal damage index. It was calculated as the sum of the maximum damage in three fundic area (histological grading of gastric mucosal lesions).*

*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. x 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 dysmutase 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 dysmutase, 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 dysmutase scavenges the generated superoxide radicals, in that way the pre-treatment with SOD was effective.

Das et al[2] 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.

REFERENCES

1. Itoh M, Guth P H. Role of oxygen-derived free radicals in haemorrhagic shock-induced gastric lesions in the rat. Gastroenterology 1985; 88:1162-7.
2. Das D, Bandyopadhyay D, Bhattacharjee M, Banerjee R K. Hydroxyl radical

is the major factor in stress-induced gastric ulceration. Free Radic Biol Med 1997; 23:8-18.

3. Perry M A, Wadhwa S, Parks D A, Pickard W, Granger D N. Role of oxygen radicals in ischemia-induced lesions in the cat stomach.Gastroenterology 1986; 90:362-7.
4. Takeuchi K, Ohno T, Okabe S. Variation of gastric transmucosal potential difference and lesions formation during hemorrhagic shock in the rat. Gastroenterology 1986; 91:1113-23.
5. Zavagno G, Cagol P P, Da Pian P P, Vallongo P, Ishaq-Waris K, Lise M. Variations in transmucosal gastric potential difference during hemorrhagic shock in the rat. Eur Surg Res 1985; 17:38-43.
6. Parks D A, Granger D N. Ischemia-induced vascular changes: role of xanthine oxidase and hydroxyl radicals. Am J Physiol 1983; 245:G285-9.
7. Klebanoff S J. Phagocytic cells: products of oxygen metabolism. In: Gallin I I, Goldstein I M, Snyderman R, editors. Inflammation: Basic Principles and Clinical Correlates. New York: Raven, 1988:391-444.
8. Klebanoff S J, Waltersdorph A M. Inhibition of peroxidase-catalyzed reactions by deferoxamine. Arch Biochem Biophys 1988; 264:600-6.
9. Granger D N, McCord J M, Parks D A, Hallmarth M E. Xanthine oxidase inhibitors attenuate ischemia-induced vascular permeability changes in the cat intestine. Gastroenterology 1986; 90:80-4.
10. Grisham M B, Hernandez L A, Granger D N. Xanthine oxidase and neutrophil infiltration in intestinal ischemia. Am J Physiol 1986; 251(Gastrointest Liver Physiol 14):G567-74.
11. Zöllei I, Karácsony G, Baltás B, Nagy S. Role of oxygen-derived free radicals in haemorrhagic shock-induced gastric mucosal lesions of rats. Acta Physiol Hung 1989; 73:357-62.
12. Schoenberg M H, Muhl E, Sellin D, Younes M, Schildberg F W, Haglund U. Posthypotensive generation of superoxide free radicals—possible in the pathogenesis of intestinal mucosal damage. Acta Chir Scand 1984; 150:301-9.
13. Bulkley G B. Free radical-mediated reperfusion injury: selective review. Br J Cancer 1987; 55(Suppl VIII):66-73.
14. Parks D A, Bulkley G B, Granger D N. Role of oxygen-derived free radicals in digestive tract diseases. Surgery 1983; 94:415-22.
15. Parks D A, Bulkley G B, Granger D N. Role of oxygen free radicals in shock, ischemia, and organ preservation. Surgery 1984; 94:428-32.
16. Parks D A, Bulkley G B, Granger D N, Hamilton S R, McCord J M. Ischemic injury in the cat small intestine: role of superoxide radicals. Gastroenterology 1982; 82:9-15.
17. Parks D A, Granger D N. Contribution of ischemia and reperfusion to mucosal lesion formation. Am J Physiol 1986; 250:G749-53.
18. Fehér J, Pollák Z, Streter L, Tonesev H, Cornides Á, Gógi Á, et al. Experimental models for study of hepatoprotection. Acta Physiol Hung 1984; 64:401-7.
19. Sulyok S, Pollák Z, Fehár E, Kemenes I, Kántor L, Fehér J. Liver lipid peroxidation induced by cholesterol and its treatment with a dihydroquinoline type free radical scavenger in rabbits. Acta Physiol Hung 1984; 64:437-42.
20. Török B, Röth E, Bar V, Pollák Z. Effects of antioxidant therapy in experimentally induced heart infarcts. Basic Res Cardiol 1986; 81:167-79.
21. Stefanini M H, De Martino C, Zamboni L. Fixation of ejaculated spermatozoa for electron microscopy. Nature 1967; 216:173-4.
22. Arvidsson S, Falt K, Haglunnd U. Acute gastric mucosal ulceration in septic shock. Acta Chir Scand 1984; 150:541-7.
23. Cunningham S K, Keaveny T V. Effect of a xanthine oxidase inhibitor on adenine nucleotide degradation in haemorrhagic shock. Eur Surg Res 1978; 10:305-13.
24. Younes M, Schoenberg M H, Jung H, Fredholm B B, Haglund U, Schildberg F W. Oxidative tissue damage following regional intestinal ischaemia and reperfusion in the cat. Res Exp Med 1984; 184:259-64.
25. Zöllei I, Asakawa H, Karácsonyi S. Histamine release and SOD, allopurinol and ranitidine pretreatment in haemorrhagic shock in the rat. Circ Shock 1991; 34:157.

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Szent-Györgyi Albert Orvostudományi Egyetem Sebészeti Klinika (igazgató: Balogh Ádám egyetemi tanár)

# A végbélrákok sebészi kezelésének javuló eredményeiről

Zöllei István
Balogh Ádám

# Improvement results in surgical treatment of rectal cancer

**A szerzők bemutatják, hogy 1985. január 1-től 1997. december 31-ig 1799 vastag- és végbélműtétet végeztek intézetükben. A vizsgált 13 évet két 5 éves és egy 3 éves periódusra bontották fel. Az adatok közül kiemelik, s külön elemzik a 442 elektív, radikális végbélműtét adatait. Az első időszakban 189, a másodikban 152, míg a harmadikban 101 műtét történt. A betegek életkori és nemek közötti megoszlása hasonló volt, s így az egyes csoportok adatai összehasonlíthatóak. Megállapították, hogy a korai műtéti halálozás minden csoportban alacsony volt. A varrógépek kiterjedt használatának következtében a sphincter-megőrző műtétek részaránya 45%-ról 58%-ra emelkedett, ugyanakkor a végbélkiirtás részaránya 55%-ról 42%-ra csökkent. A legnagyobb változás a végbél középső harmadában elhelyezkedő tumorok sebészi ellátásában történt. A szövődmények elemzésekor megállapították, hogy a sphincter-megőrző műtétek végzésekor bevezetett dupla varrógépes technika és a retrorectális, infraperitoneális drénezés a második és a harmadik periódusban lecsökkentette a sebgennyedések és a reoperációk számát.**

Kulcsszavak: végbélrák, sphincter-megőrzés, szövődmények

***Authors presented that 1799 colorectal operations were performed between 1st Jan. 1985 till 31st Dec. 1997 in their surgical department. The examined 13 years were divided into two 5 years and one 3 years periods. Authors analysed the results and complication rates of their 442 elective radical rectal operations. 189 were in the first and 152 were in the second and 101 in the third period. Distributions of age and sex were similar, and the groups were comparable. The total mortality rate did not change significantly during the examined time. The rate of anterior rectal resections has improved due to the excellent staplers from 45% to 58% and the rate of abdomino-perineal rectal extirpation has decreased from 55% to 42% at the same time. The largest differences were found among the operations of middle part of rectum. Strong difference was found in the complications rates between the first and the second-third periods among the patients with anterior rectal resections. Less wound infections were due to the double stapler technique and less reoperations were performed due to the infraperitoneal-retrorectal drainage in the second-third period.***

*Keywords:* rectal cancer, sphincter-saving rate, complications

A végbélrákok növekedésének és terjedésének tanulmányozása alapján *Miles* a végbélkiirtást (az abdominoperinealis exstirpatiot) javasolta a végbélrákok sebészi kezelésére, hiszen véleménye szerint csak így lehetséges a minden irányban terjedő malignus daganat radikális eltávolítása. A 40-es években ez a szemlélet megváltozott. Dixon kidolgozta [7] az elülső reszekciót a rectosigmoideális tumorok eltávolítására, majd ezt az eljárást kiterjesztették a végbél felső harmad tumorainak kezelésére. Ezen gyakorlat szerint a felső harmad tumorait reszekcióval, a középső és alsó harmadban levőket pedig végbélkiirtással gyógyították. Az utóbbi 20 év alatt a colorectális sebészetben új műtéti eljárásokat dolgoztak ki vagy újítottak fel a világon, s ezek hatására megváltozott a fenti taktika. A varrógépek újabb és újabb fejlesztése [4, 5, 6, 9, 10], valamint a kettős varrógép („double stapler") technikának [16, 20, 22, 24] az alkalmazása egyre biztonságosabbá és általánosabbá tette a reszekciós eljárást, lerövidítette a műtéti időt [21]. Az abdomino-sacralis [8], az abdomino-transsphincterikus [14] behatolásból végzett műtétek és a coloanalis [10, 15, 19] anasztomózis-készítés új technikája még az alsó harmadi tumorok esetében is lehetővé teszik a végbél záróizomzatának megőrzését. Napjainkban már a középső harmadban ülő malignus daganatok kezelésében is az elülső reszekció vált általánossá, s a klasszikus abdomino-perinealis végbélkiirtás részaránya lecsökkent [11, 22, 23]. Tanulmányunkban azt a célt tűztük ki, hogy megvizsgáljuk az elektív, radikális végbélműtétek taktikájának és eredményeinek a változásait a Szent-Györgyi Albert Orvostudományi Egyetem Sebészeti Klinikájának beteganyagán.

Közlésre érkezett: 1998. február 25.

## Beteganyag és módszer

A Szent-Györgyi Albert Orvostudományi Egyetem Sebészeti Klinikáján 1985. január 1. és 1997. december 3. között 1799 vastag- és végbélműtét történt. A beteganyag feldolgozása során szétválasztottuk az elektív radikális, az elektív palliatív és a sürgősségi műtétek csoportját. Az *I. táblázat* az 1218 elektív radikális műtét megoszlását mutatja be. A műtétek korai mortalitása 2,3% volt, amely megfelel a nemzetközi követelményeknek is. Tanulmányunkban a továbbiakban a 442 elektív, radikális végbélrák miatt végzett műtét adatainak elemzésével foglalkozunk.

A vizsgált csoportokban a nemek megoszlása, az átlagéletkor és még a testsúlyok megoszlása sem mutatott jelentős eltérést, így azok összehasonlíthatók voltak.

I. táblázat
Az elektív, radikális vastagbélműtétek típus szerinti felosztása
Betegek száma: 1218

| | 1985–89 (5 év) | 1990–94 (5 év) | 1995–97 (3 év) | Összesen |
|---|---|---|---|---|
| Jobb o. hemicolectomia | 93 | 101 | 59 | 253 |
| Vastagbél reszekció | 73 | 90 | 51 | 214 |
| Sigma reszekció | 110 | 137 | 62 | 309 |
| Rectum műtétek | 189 | 152 | 101 | 442 |
| Összesen | 465 | 480 | 273 | 1218 |

## Műtéti előkészítés, antibiotikum és thrombosis profilaxis

A vastagbélsebészet egyik legnagyobb veszélyforrása maga a bélben felhalmozódott bélsár. Ennek a műtét előtti kiürítésére, azaz a bél kitisztítására a hagyományos beöntések mellett újabb és újabb eljárásokat vezettek be. A vizsgálat első szakaszában, ha nem volt bélszűkület, a nemzetközileg elfogadott módszer szerint, a duodenum szondán keresztül bejuttatott 8–10 liternyi folyadékkal bélátmosást [17] végeztünk, de ezt a beteg szervezetének, elsősorban a keringés megterhelése miatt fokozatosan elhagytuk. A második és harmadik szakaszban a béltisztítást ozmotikus hashajtót tartalmazó 3 liter folyadék szájon át való elfogyasztásával értük el. A részleges elzáródás fennállásakor, vagy a hashajtás-bélátmosás során jelentkező erős hasi görcsök esetén a hagyományos beöntésekre tértünk át.

Az antibiotikum profilaxis alkalmazása rendkívül fontos a vastagbélsebészetben. A szerzők intézetében az országban az elsők között vezették be az úgynevezett „one shot", azaz az egyszeri antibiotikum alkalmazását [17]. A vizsgált beteganyag közel felében ezt a módszert, a másik felében pedig a 24 órás antibiotikum profilaxist alkalmaztuk [2].

A vizsgált periódusban a thrombosis profilaxis a kis molekulasúlyú heparin-származékokkal (Calciparin, Fragmin, Fraxiparin) történt.

## Műtéti technika

Klinikánkon korábban a kézi anasztomózis-készítés mellett SPTU körkörös szovjet varrógépet is használtunk a végbélreszekciók során [4, 5]. Az értékelt 1. vizsgálati szakaszban öt ilyen beteg adatai is szerepelnek, de ezek száma olyan kicsi, hogy nem befolyásolták a kiértékelést. A rectum reszekciós műtétek során a továbbiakban kézi anasztomózist csak kivételesen alkalmaztunk. Erre legtöbbször a varrógépek használata során jelentkező technikai hibák esetében került sor. Az első szakaszban az EEA amerikai körkörös varrógépet úgy alkalmaztuk, hogy mind a distális, mind a proximális bélvégre nyitott állapotban helyeztük be a körkörös dohányzacskó varratot. A 2. és a 3. periódusban a kettős varrógép technikát vezettük be, azaz a distális bélvéget egyenes varrógéppel lezártuk a reszekció során, s a varratsoron keresztül vezettük fel a szétszedhető amerikai varrógép testében levő nyársat. Ezzel a „zárt" módszerrel a műtéti terület tisztaságát lehetett fokozni, amely a posztoperatív szeptikus szövődmények csökkenését eredményezte. A 3. periódusban különösen ügyeltünk a teljes mesorectum exstirpatio (total mesorectal excision, „TME") helyes kivitelezésére [1]. Fontos változás volt az első szakaszban alkalmazott hasűrön keresztüli drénezési módszer megváltoztatása. A második és harmadik periódusban az úgynevezett retrorectális, infraperitoneális drénezést alkalmaztuk. Az anasztomózis felett a peritoneumot gondosan zártuk. Részleges tehermentesítés céljából a végbélnyíláson át felvezetett drént használtunk, amely segítségével az anasztomózis épségének vizsgálatára rutinszerűen festékpróbát végeztünk. Csak kivételesen készítettünk, másokhoz hasonlóan [12], tehermentesítő sztomát.

Az abdomino-perinealis rectum exstirpatiot mindhárom vizsgált időszakban a Lloyd-Davies által javasolt két munkacsoportos módszerrel végeztük.

## Eredmények

A szövettani eredmények vizsgálatakor sajnálattal kellett megállapítanunk, hogy a vizsgált mindhárom időszakban a Dukes C stádiumú tumorok az esetek 2/3-át képviselték. A Dukes B stádium csak 20–25% között mozgott, míg a Dukes A stádium alig érte el a 10%-ot.

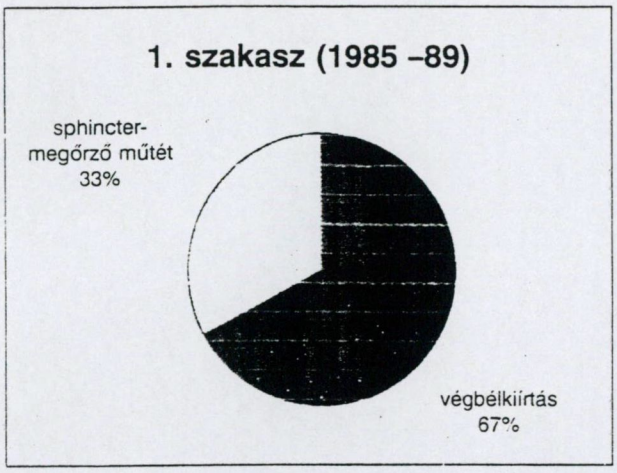
A *II. táblázat* adatai a végbélműtétek megoszlását és a korai mortalitás adatait mutatja. A mortalitás adataiban nem volt szignifikáns eltérés az egyes csoportokon belül. A műtéti megoszlás elemzésekor megállapíthatjuk, hogy az első szakaszban több végbélkiirtás történt, mint reszekció, de a 2. és a 3. periódusban ez az arány megfordult a sphincter-megőrző műtétek javára. Az *1. ábra* adatai szerint a meghatározó változás a középső harmadban elhelyezkedő tumorok kezelésében történt. Míg az első periódusban a végbél középső harmad tumorainak csak 33%-át reszekáltuk, addig ez az arány a második és harmadik szakaszban 72%-ra emelkedett. Az alsó harmadban levő tumorok miatt elvi meggondolás alapján abdomino-perineális végbélkiirtást végeztünk.

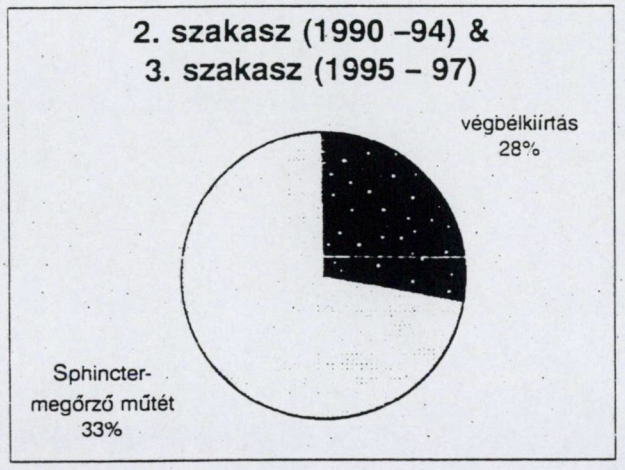
II. táblázat
Az elektív végbélműtétek korai halálozási aránya

| | 1985–89 (5 év) | | 1990–1994 (5 év) | | 1995–1997 (3 év) | | Összesen | |
|---|---|---|---|---|---|---|---|---|
| Abdomino-perinális rectum exstirpatio | 3/104 | 2,9% | 2/64 | 3,0% | 2/44 | 4,5% | 7/212 | 3,3% |
| Resectio anterior recti | 2/85 | 2,35% | 2/88 | 2,27% | 1/57 | 1,75% | 5/230 | 2,17% |
| Összesen | 5/189 | 2,64% | 4/152 | 2,63% | 3/101 | 2,97% | 12/442 | 2,7% |

A *III. táblázat* a végbélkiirtás utáni műtéti szövődményeket mutatja. A vizsgált periódusban jelentősebb javulás a húgyúti és a gáti fertőzésekben jött létre. A reoperációk részaránya és a korai műtéti mortalitás a harmadik periódusban enyhén emelkedett, de az emelkedés mértéke nem volt jelentős.

A *IV. táblázat* a reszekciós műtétek korai szövődményeit tartalmazza. A mortalitásban javulás mutatható ki, de a javulás mértéke nem volt meghatározó. Figyelemre méltó javulás jött létre a húgyúti fertőzések és a sebfertőzések terén. A sebfertőzések csökkenését nagy valószínűséggel a kettős varrógépes technika alkalmazása eredményezte. Ugyancsak örvendetes javulás jött létre a második és harmadik periódusban a reoperációk vonatkozásában. A műtéti technika leírásakor említettük, hogy ebben a két periódusban retrorectális, infraperitoneális drént alkalmaztunk a kismedence lezárt területének drenálására. Ez a drén jelezte a klinikailag is észlelhető varratelégtelenséget, s ugyanakkor több eset-




1. ábra

ben megakadályozta az általános hashártyagyulladás kialakulását, s így a beteget nem kellett reoperálni, a kisebb fokú varratelégtelenség a konzervatív kezelés, szívó-öblítő rendszer alkalmazásával gyógyult.

III. táblázat
A végbélkiirtás utáni szövődmények

| | 1985–89 (5 év) No 104 | | 1990–94 (5 év) No 64 | | 1995–97 (3 év) No 44 | | Összesen No 212 | |
|---|---|---|---|---|---|---|---|---|
| Szív-tüdő | 4 | 3,85% | 2 | 3,1% | 2 | 4,5% | 8 | 3,8% |
| Gyomor-bél vérzés | 3 | 2,9% | 2 | 3,1% | 1 | 2,27% | 6 | 2,83% |
| Húgyúti fertőzés | 11 | 10,5% | 5 | 7,8% | 3 | 6,8% | 19 | 9,0% |
| Subileus | 10 | 9,6% | 6 | 9,3% | 4 | 9,0% | 20 | 9,4% |
| Hasfali infekció | 8 | 7,7% | 4 | 6,25% | 3 | 6,8% | 15 | 7,0% |
| Gáti infekció | 12 | 11,5% | 6 | 9,3% | 4 | 9,0% | 22 | 10,4% |
| Reoperáció | 2 | 1,9% | 1 | 1,5% | 3 | 4% | 5 | 2,35% |
| Halálozás | 3 | 2,9% | 2 | 3,0% | 2 | 4,5% | 7 | 3,3% |

IV. táblázat
A sphincter-megtartó rectumműtétek szövődményei

| | 1985–89 (5 év) No 85 | | 1990–94 (5 év) No 88 | | 1995–97 (53év) No 57 | | Összesen No 230 | |
|---|---|---|---|---|---|---|---|---|
| Szív-tüdő | 3 | 3,5% | 3 | 3,4% | 2 | 3,5% | 8 | 3,48% |
| Gyomor-bél vérzés | 3 | 3,5% | 2 | 2,3% | 2 | 3,5% | 7 | 3% |
| Húgyúti fertőzés | 8 | 9,4% | 6 | 6,8% | 3 | 5,26% | 17 | 7,4% |
| Subileus | 8 | 9,4% | 7 | 7,9% | 4 | 7% | 19 | 8,2% |
| Sebfertőzés | 10 | 11,8% | 5 | 5,7% | 3 | 5,26% | 18 | 7,8% |
| Varrat-elégtelenség | 4 | 4,7% | 4 | 4,5% | 2 | 3,5% | 10 | 4,35% |
| Reoperáció | 3 | 3,5% | 1 | 1,13% | 1 | 1,75% | 5 | 2,17% |
| Halálozás | 2 | 2,35% | 2 | 2,27% | 1 | 1,75% | 5 | 2,17% |

## Megbeszélés

A vastag- és végbélsebészet az utóbbi 20 év alatt jelentős fejlődésen ment át világszerte, s hazánkban is [11, 16, 20, 23]. A fejlődést sok-sok tényező együttes hatása okozta. Javult a műtéti érzéstelenítés, a belek preoperatív előkészítése és a stressz-ulcusok elleni védekezés. Általános gyakorlattá vált az antibiotikum és a thrombosis profilaxis. A varróanyagok és varrógépek folyamatos fejlesztése egyre biztonságosabbá tette a végbélrákok sebészetét is.

A végbélrák kezelésében bár van olyan irányzat, amely az alsó harmadban levő Dukes A stádiumú tumorok helyi kimetszését is elégségesnek tartja [13, 18], de az általánosan elfogadott módszer a reszekció vagy végbélkiirtás. Klinikánkon lokális kimetszést csak az in situ végbélrák esetében alkalmaztunk. A modern körkörös varrógépek használata [4, 5, 6, 9, 10], a dupla varrógépes technika bevezetése jelentős mértékben növelte a sphincter-megőrző műtétek részarányát [16, 20, 22, 24]. A rectum felső harmadában ülő tumorok esetében már korábban kizárólagossá vált, s az utóbbi években a középső harmadban ülő tumorok esetében is jelentősen emelkedett a reszekciók részaránya [11, 22, 23]. Egyes munkacsoportok az abdomino-sacrális [8] és abdomino-transsphincticus [14] reszekciók alkalmazásával már az alsó harmadi tumorok esetében is javasolják a sphincter-megőrző műtétek végzését.

A SZOTE Sebészeti Klinikáján az elektív radikális végbélműtétek nemzetközileg elfogadható szinten (alacsony korai mortalitás és posztoperatív szövődményráta) történnek. A vizsgált időszakban a körkörös varrógépek kiterjedt használatával és a dupla varrógépes technika bevezetésével nemcsak a sphincter-megőrző műtétek részaránya emelkedett, de csökkent a posztoperatív morbiditás és a reoperációk száma is. Az alsó harmadban ülő végbélrákok kezelését elvi okokból abdomino-perineális végbélkiirtással végeztük, azaz nem alkalmaztuk a fentebb említett abdomino-sacrális reszekciót. A lokálisan kiterjedt végbélrákok miatt végzett, több szervet is érintő műtéteinkről más tanulmányban [3] számoltunk be. Az utolsó vizsgálati periódusban végzett TME helyi recidívát csökkentő és túlélést növelő hatását az utóvizsgálatok bizonyíthatják.

## IRODALOM


1. *Balogh Á., Varga L., Lázár Gy., Höhn J., Paszt A., Furák J.:* „Harmonic scalpellel" végzett mesorectum excisio a rectum carcinoma műtéteinél. Magy. Seb. *51*, 177 (1998).
2. *Balogh Á., Lázár Gy., Zöllei I., Szederkényi E.:* The value of augmentin prophylaxis in colorectal surgery: a double blind randomized study. Br. J. Surg. 85, Suppl. *2*, 24 (1998).
3. *Balogh Á., Zöllei I., Lázár Gy.:* Multiple organ resections for locally advanced rectal cancer. Eur. J. Surg. Oncol. *22*, 434 (1996).
4. *Baradnay Gy., Nagy A., Zöllei I.:* Gépi varratok a vastagbélsebészetben. Orv. Hetil. *126*, 909 (1985).
5. *Baradnay Gy., Nagy A., Zöllei I.:* Erfahrungen mit dem sowjetischen Klammernahgerat. Zbl. Chir. *110*, 108 (1985).
6. *Bartha I., Bodrogi T., Németh A., Hajdu J.:* Szemléletváltozás a végbélrák sebészeti kezelésében. Magy. Seb. *46*, 373 (1993).
7. *Dixon C. F.:* Surgical removal of lesions occuring in the sigmoid and rectosigmoid. Amer. J. Surg. *12*, 46 (1939).
8. *Eng K., S. A. Localio:* Abdominosacral resection for midrectal cancer. Hepato-Gastroenterol. *39*, 207 (1992).
9. *Goligher J. C., W. R. Lee:* Experience with the Russian model 249 suture gun for anastomosis of the rectum. Surg. Gyn. Obstet. *148*, 517 (1979).
10. *Goligher C.:* Use of the circular stapling gun with perianal insertion of anorectal purse-string suture for constructing of very low colorectal or coloanal anastomoses. Br. J. Surg. *66*, 501 (1979).
11. *Görög D., Tóth A., Weltner J.:* A végbélrák sebészi kezelése: reszekció vagy exstirpáció? Magy. Seb. *48*, 174 (1995).
12. *Heald R. J.:* Towards fewer colostomies – impact of circular stapling devices on the surgery of rectal cancer in a district hospital. Br. J. Surg. *67*, 198 (1979).
13. *Hildebrandt U., G. Schüder, G. Feifel:* Preoperative staging of rectal and colonic cancer. Endoscopy *26*, 810 (1994).
14. *István G., Berki I., Kiss S., Faller J.:* Abdomino-transsphincticus végbélreszekció: újabb lehetőség a mélyen elhelyezkedő végbélrák záróizom-megtartó sebészi kezelésére. Orv. Hetil. *139*, 293 (1998).
15. *István G., F. Lazorthes, P. Chiotasso, M. Cherubin, B. Thierry:* Végbélreszekció és coloanal anasztomózis eredményei a distális elhelyezkedésű végbélrák sphinctermegtartó sebészi kezelésében. Magy. Seb. *49*, 267 (1996).
16. *Köves I., Vámosi-Nagy I., Besznyák I.:* 360 kézi és gépi úton, Dixon szerint végzett rectum reszekció értékelése. Magy. Seb. *48*, 187 (1995).
17. *Nagy A., Zöllei I., Garas F., Karácsonyi S.:* Az ortográd bélátmosás és ultrarövid iv. antibiotikum profilaxis hatása a vastagbélműtöttek gyógyulására és kezelésük költségeire. Quarterly Bulletin of the Hung. Gastroent. Society *4*, 44 (1987).
18. *Nagy A., G. Buess:* A transanalis endoszkópos műtéttechnika (sec. Buess) magyarországi első eredményei. Orv. Hetil. *137*, 2839 (1996).
19. *Parks A. G.:* Transanal technique in low rectal anastomosis. Proc. R. Soc. Med. *65*, 975 (1972).
20. *Weltner J., Németh Zs., Bursics A., Friedmann G., Ladányi A.:* Változások a colorectális rákok kezelésében. Magy. Seb. *51*, 343 (1998).
21. *Zöllei I.:* Cost-benefit problems in colorectal surgery in Hungary. Br. J. Surg. *85*, Suppl. 2., 23 (1998).
22. *Zöllei I., Balogh Á., Lázár Gy.:* Changes of tendency of rectal surgery. Br. J. Surg. *83*, Suppl. 2, 38 (1996).
23. *Zöllei I., Balogh Á.:* Improvement of results in the colorectal surgery. Digestion *59*, Suppl. 3, 747 (1998).
24. *Zöllei I., Balogh Á., Lázár Gy.:* Efforts for decreasing the complication rates in rectal surgery. Int. J. Colorect. Dis. *12*, 190 (1997).



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ORIGINAL ARTICLE

# Betaine-Palmitate Reduces Acetylsalicylic Acid-induced Gastric Damage in Rats

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Zöllei I, Szabó A, Kaszaki J, Tiszlavicz L, Ghyczy M, Boros M. Betaine-palmitate reduces acetylsalicylic acid-induced gastric damage in rats. Scand J Gastroenterol 2001;36:811–816.

**Background:** Acetylsalicylic acid (ASA)-induced gastric injury is reduced when ASA is administered along with phosphatidylcholine. The hydrolysis of endogenous phosphatidylcholine leads to the production of betaine, which may participate in the maintenance of cellular homeostasis. The present aims were to investigate the effects of exogenous betaine and its palmitic acid complex (betaine-palmitate) in the protection of the gastric mucosa in ASA-induced subacute damage. **Methods:** Repeated doses of ASA were given intragastrically to male Wistar rats. Control rats were given vehicle only, while treated animals were challenged with ASA or with ASA along with betaine, palmitic acid or betaine-palmitate. The gastric mucosa was examined after 3 days and the nature of any microscopic mucosal injury was assessed by histology. The extent of macroscopic damage, changes in permeability (assessed by Evans blue method) and tissue ATP concentrations were determined in separate series. **Results:** ASA induced a significant fall in the ATP content of the mucosa, which was not affected by the other drugs used in the study. However, the ASA-induced mucosal permeability increase could be completely reversed by betaine-palmitate supplementation. The extent of severity of the macroscopic and microscopic lesions was 33% and 2.45, respectively, for ASA, as compared with 15% and 2.2 for betaine, 14% and 1.9 for palmitic acid and 3% and 1.4 for betaine-palmitate. **Conclusions:** Betaine-palmitate affords a significant protective effect against ASA-induced injury, without influencing the ATP synthesis, and this suggests that the defence is due to its ability to prevent secondary damage.

**Key words:** Aspirin; acetylsalicylic acid; ATP; betaine; betaine-palmitate; gastric mucosa; injury; permeability; rat

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Acetylsalicylic acid (ASA) is used extensively for the treatment of inflammation and for pain alleviation in several acute and chronic diseases. While the clinical benefits of this compound are evident, its therapeutic utility is limited by the high incidence of mucosal side effects characterized by disruption of the mucosal permeability barrier (1). Although the pathomechanism of ASA-induced gastric erosion and ulceration is still unclear, a number of data suggest that the phospholipids of the gastric mucus gel play an important role in this action. Phosphatidylcholine containing palmitic acid as hydrophobic moiety in the molecule is among the major phospholipids of the gastric mucus. It has been demonstrated that ASA attenuates the surface hydrophobicity of the gastric mucosa by destabilizing phosphatidylcholine within the mucus layer (2). Similarly, it has been observed that non-steroidal antiinflammatory drug (NSAID)-induced mucosal injury could be prevented when the drugs were chemically associated with phosphatidylcholine before administration (3). These observations suggest that the ability of phosphatidylcholine to inhibit mucosal injury after NSAID administration is related to a function in the maintenance of the defensive barrier of the gastric mucosa.

On the other hand, it has been proposed that the multifactorial ulcer-producing actions of ASA are initiated by the lowering of adenosine triphosphate (ATP) generation in the affected tissues (4). After ingestion, ASA is hydrolysed rapidly to salicylate, a potent uncoupler of mitochondrial oxidative phosphorylation (4). The mitochondria, which play a crucial role in maintaining the cell ATP-dependent processes, are therefore considered to be potential targets of ASA-induced toxicity. The phospholipase D-catalyzed hydrolysis of membrane phosphatidylcholine to phosphatidic acid and choline and the subsequent oxidation of choline leads to the production of endogenous (carboxymethyl)trimethylammonium hydroxide (betaine). The physiological role of endogenous betaine is not well defined, but the cytoprotective effect of exogenous betaine in ethanol-induced liver steatosis and ischaemia-reoxygenation injury has recently been demonstrated (5, 6).

Table I. The numbers of animals ($n$) used for each data point in the subsequent Figures are noted in this Table. See Materials and Methods for a description of the experiments

| Experimental series | Group 1 Control | Group 2 ASA + vehicle | Group 3 ASA + betaine | Group 4 ASA + betaine-palmitate | Group 5 ASA + palmitic acid |
|---|---|---|---|---|---|
| Series 1 Histology and planimetry | 5 | 5 | 5 | 5 | 5 |
| Series 2 ATP measurements | 8 | 7 | 7 | 8 | 8 |
| Series 3 Permeability measurements | 7 | 8 | 7 | 8 | 8 |
| Total ($n$) | 20 | 20 | 19 | 21 | 21 |

In the present study, we have assessed the ability of ASA to interfere with ATP synthesis and to cause changes in the structural and functional integrity of the rat gastric mucosa. In this context, we set out to establish whether exogenous betaine administration can protect the gastric mucosa under highly ulcerogenic conditions. The study extended to the effects of betaine complexed with palmitic acid, a long-chain fatty acid involved in the maintenance of the hydrophobic properties of the gastric mucosa. Palmitic acid was also selected as a permeation enhancer, with regard to its ability to bind and incorporate into the gastric mucin (7, 8).

We report here that this novel treatment modality offers significant protection against ASA-induced gastric damage and mucosal permeability changes in the rat.

## Materials and Methods

The experiments were performed in accordance with the NIH Guidelines (Guide for the Care and Use of Laboratory Animals) and the study was approved by the Animal Welfare Committee of the University of Szeged. One-hundred-and-one male Wistar rats (average weight 250 g) were housed in an environmentally controlled room under a 12-h light – 12-h dark cycle. The animals were kept on a standard laboratory diet and then on a carbohydrate-rich diet (bread rolls) for 3 days prior to the experiments.

### *Induction of subacute gastritis*

The animals were randomly allotted into five groups. Those in group 1, which served as vehicle-treated control, received 15 ml/kg buffered 0.11 M potassium hydroxide (KOH) via a flexible oesophageal tube under light inhalation anaesthesia. The procedure was repeated three times daily on three consecutive days.

In groups 2–5, subacute gastritis was induced. The animals were gavaged with ASA solution (Reanal, Budapest, 200 mg/kg in a volume of 10 ml/kg) 3 times daily for 3 days. During this intragastric ASA treatment, the animals additionally received 5 ml/kg vehicle (group 2), 37.5 mg/kg betaine in a volume of 5 ml/kg (group 3), 100 mg/kg betaine-palmitate in 5 ml/kg (group 4), or 62.5 mg/kg palmitic acid in 5 ml/kg (group 5). Betaine-palmitate was freshly prepared according to the following method: 10 mmol of betaine was dissolved in 30 ml methanol and mixed with 10 mmol of palmitic acid dissolved in 30 ml acetone for 10 min. The solution was evaporated to dryness, and the residue was washed with diethyl ether, ground, washed again and filtered off. Stock solutions of ASA (9) and test agents were made on the day of use and administered in a 1:2 molar ratio. Other betaine-fatty acid complexes were also tested in pilot experiments (data not shown).

On day 3, the animals in groups 2–5 were randomly assigned into 3 experimental series. In series 1, the abdomen was opened under ether anaesthesia exactly 2 h after the last treatment, and the stomach was removed, cut along the longer curvature and gently washed in saline. Computer-assisted planimetric analysis and histological evaluation were subsequently performed. Series 2 and 3 were used for tissue ATP and microvascular permeability measurements, respectively. The numbers of animals used for each series of studies are given in Table I.

### *ATP measurement*

Whole-thickness gastric samples were taken by means of a freeze-clamp technique. The tissues were immediately cooled in liquid nitrogen and stored at −80 °C until processing. Samples were homogenized in 6% trichloroacetic acid (250 mg/ml) and centrifuged at 5000$g$ for 10 min. The supernatant was neutralized with an equal volume of 0.4 M potassium hydrogencarbonate solution and further centrifuged at 5000$g$ for 10 min. The ATP concentration was measured spectrophotometrically according to Lamprecht & Trautschold (10). The method is based on the principle that beta-nicotinamide adenine dinucleotide phosphate is used up in an enzymatic reaction catalyzed by glucose-6-phosphate dehydrogenase and hexokinase in an ATP-dependent manner.

### *Vascular permeability index*

Changes in the permeability of the gastric mucosa were determined 2 h after the last intragastric treatment. Microvascular permeability was determined by using Evans blue (Sigma Chemicals), which binds rapidly to albumin and migrates with it. Briefly, the animals were anaesthetized, the external jugular vein was cannulated and Evans blue (a

10 mg/kg bolus in 1 ml/kg saline) was injected iv. Thirty minutes later, a blood sample was taken from the right ventricle. The stomach was rapidly excised, the mucosal layer was scraped off with a microscope slide, and the scrapings were put in 1 ml of formamide and homogenized in a glass Potter homogenizer for 1 min. The homogenate was incubated at room temperature for 18 h and then centrifuged at 5000*g* for 60 min. The absorbances of the supernatant and serum were determined at 650 nm against a formamide blank with a UV-1601 spectrophotometer (Shimadzu, Japan). The protein contents of the samples were determined by the procedure of Lowry et al. (11). Gastric microvascular permeability was expressed as the permeability index (PI), defined as the ratio of the concentration of Evans blue in the mucosa to the concentration in the serum: PI = (Evans blue concentration in tissue)/(Evans blue concentration in plasma).

*Computer-assisted planimetric analysis*

The stomach was removed, cut open along the greater curvature and gently rinsed with isotonic saline, and SVHS video images of the gastric mucosa were taken. The pictures were digitized, and quantitation of the macroscopic mucosal damage (dark parts of the stomach) was performed off-line by analysis of videotaped images using a computer-assisted image analysis system (IVM Pictron, Budapest, Hungary). The damaged area was expressed as a percentage of the total mucosal area.

*Histology*

Tissue biopsy samples for light microscopy were fixed in ice-cold neutral formalin, and the fixed tissue was attached to a hard cardboard backing to ensure the optimal longitudinal direction of the section. The samples were embedded in paraffin, sectioned (6 μm) and stained with haematoxylin-eosin. Mucosal damage was assessed on the scale of Arvidsson et al. (12), with the following criteria: grade 0, normal mucosa; grade 1, oedema just beneath the superficial epithelium; grade 2, disappearance of the surface epithelial cells; grade 3, damage to the upper half of the glandular cells of the gastric crypts; grade 4, disappearance of the glands. The gastric mucosal damage index was calculated as the average of the damage in three different areas of the fundus.

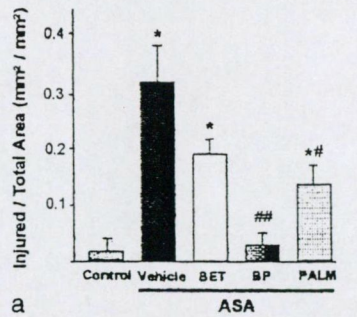
*Statistical analysis*

The Kruskal-Wallis test was used for the estimation of stochastic probability in intergroup comparisons. The Friedman test followed by Dunnett's method was applied for multiple comparisons with a control. Mean ± $s_{\bar{x}}$ values are given. *P* values less than 0.05 were considered significant.

## Results

*Gastric morphological changes*

The effects of oral ASA administration for 3 days on the gastric morphology are presented in Figs 1 and 2. The ASA



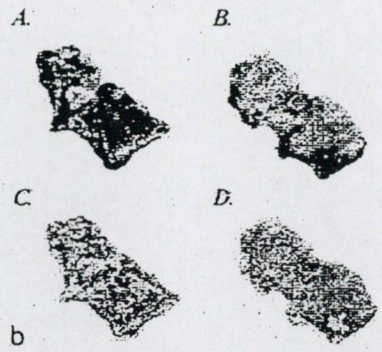




Fig. 1a. Effects of betaine (BET, open bar), betaine-palmitate (BP, shaded bar) and palmitic acid (PA, grey bar) on macroscopic damage in the gastric mucosa. Subacute gastritis was elicited by oral administration of ASA three times a day for 3 days. Data are means ± $s_{\bar{x}}$. $^*P < 0.01$ versus control, # $P < 0.05$ versus vehicle + ASA.

Fig. 1b. Representative images of ASA-induced gastric mucosal damage. ASA administration for 3 days (*A*). Betaine-palmitate (BP) was given orally at the time of ASA administration (*B*). Planimetric assessment of the extent of damage was performed by means of a computer-assisted analysis of digitized video images. Native images of injury caused by ASA (*A*) or ASA + BP (*B*); and injured areas as marked by the image-analysis software for ASA (*C*) and ASA + BP (*D*) treatment, respectively.

treatment induced severe macroscopic mucosal damage, the area of the gastric erosions reaching more than 30% of the total surface of the stomach. Patchy erosions and ulcerations were generally present, but large confluent, damaged areas were also observed occasionally (Fig. 1).

The biopsy samples from the vehicle-treated control group exhibited an average grade of injury of approximately 0.2. The 3-day long ASA administration induced severe microscopic tissue damage and resulted in an injury grade of 2.45 (Fig. 2). Exfoliation of the surface epithelium, denudation, subepithelial oedema, and even disintegration of the upper half of the glandular cells of the gastric crypts were apparent in each of the sections. Scattered inflammatory infiltrations and spotty areas of deeper mucosal necrosis appeared at times.

When betaine-palmitate was administered in combination with ASA, the area of macroscopic damage was significantly reduced ($P < 0.01$; Fig. 1a, b). Palmitic acid alone was also protective, but to a lesser extent than betaine-palmitate. In the betaine-treated group, there was a tendency toward less

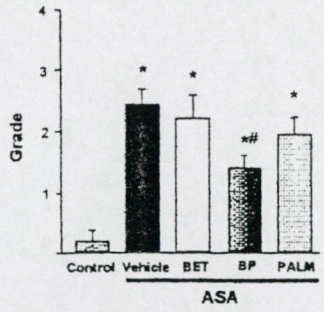



Fig. 2. Effects of betaine (BET, open bar), betaine-palmitate (BP, shaded bar) and palmitic acid (PA, grey bar) on the histological scores of injury. The gastric mucosal damage index was calculated as the average of the damage scores measured in three areas of the fundus. Data are presented as means ± $s_{\bar{x}}$. *$P < 0.01$ versus control group.

extensive injury, but the difference in extent of macroscopic mucosal damage from that for the vehicle-treated control group was statistically not significant.

Similarly, the area of microscopic damage was significantly reduced when betaine-palmitate was given in conjunction with ASA ($P < 0.05$). Betaine or palmitic acid alone failed to ameliorate the microscopic tissue injury. The average grade of tissue damage was reduced, but these groups each exhibited damage with a grade of approximately 2, a value statistically not significantly different from that for the ASA-treated group.

*Permeability changes*

ASA induced a significant increase in microvascular permeability as compared to the control group. VPI, a measure of the dysfunction of the microvascular component of the gastric mucosa, was significantly reduced after either betaine-palmitate or palmitic acid treatment as compared to the ASA-treated group (Fig. 3). Betaine administration alone was ineffective in reducing the degree of ASA-induced VPI increase.

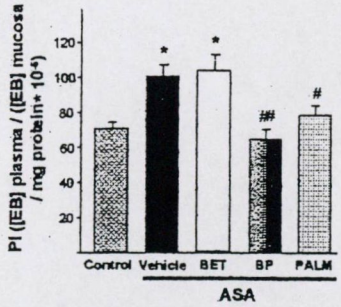



Fig. 3. Effects of betaine (BET, open bar), betaine-palmitate (BP, shaded bar) and palmitic acid (PA, grey bar) on the microvascular permeability index (PI) of the gastric mucosa. Data are presented as means ± $s_{\bar{x}}$. *$P < 0.05$ versus control, # $P < 0.05$ versus vehicle + ASA-treated group.

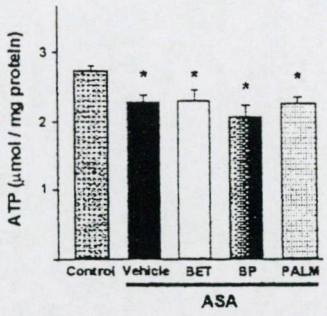



Fig. 4. Effects of betaine (BET, open bar), betaine-palmitate (BP, shaded bar) and palmitic acid (PALM, grey bar) on the ATP content of the gastric mucosa during subacute gastritis. Data are presented as means ± $s_{\bar{x}}$. *$P < 0.01$ versus control group.

*Tissue ATP changes*

The in vivo interference of the 3-day ASA treatment with the ATP production of the rat gastric mucosa was evaluated. There was a statistically significant fall in the ATP content of the mucosa after ASA treatment, and this change was not affected by the drugs used in the study (Fig. 4). The mucosal ATP content decreased to mean values of 2.3, 2.0 and 2.2 μmol/mg protein, values statistically not significantly different from the corresponding value for the ASA-treated group.

## Discussion

Despite the continuously growing body of information, the exact pathophysiology of ASA-induced gastric ulceration remains uncertain. It appears that several major pathways contribute to the development of damage in the stomach, including a reduction of the gastric mucosal blood flow, suppression of gastric prostaglandin synthesis, topical irritation on the epithelium, impairment of the barrier properties of the mucosa and interference with mucosal repair (13). The hypothesis that ATP depletion is primarily responsible for mucosal injury has received much support over the past few years (14, 15). After ingestion, ASA is hydrolysed rapidly to salicylate, a potent uncoupler of mitochondrial oxidative phosphorylation (4, 16). In the present study we provided evidence that the 72-h ASA treatment significantly depleted the mucosal ATP stores with a concomitant disruption of the gastric mucosal barrier.

In our experiments, betaine, palmitic acid and betaine-palmitate administration did not affect the decreased ATP synthesis of the gastric tissues. Nevertheless, betaine-palmitate treatment was associated with a marked reduction in the severity of the mucosal structural injury and significantly attenuated the ASA-induced gastric permeability changes. A prudent explanation would be that betaine-palmitate does not have the capacity to modulate the mitochondrial ATP synthesis, but effectively blocks a secondary pathway downstream from the mitochondrial dysfunction and energetic derangement. The question therefore arises as to which

process may be of critical significance in the mechanism of mucosal protection after betaine-palmitate treatment.

The inhibition of oxidative phosphorylation and the impairment of ATP generation lead to the loss of epithelial and endothelial cell tight junctions and to an increased mucosal permeability. It has been shown that functional changes in the mucosa, such as permeability alterations, precede histologically manifested tissue damage (17), and the literature data suggest that gastric mucosal microvessels are among the major targets for aspirin-induced injury (18). Tarnawski et al. (18) demonstrated significant damage to both superficial and deeper microvessels after ASA treatment, preceding the development of deeper tissue lesions. An increase in mucosal permeability leads to the movement of fluid into the interstitium, and the movement of luminal constituents toward the lamina propria. The presence of acid in the lumen of the stomach will also contribute to the pathogenesis of NSAID-induced ulcers and bleeding in a number of ways, such as interference with haemostasis and impairment of the process of mucosal integrity restitution. Similarly, several data suggest an important role for neutrophil leukocytes in mediating the second line of attack during ASA-induced injury (19). In this respect, it has been shown that a mixture of phospholipids (phosphatidylcholine and phosphatidylglycerol in a 7:3 ratio) inhibits the respiratory burst and superoxide generation of human neutrophils (20).

The present study did not establish an exact mechanism by which betaine-palmitate or its components, betaine and palmitic acid, protects the gastric mucosa against the damaging action of ASA. Previous experimental evidence has pointed to the importance of the intracellular betaine supply in cellular homeostasis. In particular, betaine confers considerable stress tolerance in high-osmolarity media, elevates the level of S-adenosylmethionine, and protects cell components in adverse conditions (6, 21–24). It was recently shown that orally administered betaine increased the number of mitochondria and the volume density of rough endoplasmic reticulum in the liver cell cytoplasm in rats, and the administration of betaine reduced the toxic effects of carbon tetrachloride on the cellular organelles (25).

In the mitochondria, the presence of a choline transporter in the mitochondrial inner membrane provides a potential site for the control of choline oxidation and hence for the supply of endogenous betaine (26). Betaine synthesized within the mitochondrial matrix is transported across the mitochondrial inner membrane by simple diffusion, and it has been shown that adrenergic stimulation activates the generation of endogenous betaine in the cardiac myocytes (27). The source of endogenous betaine appears to be the hydrolysis of membrane phosphatidylcholine to phosphatidic acid and choline by phospholipase D, with subsequent oxidation of the choline to betaine (28). From the aspect of constant betaine formation, the replenishment of exhausted endogenous supplies would offer a possible explanation for the protective effect of exogenous betaine-palmitate. It was recently demonstrated that the osmosensitive uptake of betaine in the hepatic stellate cells is mediated by an amino acid transport system, and hypo-osmotic cell swelling induces a rapid betaine efflux (29). The sinusoidal endothelial cells release osmolytes upon vasopressin and glucagon stimulation (30). Taken together, these results suggest that betaine may participate in the mechanisms of regulatory control of the epithelial tight junctions or endothelial cell volume homeostasis in the gastric mucosa. However, identification of the underlying mechanism is still necessary, and characterization of this process requires an in-depth investigation.

In our study, relatively high concentrations of ASA were used to evaluate the protective effect of betaine. Dunjic et al. (31) recently demonstrated that exogenously administered phosphatidylcholine prevented NSAID-induced acute lesions, whereas no protective effect was exerted after 72 h. They suggested that the incomplete protection might be due to the complex pathogenesis, which requires activation of several levels in the mucosal defence. It is in this context that our approach may offer a way to strengthen an important level of the insufficient or weakened endogenous mucosal protection. We propose that the better efficacy of betaine-palmitate treatment is related to the better biological availability of betaine in the betaine-palmitate form. It is suggested that complexing betaine with palmitic acid to form betaine-palmitate allowed the specific agent to reach and maintain an optimal concentration at the site of action.

In conclusion, the results of the present study are consistent with the hypothesis that an ATP depletion accompanies the evolution of mucosal ulceration. Betaine-palmitate administration significantly prevented ASA-induced disruption of the mucosal barrier. The effectiveness of the applied treatment regimen points to a novel therapeutic and preventive approach in ameliorating ASA-induced mucosal damage.

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## References

1. Davenport HW. Gastric mucosal injury by fatty and acetylsalicylic acids. Gastroenterology 1964;46:245–54.
2. Goddard PJ, Hills BA, Lichtenberger LM. Does aspirin damage canine gastric mucosa by reducing its surface hydrophobicity? Am J Physiol 1987;252:G42 1–30.
3. Leyck S, Dereu N, Etschenberg E, Ghyczy M, Graf E, Winkelmann J, et al. Improvement of the gastric tolerance of non-steroidal anti-inflammatory drugs by polyene phosphatidylcholine (Phospholipon 100). Eur J Pharmacol 1985;117:35–42.

4. Mingatto FE, Santos AC, Uyemura SA, Jordani MC, Curti C. In vitro interaction of nonsteroidal anti-inflammatory drugs on oxidative phosphorylation of rat kidney mitochondria: respiration and ATP synthesis. Arch Biochem Biophys 1996;334: 303–8.
5. Weinstein M, Haussinger D. Cytoprotection by the osmolytes betaine and taurine in ischemia-reoxygenation injury in the perfused rat liver. Hepatology 1997;26:1560–6.
6. Barak AJ, Beckenhauer HC, Badakhsh S, Tuma, DJ. The effect of betaine in reversing alcoholic steatosis. Alcohol Clin Exp Res 1997;21:1100–2.
7. Gong DH, Turner B, Bhaskar KR, Lamont JT. Lipid binding to gastric mucin: protective effect against oxygen radicals. Am J Physiol 1990;259:G681–6.
8. Slomiany BL, Takagi A, Liau YH, Jozwiak Z, Slomiany A. In vitro acylation of rat gastric mucus glycoprotein with [3H]palmitic acid. J Biol Chem 1984;259:11997–20000.
9. Trost LC, Lemasters JJ. The mitochondrial permeability transition: a new pathophysiological mechanism for Reye's syndrome and toxic liver injury. J Pharmacol Exp Ther 1996; 278:1000–5.
10. Lamprecht W, Trautschold I. Adenosine 5'-triphosphate. Determination with hexokinase and glucose 6-phosphate dehydrogenase. In: Bergmeyer H-U, editor. Methods of enzymatic analysis, vol. 4. New York: Verlag Chemie, Weinheim and Academic Press; 1976. p. 2101–9.
11. Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. Protein measurement with the phenol reagent. J Biol Chem 1951;193: 265–75.
12. Arvidsson S, Falt K, Haglund U. Acute gastric mucosal ulceration in septic shock. Acta Chir Scand 1984;150:541–7.
13. Davies NM. Review article: non-steroidal anti-inflammatory drug-induced gastrointestinal permeability. Aliment Pharmacol Ther 1998;12:303–20.
14. Hayllar J, Macpherson A, Bjarnason I. Gastroprotection and nonsteroidal anti-inflammatory drugs (NSAIDS). Rationale and clinical implications. Drug Saf 1992;7:86–105.
15. Fosslien E. Adverse effects of nonsteroidal anti-inflammatory drugs on the gastrointestinal system. Ann Clin Lab Sci 1998;28: 67–81.
16. Petrescu I, Tarba C. Uncoupling effects of diclofenac and aspirin in the perfused liver and isolated hepatic mitochondria of rat. Biochim Biophys Acta 1997;1318:385–94.
17. Haglund U. Gut ischaemia. Gut 1994;35 Suppl 1:S73–6.
18. Tarnawski A, Stachura J, Gergely H, Hollander D. Gastric microvascular endothelium: a major target for aspirin-induced injury and arachidonic acid protection. An ultrastructural analysis in the rat. Eur J Clin Invest 1990;20:432–40.
19. Anthony A, Sim R, Dhillon AP, Pounder RE, Wakefield AJ. Gastric mucosal contraction and vascular injury induced by indomethacin precede neutrophil infiltration in the rat. Gut 1996;39:363–8.
20. Chao W, Spragg RG, Smith RM. Inhibitory effect of porcine surfactant on the respiratory burst oxidase in human neutrophils. Attenuation of p47phox and p67phox membrane translocation as the mechanism. J Clin Invest 1995;96:2654–60.
21. Huang J, Hirji R, Adam L, Rozwadowski KL, Hammerlindl JK, Keller WA, et al. Genetic engineering of glycinebetaine production toward enhancing stress tolerance in plants: metabolic limitations. Plant Physiol 2000;122:747–56.
22. Miller TJ, Hanson RD, Yancey PH. Developmental changes in organic osmolytes in prenatal and postnatal rat tissues. Comp Biochem Physiol A Mol Integr Physiol 2000;125:45–56.
23. Barak A, Beckenhauer HC, Junnila M, Tuma, DJ. Dietary betaine promotes generation of hepatic S-adenosylmethionine and protects the liver from ethanol-induced fatty infiltration. Alcohol Clin Exp Res 1993;17:552–5.
24. Barak AJ, Beckenhauer HC, Tuma DJ. S-adenosylmethionine generation and prevention of alcoholic fatty liver by betaine. Alcohol 1994;11:501–3.
25. Junnila M, Rahko T, Sukura A, Lindberg LA. Reduction of carbon tetrachloride-induced hepatotoxic effects by oral administration of betaine in male Han-Wistar rats: a morphometric histological study. Vet Pathol 2000;37:231–8.
26. Kaplan CP, Porter RK, Brand MD. The choline transporter is the major site of control of choline oxidation in isolated rat liver mitochondria. FEBS Lett 1993;321:24–6.
27. Meister A, Kleuser BP, Ollert MW, Gercken G. Betaine generation in cardiac myocytes after adrenergic activation of phosphatidylcholine hydrolysis. J Mol Cell Cardiol 1996;28: 1109–18.
28. Porter RK, Scott JM, Brand MD. Choline transport into rat liver mitochondria. Characterization and kinetics of a specific transporter. J Biol Chem 1992;267:14637–46.
29. Peters-Regehr T, Bode JG, Kubitz R, Haussinger D. Organic osmolyte transport in quiescent and activated rat hepatic stellate cells (Ito cells). Hepatology 1999;29:173–80.
30. Weinstein M, Peters-Regehr T, Kubitz R, Fischer R, Holneicher C, Monnighoff I, et al. Release of osmolytes induced by phagocytosis and hormones in rat liver. Am J Physiol 2000;278:G227–33.
31. Dunjic BS, Axelson J, Ar'Rajab A, Larsson K, Bengmark S. Gastroprotective capability of exogenous phosphatidylcholine in experimentally induced chronic gastric ulcers in rats. Scand J Gastroenterol 1993;28:89–94.

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