The control of gastrointestinal bleeding
Clinical and experimental studies

Ph.D. thesis

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Background

In the current health care system, the average age of in-patients and the number of elderly people in the surgical wards has been steadily increasing. The introduction of geriatrics was followed by the establishment of geriatric surgery. Due to modern anaesthesia, elder patients safely recover even from large-scale interventions. However, they frequently experience serious complications caused by the intra- and peri-operative stress and infections, the hypotensive-reperfusion states resulting from operative haemorrhage and blood transfusion, and lengthy post-operative painkiller medication.

The statistics at the beginning of the 1980's demonstrated that in case of patients above the age of 50 years undergoing colorectal surgery the most threatening postoperative complications are resulted from gastrointestinal bleeding. These complications appeared in 15-20 % of the cases, and the outcome was often fatal. The mortality rate was particularly high among patients who had to undergo re-operation due to gastrointestinal perforation or bleeding. In case of medical treatment of the elderly, it is not only the interventions but the painkillers and especially "non-steroid anti-inflammatory drugs" (NSAIDs) that lead to serious problems of similar gastrointestinal complications. Side-effects of painkillers, NSAIDs and the often serious accompanying diseases could potentially unfavourably combine and the patients' state could worsen instead of the expected recovery.

In our study, we aimed at examining the causes of these problems. In particular, we were to establish possible routes for preventing and treating post-operative gastrointestinal haemorrhagic complications. We carried out our investigation in three separate but inter-related stages. After completing the first clinical investigations, we focused our attention on the pathomechanism of gastrointestinal bleeding. We performed animal experimental studies to investigate two key elements of this pathological process.

1. Clinical Studies

1.1. Introduction

When examining the local growth and the spread of rectal cancer, Miles suggested rectal extirpation as a surgical treatment, favoring radicality. A modified version of the Miles-type surgical technique, which is still applied nowadays, results in blood loss even with very careful preparation. Blood-loss and efforts to replace what has been lost might temporarily result in milder and/or more severe hypotensive states, which are then followed by reperfusion states due to transfusion and volume correction. In the 1940's, Dixon introduced (25) the anterior resection technique for removing recto-sigmoideal tumours. Later this method was expanded to treat tumours in the upper third of the rectum. According to the latter, tumours while located in the upper third of the rectum were to be treated by resection and those in the middle and lower third regions were to be extirpated. During the past 20 years new techniques have been introduced in colorectal surgery world wide, hence the above strategy changed. The further enhancement of modern stapler machines (9,10,14,31,32) and the application of the "double stapling" technique (49,81,89,91) made the resection intervention safer and more widely accepted and it also reduced operation time (90). The abdomino-sacral (27), the abdomino-transsphinteric (42) interventions and the preparation of a new technique for colo-anal (32,43,61) anastomoses facilitates, even in case of tumours in the lower third section, gave a newer possibilities for the preservation of the rectal sphincter. Nowadays, even in the case of malignant tumours sitting in the middle third section, anterior resection has become general practice and the rate of classic abdomino-perineal rectal extirpation decreased (20,36,88,91).The above methods significantly improved the operative results with respect to preserving the anal spinchter. During the preparation of rectum tumours, however, the problem of blood-loss might still surface, which makes the occurrence of the hypotensive-reperfusion states possible. The gastrointestinal mucosal layer can be significantly damaged, which can lead to microscopic haemorrhage or considerable acute blodd loss. The other menacing type of complications in colorectal surgery is related to infections. Infections may appear locally in the wound, but may also be present in distant organs. If a septic state develops, the question of secondary, life-threatening gastrointestinal haemorrhage have to be considered.

1.1.1. The goal of this study

Because of this background, one needs to take on complex tasks during interventions: multiple prevention methods have to be applied concurrently in order to reduce the number of postoperative colorectal complications. In our first clinical study, we aimed to examine the changes in strategies and results of elective radical colorectal operations of the patients at the Surgical Department of the Medical University of Szeged, with special emphasis on the development of gastrointestinal complications.

1.2. Patients and methods

1799 colorectal operations took place since 1st January, 1985 till 31st December, 1997 at the Surgical Department of the Medical University of Szeged. These 13 years were divided into two 5-years and one 3-years periods. We examined the data of patients, and then the operations were classified as elective radical, elective palliative and emergency surgery. Table 1.1 shows the distribution of the 1218 elective radical cases according to further technical segmentations. The "early" mortality rate of operations was only 2,3%, which adheres to international requirements. In the following part of our study, we analysed information about the 442 elective radical rectal operations, as it was during these interventions that patients lost the higher amount of blood, and the need for painkillers and the danger of developing post-operative infection was the greatest.

	1985-89 (5 years)	1990-94 (5 years)	1995-97 (3 years)	**Total**
Right hemicolectomy	93	101	59	253
Colon resection	73	90	51	214
Sigmoid resection	110	137	62	309
Rectal operation	189	152	101	442
Total	465	480	273	1218

Table 1.1 The type of the elective radical colorectal operations according to segmentation (total number of patients: 1218).

1.2.1. Statistical analysis

Two-sided T-tests and Wilcoxon's tests were performed at the Department of Medical Informatics of Medical University of Szeged.

1.3. Pre-operative preparations, antibiotics and thrombosis prophylaxis

The most significant risk in colorectal surgery stems from feces deposited in the intestines. In order to cleanse or evacuate the bowels, new methods have been introduced besides the traditional enema procedures. During the first part of the examined period we carried out cleansing by injecting 8-10 litres of liquid via the duodenum tube (58), according to the international standards if no "stricture" was present. However, the procedure induces stress and influences the patient's circulatory system, thus we gradually discontinued this procedure. Later, in the second and third part of the examination, we achieved the same task by giving osmotic laxative dissolved in 3 litres of liquid orally to the patients. In case of partial bowel obstruction or strong abdominal spasms resulting from the laxative or bowel cleansing, we switched to the traditional enema methods. The application of antibiotic prophylaxis is particularly essential in colorectal surgery. In Hungary we were among the first ones to introduce the so-called "one-shot" antibiotic profilaxis (58). In approximately one half of the examined patients we applied this method while in the other half the 24-hour antibiotic prophylaxis was used (6). During the examined period, thrombosis prophylaxis was done by small molecular weight heparin derivatives (Calciparin, Fragmin, Fraxiparin).

1.4. Prevention of post-operative ulcer formation

According to clinical research studies completed at our University and Department of Surgery (8), we applied histamine H_2-receptor blocker pre-treatment in case of patients over 50 years. The applied medication included cimetidine, ranitidine and famotidine administration. We started the H_2-receptor blocker pre-treatment on the day of surgery, and continued until the seventh post-operative day. If ulcer development was indicated in previous patient's history, we applied the anti-ulcer treatment for the whole length of hospitalisation. If for some reason, bowel movement could only be initiated with great difficulties and long gastric tube suction was necessary, the protection of the abdominal mucous membranes was also achieved by locally given medication injected through the naso-gastric tube.

1.5. Operation technique

At the Department of Surgery "single-layer" circular “Soviet” staplers were used for rectum resection beside the manually-sutured anastomoses (9,10). During the first examined period,

we found data on 5 patients who underwent such a procedure. However, this number is so low that it does not have an impact on the final evaluation. In case of rectum resection, we carried out manual anastomosis only under special circumstances, namely when we encountered technical problems while using the staplers. During the first period, we applied the USA-manufactured "double-layer" circular stapler machines, we placed the circular purse-string suture for both the distal and proximal intestine in an open position. During the second and third period, we introduced the "double stapling" technique, we closed the distal intestinal part with a linear stapler during the resection and we manoeuvred the "prod" found within the dismountable USA-stapler machine through the suture. With this "closed" technique, we could enhance the cleanness of the operation site, which indeed lead to a reduction in the number of postoperative septic complications. During the third period, we paid particular attention to the proper implementation of mesorectum extirpation (total mesorectal excision, "TME") (5). Changing the "drainage" method that was applied in the first period through the abdomen, meant a significant modification as well. In the second and third periods, we applied the so-called retrorectal, infraperitoneal drainage. Above the anastomosis, we carefully closed the peritoneum. In order to have partial decompression, we used a drain lead through the anus, with the help of which we carried out routine dye-test methods to test the soundness of the anastomosis. Similar to others (39), we prepared decompression stoma only in very special cases. In each periods, we carried out the abdomino-perineal rectum extirpation with the 2-group method described by Lloyd-Davies. We reported on operations regarding multiple organs due to locally spread-out rectum tumours in another study (7).

1.6. Results

When examining the histological results, we had to realise that throughout the examined 13-years tumours in the Dukes C stage represented 2/3 of the cases. The Dukes B stage occurred only 20-25% and the Dukes A stage hardly reached 10%. Table 1.2 demonstrates the distribution of the colorectal operations and gives information on "early" mortality. There was no significant difference in the mortality rate among the various groups. Analysing the distribution of interventions, we found that there were more rectum extirpation procedures than rectum resections in the first period, while this ratio was reversed in the 2nd and 3rd period favoring the sphincter-preserving methods.

	1985-89 (5 years)		1990-1994 (5 years)		1995-1997 (3 years)		**Total**	
Rectal extirpation	3/104	2,9%	2/64	3,0%	2/44	4,5%	7/212	3,3%
Rectal resection	2/85	2,35%	2/88	2,27%	1/57	1,75%	5/230	2,17%
Total	5/189	2,64%	4/152	2,63%	3/101	2,97%	12/442	2,7%

Table 1.2 The early mortality rate of elective rectal operations

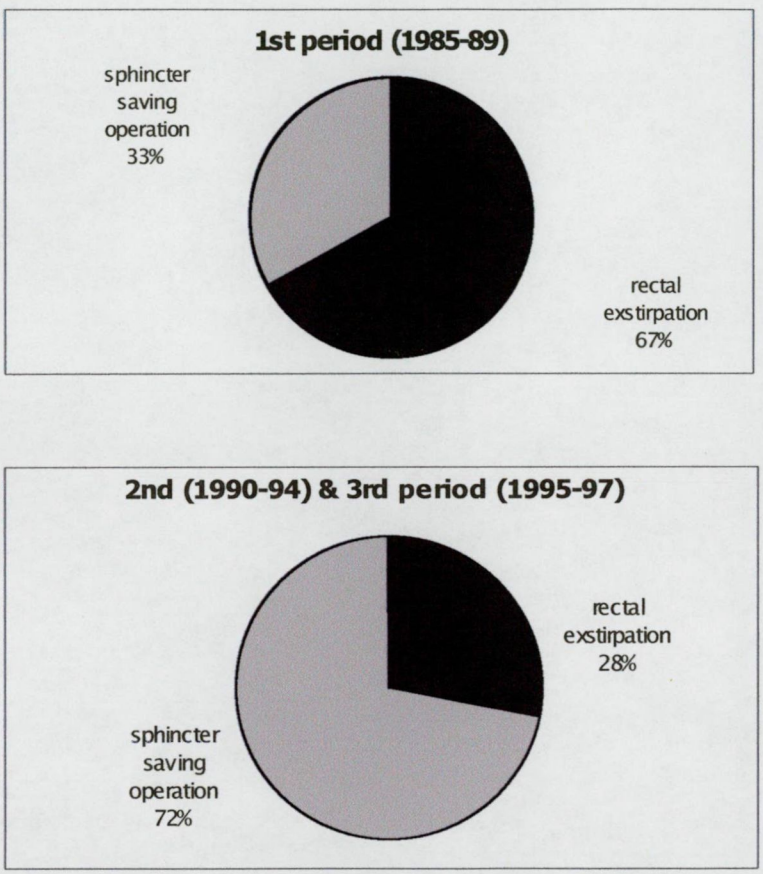

Figure 1.1 Changes in the distribution of operations on rectal tumours in the middle third.

According to Figure 1.1, it was in case of tumours located in the middle third that the significant changes occurred. While in the first period, we only resected 33% of rectal tumours in the middle section, this ratio increased to 72% in the second and third period. For the treatment of tumours in the lower third of the rectum, we carried out, as a matter of principle, abdomino-perineal rectum extirpation.

Table 1.3 presents postoperative complications of rectal extirpation. During the examined time frame, there was an improvement in the urinary and perineal infections. The rate of re-operations and "early" mortality slightly increased in the third period, but this increase was not significant.

	1985-89 (5 years) No 104		1990-94 (5 years) No 64		1995-97 (3 years) No 44		**Total** No 212	
Heart-Lungs	4	3,85%	2	3,1%	2	4,5%	8	3,8%
Gastric haemorrhage	3	2,9%	2	3,1%	1	2,27%	6	2,83%
Urine infection	11	10,5%	5	7,8%	3	6,8%	19	9,0%
Bowel obstruction	10	9,6%	6	9,3%	4	9,0%	20	9,4%
Abdominal wound infection	8	7,7%	4	6,25%	3	6,8%	15	7,0%
Perineal wound infection	12	11,5%	6	9,3%	4	9,0%	22	10,4%
Re-operation	2	1,9%	1	1,5%	3	4,%	5	2,35%
Early mortality	3	2,9%	2	3,0%	2	4,5%	7	3,3%

Table 1.3 Postoperative complications of rectum extirpation.

Table 1.4 contains information about the "early" complications of anterior resections. An improvement in mortality can be seen, but the rate of improvement is statistically not significant. Remarkable improvement can be noted in case of urinary and perineal infections. Reduction in the number of infected wounds resulted, most probably, from applying the "double stapling" technique. Great improvement can also be demonstrated with respect to the number of re-operations in the second and third time period. When describing technical details of the operations, we mentioned that during these two periods we placed a drain behind the rectum, into the infraperitoneal region to evacuate the closed area of the "small pelvis". This drain indicated the presence of anastomotic leak and at the same time prevented the development of general peritonitis. In this manner, several patient were saved from re-operation, since smaller-scale anastomotic leakages healed via conservative treatment.

	1985-89 (5 years) No 85		1990-94 (5 years) No 88		1995-97 (3 years) No 57		**Total** No 230	
Heart-Lungs	3	3,5%	3	3,4%	2	3,5%	8	3,48%
Gastric haemorrhage	3	3,5%	2	2,3%	2	3,5%	7	3%
Urine infection	8	9,4%	6	6,8%	3	5,26%	17	7,4%
Bowel obstruction	8	9,4%	7	7,9%	4	7%	19	8,2%
Wound infection	10	11,8%	5	5,7%	3	5,26%	18	7,8%
Anastomosis leak	4	4,7%	4	4,5%	2	3,5%	10	4,35%
Re-operation	3	3,5%	1	1,13%	1	1,75%	5	2,17%
Early mortality	2	2,35%	2	2,27%	1	1,75%	5	2,17%

Table 1.4 Complications of the sphincter-preserving anterior resections.

Table 1.3 and 1.4 also contain information on gastrointestinal bleeding. The results show that the applied preventive treatment (H_2-receptor antagonists) and all the assemblies of methods including improved surgical cleanness during operation, reduced intervention time and infection control were able to keep the ratio of post-operative gastrointestinal haemorrhage and mucosal erosion on low level in certain groups. The rate of gastrointestinal complications was around 3%, and there was no significant difference in that value among groups.

1.7. Discussion

During the past 20 years, colorectal surgery has gone through significant improvement world- and nation-wide as well (36,49,81,88). Such an improvement resulted from the joint influence of many factors. There has been improvement in anaesthesia, pre-operative preparation of the bowels and prophylactic treatment against stress-ulcer development. Antibiotics and thrombosis prophylaxis have become standard procedures. Continuous development of staples and staplers lead to safer rectal surgery, as well. Although there is a trend in the treatment of rectal tumours which considers even the local excision of Duke A-type tumours in the lower third satisfactory (40,59), the commonly accepted method is either anterior resection or extirpation. At our institution, we only applied local excision in case of *in situ* rectal cancer. The use of modern circular staplers (9,10,14,31,32) and the introduction of the double stapling technique significantly increased the ratio of sphincter-saving operations

(49,81,89,91). In case of tumours situated in the upper third of the rectum, it had become exclusive and in the past couple of years in case of tumours located in the middle third the rate of anterior resections increased (8,13,24,25). Some groups also suggest sphincter-preserving interventions, abdomino-sacral (27) and abdomino-transsphincteral (42) resections, in case of rectal cancers of lower third.

At the Department of Surgery of the Medical University of Szeged, the elective radical colorectal operations were carried out according to international standards (with respect to low mortality rate and post-operative complications) during the examined time period. The widespread use of circular staplers and the introduction of the "double stapling" technique, was not only increased the rate of sphincter-preserving interventions, but decreased the post-operative morbidity and the number of re-operations also. The peri-operative routine preventive treatment against gastro-duodenal ulcers which was introduced at the beginning of the 1980's proved to be successful. This method gives excellent results even according to the international literature. Nevertheless, although the occurrence of the ulcer complications was reduced to 3%, its occurrence could not be completely removed, even with the application of the most careful modern pre-operative bowel cleansing, the reduction in operation time and the modern and effective antibiotics prevention methods. Thus after analysing our clinical cases, we turned our attention to the questions of the pathophysiology of post-operative gastrointestinal bleedings. In order to find an answer to the emerging questions, we planned and outlined several animal experiments.

2. Protection of the gastric mucosa by antioxidant pre-treatment (animal experiments)

2.1. Introduction

Hypoxic/ischemic tissue injury results not only by the lack of oxygen as it was previously believed. Today it is clear that tissue injury associated with hypoxia occurs to a large extent in the post-hypoxic re-oxygenation 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 (21, 44, 67, 75, 85). It has been shown that the cytotoxic effects of these radicals result from the increased peroxidation of the lipid components of cellular and mitochondrial membranes (21,62). On the other hand, activation of polymorhonuclear neutrophil leukocytes is also accompanied by the release of oxygen-derived free radicals (47,48). Ischaemia-reperfusion injury to the intestine and stomach can be reduced by various antioxidants (34, 35, 67, 86). Many authors have found that treatment with free radical-scavenging enzymes or an agent inhibiting free radical synthesis significantly protected against gastric damage induced by haemorrhagic shock. Parks and Granger (62) demonstrated that the increased intestinal vascular permeability produced by ischaemia was largely prevented by pre-treatment with either allopurinol (xanthine oxidase inhibitor) or dimethyl sulphoxide (a hydroxyl radical scavenger). Treatment with allopurinol or superoxide dismutase (SOD) provides beneficial results in hypoperfusion of different organs (44,62,67,71,86). Allopurinol as well as superoxide dismutase 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 (17,63,64,65,66,). A synthetic antioxidant, MTDQ-DA (6,6'-methylene-bis 2,2-dimethyl-4-methane sulfonic acid sodium-1,2 dihydroquinoline; trade name: Kontrad, Hungary) has been found to inhibit lipid peroxidation and to exert radicals scavenger action in liver and heart (28,73,79). MTDQ-DA having three functional groups also helps to scavenge the superoxide radicals and to block the Haber-Weiss reaction.

The link between oxygen-derived free radicals and histamine release has been established by in vitro system (53,54). Histamine release has been demonstrated in haemorrhagic shock (60). According to some observation oxygen-derived free radicals may cause histamine release

through the activation of macrophages. A local histamine release was found *in vivo* after intestinal ischaemia and reperfusion (16). These data suggest that oxygen-derived free radicals may play an important role of histamine release during and after the shock period and the gastric mucosal lesions formations.

2.1.1. The goals

In these studies a haemorrhagic shock-reperfusion model was used to produce gastric mucosal lesions. We decided to examine the increased activity of the non-specific endogenous peroxidase enzyme histochemically and to compare the protective effect of different pre-treatment such as H_2 receptor blocker cimetidine (Histodil, Richter Gedeon Co., Hungary), the xanthine oxidase inhibitor allopurinol (Zyloric, Wellcome Co., England) and a superoxide-scavenger drug, MTDQ-DA (Kontrad, Human Co., Hungary) against the gastric mucosal lesions induced by haemorrhagic shock in the rat.

In the second series the goal of our study was to investigate whether there is any histamine release during the shock period and after the re-infusion the shed blood when the oxygen-derived free radicals damage the membranes and to compare the protective effect of allopurinol (Ziloric, Wellcome Comp., England), superoxide dismutase (SOD, from bovine liver, S4761, Sigma Chem. Comp.) and a H_2-receptor antagonist, ranitidine (Zantac, Glaxo Co.).

2.2. Materials and methods

2.2.1. Animal preparation

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

2.2.2. 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 re-infused. Twenty minutes later the experimental rat was sacrificed by thoracotomy and the stomach was removed. One minute before sacrifice 1 ml of 1% Evans blue dye was injected into 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's fixative (74).

2.2.3. Drug studies

In the first experimental study seventy rats were divided into six groups. The animals of the first group were operated on, but there were no shock period (control group). The other groups had experienced shock period. Different drugs were given at the same time when rats received the pentobarbital sodium intra-peritoneally:

Control group:	sham-operated group,
Shock group:	no pre-treatment,
Cimetidine groups:	50 mg/kg pre-treatment,
	100 mg/kg pre-treatment,
Xanthine oxidase inhibitor:	50 mg /kg allopurinol pre-treatment,
Superoxide scavenger:	50 mg/kg MTDQ-DA pre-treatment.

In the second experimental study 67 rats were divided into seven groups. The different drugs were given intra-peritoneally at the same time when the animals received the pentobarbital sodium:

Shock group:	no pre-treatment,
Ranitidine groups:	1 mg/kg,
	2 mg/kg,
	4 mg/kg,
	8 mg/kg pre-treatment,
Xanthine oxidase inhibitor:	50mg/kg allopurinol pre-treatment,
Superoxide dismutase group:	20000 U/kg SOD pre-treatment.

2.2.4. Measurement of gastric lesions

Photographs of the stomachs were taken after the formalin fixation. After magnifying 3 times an independent observer measured the areas of gastric lesions and the total gastric mucosal surface with grid (results are given in square-mm).

2.2.5. Statistical analysis

For comparison of the damaged are multiple variance analysis and Scheffe's test were performed at the Department of Medical Informatics of Medical University of Szeged. For changes of histamine plasma levels Wilcoxon's rank sum test was carried out for estimation of stochastic probability of the intergroup comparison (Asahikawa Medical College). $P<0.05$ were considered significant.

2.2.6. Enzyme study

For demonstration of endogenous peroxidase activity, 3-4 μm 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) staining.

2.2.7. Histological grading

Formalin fixed specimens stained with haematoxylin-eosin were used for grading on a 0-4 semiquantitative scal. The lesions were classified according to the method of Arvidsson (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.

2.2.8. Measurements of plasma histamine levels

Histamine levels were determined in the shock group and allopurinol, SOD and ranitidine (8mg/kg) pre-treated groups. 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. Arterial blood from carotid artery was taken into cooled centrifuge tubes and immediately centrifuged at 4 °C for 10 min with 400 g (Kokusan H-500 R) The plasma was separated and stored below –20 °C for a maximum of 3 weeks before analysis. Histamine plasma levels were determined by radioimmunoassay (Immunotech S.A., France). The detection was carried out by a 1282 Compugamma LKB WALLAC instrument.

2.3. Results

In this experiment Evans blue was used to enhance the gastric mucosal lesions. The dye was applied intraarterially to demonstrate the increased permeability of membranes. Figure 2.1 shows a picture of a stomach of a rat from the shock group. The dark areas represent the gastric mucosal lesions.

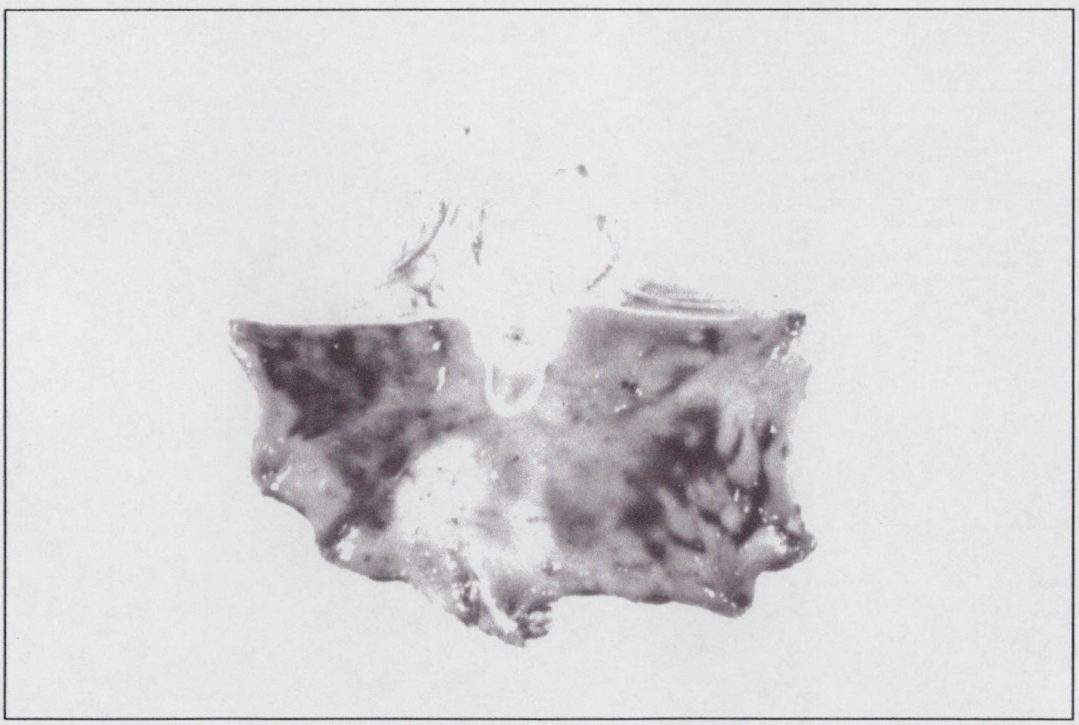

Figure 2.1 Photomicrograph of a stomach of the "shock" group. Dark areas represent the gastric mucosal lesions.

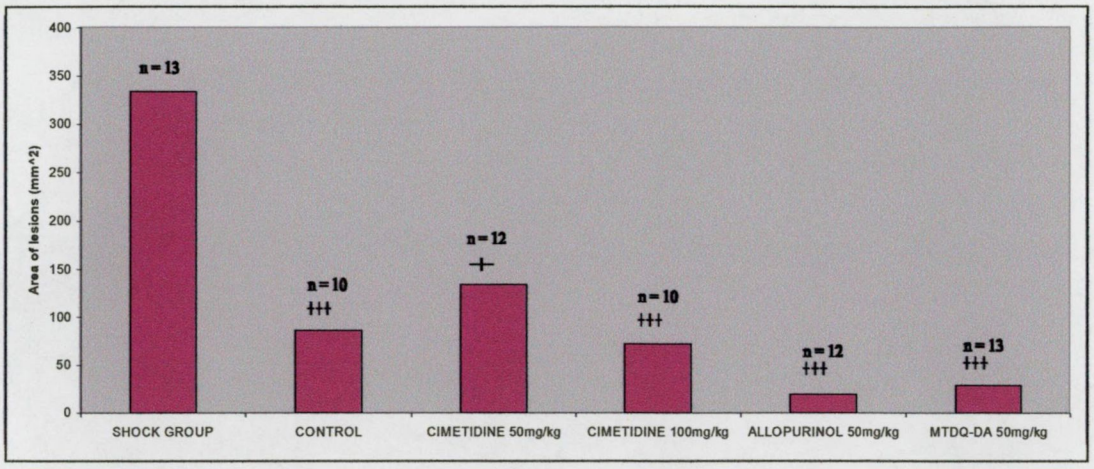

Figure 2.2 Effect of cimetidine, allopurinol and MTDQ-DA on areas of gastric mucosal lesions induced by haemorrhagic shock.

\+ P<0.01 vs. Shock group; +++ P < vs. Shock group

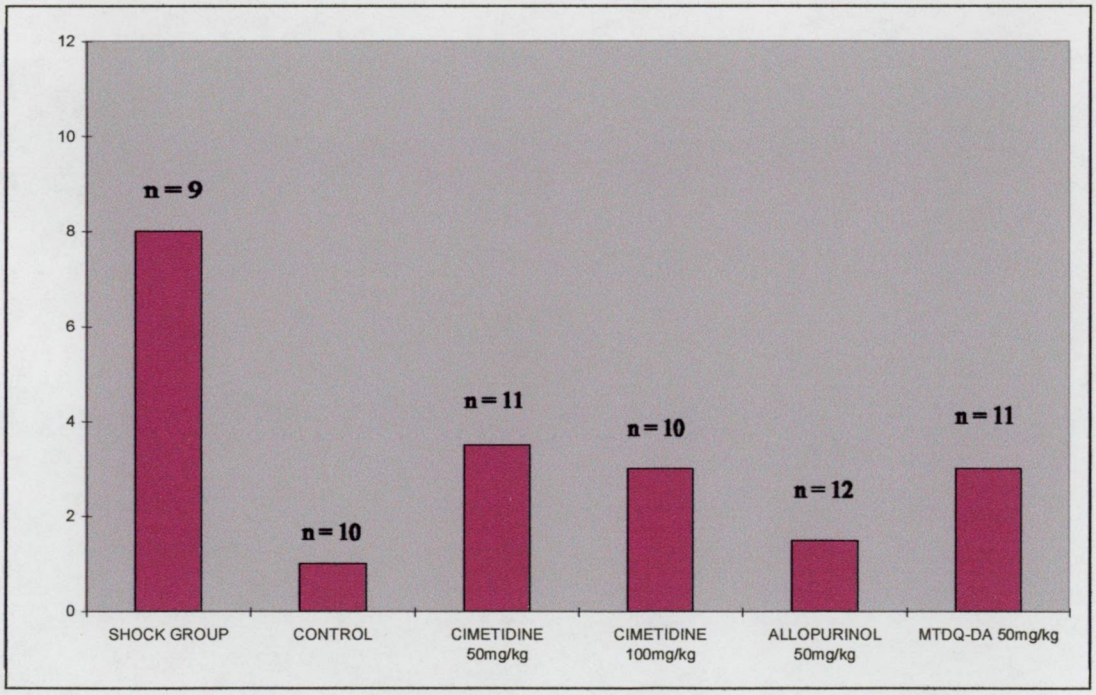

Figure 2.3 Changes of gastric mucosal damage index. It was calculated as the sum of the maximum damage in three fundic area (histological grading of gastric mucosal lesions).

Figure 2.2 shows the effect of different drugs on area of gastric lesions induced by haemorrhagic shock-reperfusion in the first experimental study. The damaged areas of stomachs of shock group were significantly larger as compered to the control group. Allopurinol, MTDQ-DA and high dose cimetidine pre-treatment were effective against these gastric mucosal lesions. The effect was highly significant ($P<0.001$). The smaller dose of cimetidine was effective but to a lesser extent ($P<0.01$).

Figure 2.3 shows the changes of gastric mucosal damage index in the different groups in the first experimental study. The median values of mucosal damage index were as follows: shock group: 8; control group: 1; allopurinol: 1,5; MTDQ-DA and cimetidine 100 mg/kg-treated groups: 3; cimetidine 50 mg/kg: 3,5, respectively. The histological examination also proved that allopurinol, MTDQ-DA and the higher dose cimetidine pre-treatment were effective against the shock induced gastric mucosal lesions.

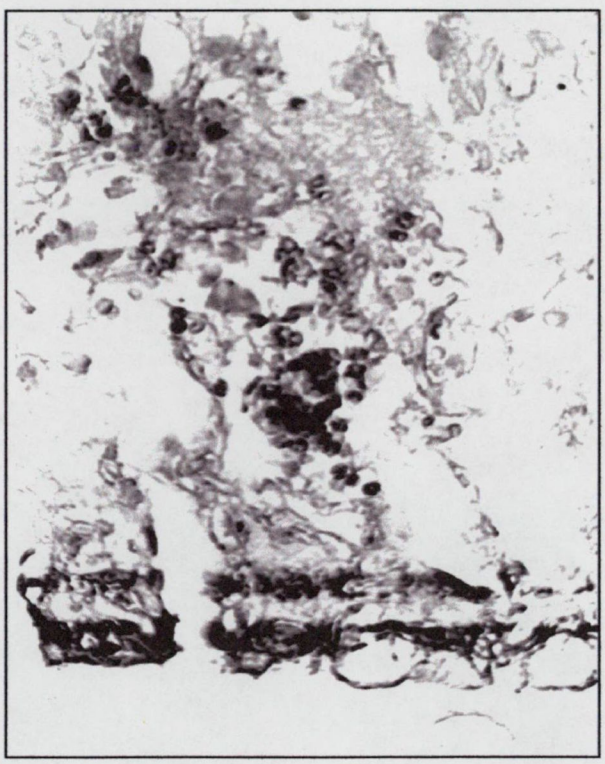

Figure 2.4 Endogenous peroxidase activity in the rat stomach. Increased endogenous peroxidase activity can be seen in the superficial layer of lamina propria and in the red blood cells within a dilated capillary. Epithelial cells are completely absent. Indirect PAP technique, x 300 (11).

Figure 2.4 shows the results of peroxidase-staining study in the shock group. An increased peroxidase activity can be seen in the superficial layer of lamina propria and in the red blood cells within dilated capillaries.

Figure 2.5 shows the effect of different drugs on area of gastric lesions induced by haemorrhagic shock-reperfusion in the second experimental study. 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 as compared to the control group. The ranitidine pre-treatment with lower drug concentration (with 1 mg/kg and 2 mg/kg) were not effective.

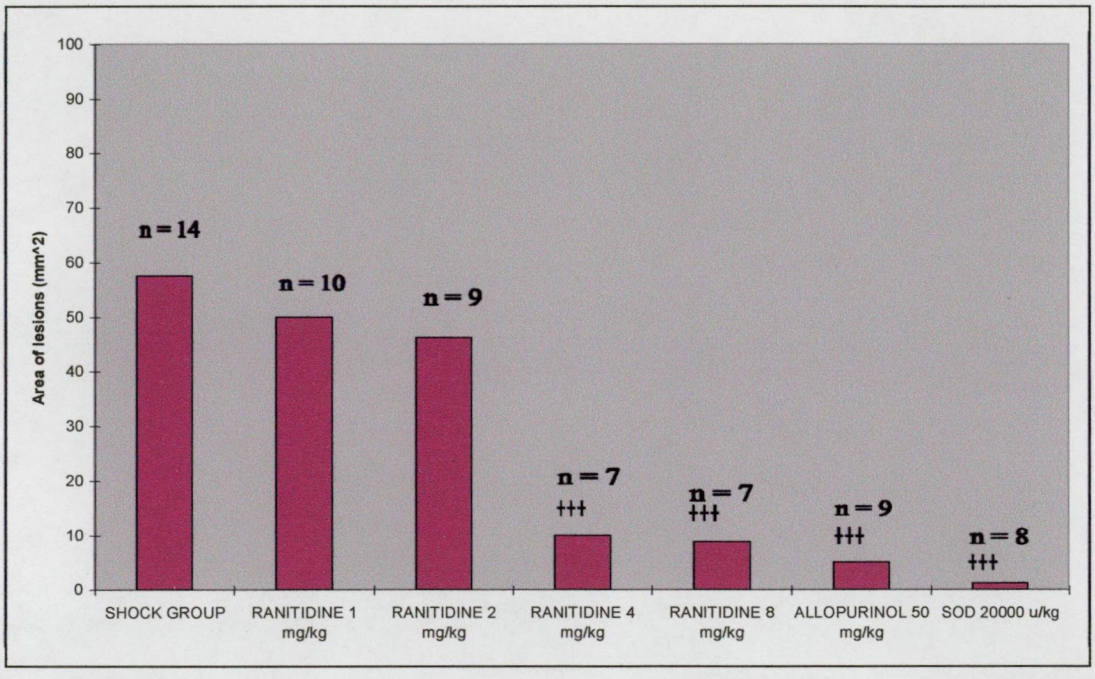

Figure 2.5 Effect of ranitidine, allopurinol and superoxide dismutase on areas of gastric mucosal lesions induced by haemorrhagic shock.

Figure 2.6 shows the disappearance of the surface epithelial cells (Grade II).

Figure 2.7 shows the changes of gastric mucosal damage index in the different groups in the second experimental study. 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 demonstrate the protective effect of allopurinol and SOD and the higher dose of ranitidine in this shock model.

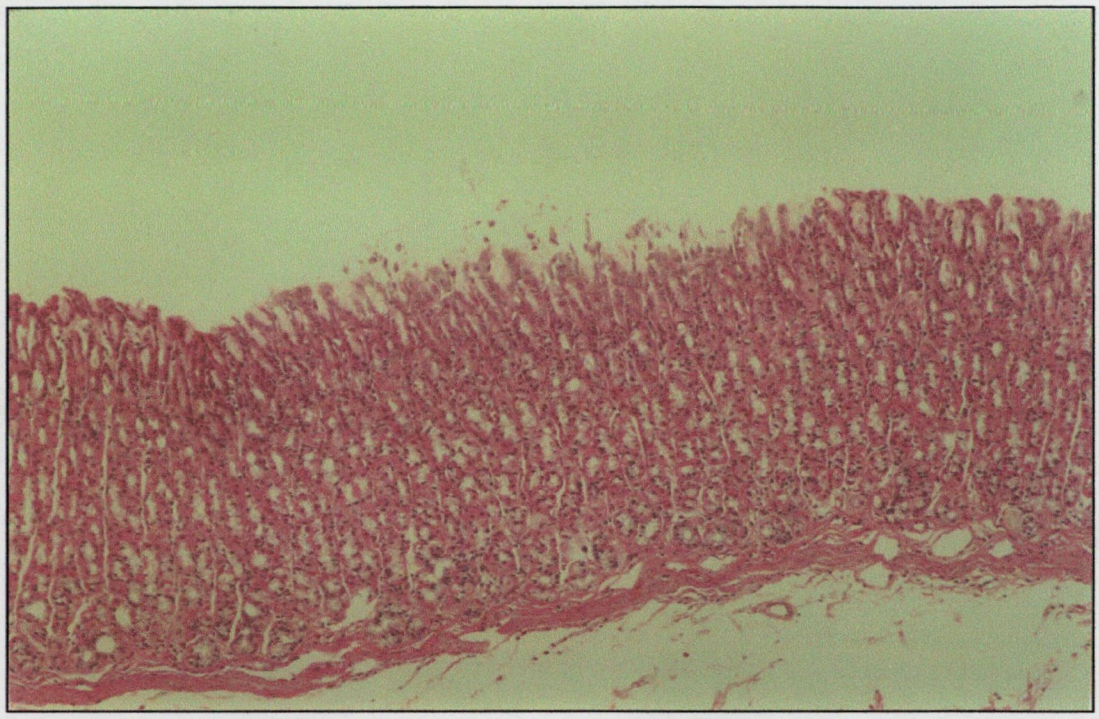

Figure 2.6 Light microscopic appearance of histological section of a stomach. Grade II shows the disappearance of the surface epithelial cells (haematoxylin-eosin) (x200).

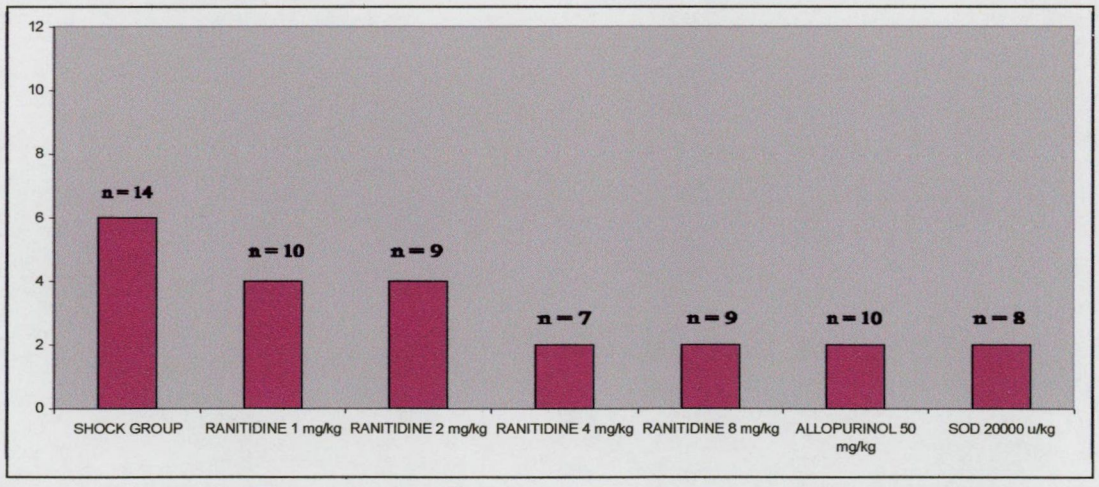

Figure 2.7 Changes of gastric mucosal damage index. It was calculated as the sum of the maximum damage in three fundic area (histological grading of gastric mucosal lesions).

Histamine levels were determined in the shock group, and the allopurinol, SOD and ranitidine (8mg/kg) pre-treated groups (Figure 2.8). In the shock 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 re-infusion of the shed blood remained essentially at the same level for five minutes, and later it decreased dramatically. Allopurinol and SOD pre-treatment did not influence significantly the values of histamine. In these groups the plasma histamine levels were slightly smaller compared to the shock group but the difference was not significant. After the re-infusion of the shed blood no histamine release was detected in these three groups. In the ranitidine pre-treated group the plasma histamine values were different as compared to the shock group. The ranitidine bolus injection caused significant histamine release immediately and then the histamine plasma level was significantly higher in this group compared to the shock except for the final value which was lower than the corresponding value in the shock group. Beside the initial histamine release another release of histamine could be observed by the end of shock period. After the re-infusion of shed blood a small increase of histamine level was found, but it was not significant

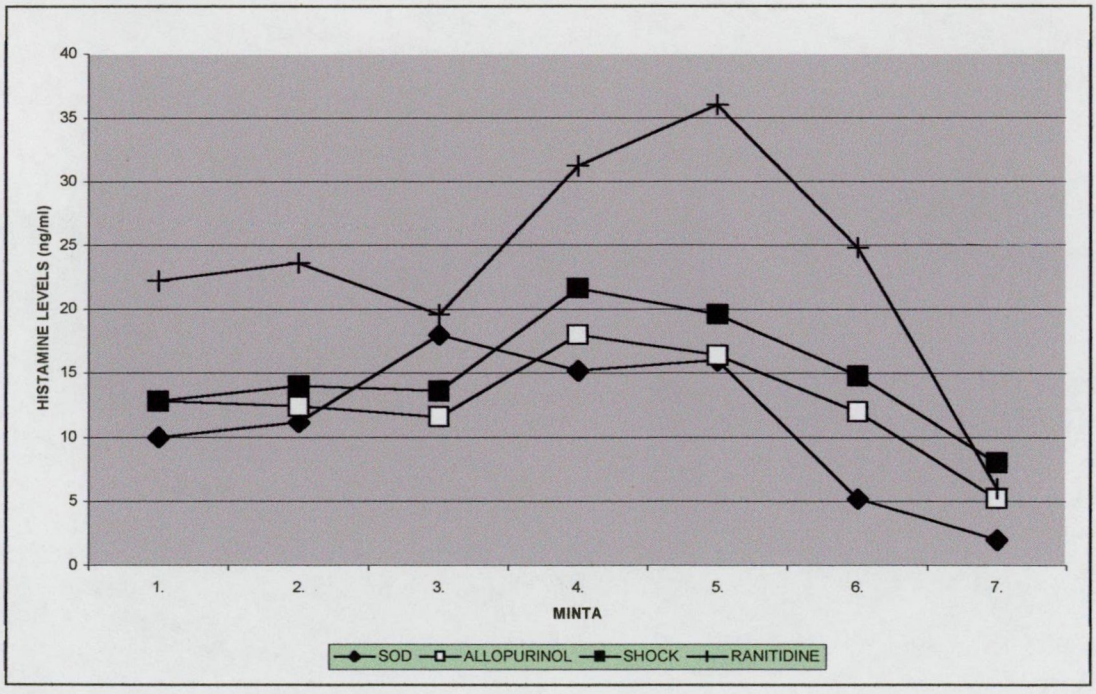

Figure 2.8 Plasma histamine level changes

2.4. Discussion

It is well-known that intestinal vascular permeability increases during hypovolaemic shock. In our study Evans blue passed 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 (19,44,62,67) against this increased vascular permeability. Allopurinol prevents oxygen-derived free radical formation, which are responsible for a large proportion of the gastric mucosal lesions observed after ischaemia. Superoxide dismutase scavenges the oxygen-derived free radicals, and it has protective effect against gastric mucosal lesions as well (17,63,64,65). Das et al. have found that hydroxyl radical is a major causative factor in stress-induced gastric ulceration, and they showed that desferrioxamine, a non-toxic transition metal ion chelator, protected the mucosa against the stress-ulceration in dose dependent manner (21).

An increased lipid peroxidation was proved by biochemical methods (determination of malondialdehyde) during reoxygenation injuries which suggested the breakdown of polyunsaturated fatty acids in the membranes. Similarly, an increased glutathione peroxidase activity was measured in the small intestine during and after the period of regional ischaemia (71,84). In our study, the presence of an increased endogenous peroxidase activity was proved in the damaged tissue histochemically in the control (no pre-treatment) group. It is possible, that the high peroxidase activity is in connection with the development of oxygen-derived free radicals. MTDQ-DA and allopurinol were found effective against the increased activity of endogenous peroxidation.

MTDQ-DA, allopurinol, SOD and the high dose ranitidine and cimetidine 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.. These results suggest that oxygen-derived free radicals have an important role in the pathogenesis of gastric lesions produced by ischaemia-reperfusion. Allopurinol pre-treatment reduces the formation of oxygen-derived free radicals. Superoxide dismutase scavenges the generated superoxide radicals, in that way the pre-treatment with SOD was effective. MTDQ-DA having three functional groups also helps to scavenge the superoxide radicals and to block the Haber-Weiss

reaction. It should be noted that we demonstrated for the first time that MTDQ-DA applied in a haemorrhagic shock model protects the gastric mucosa.

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 H_2-receptor blocker ranitidine and cimetidine were effective in high dose, but not in smaller doses in this hypovolaemic-reperfusion study in the rat against the gastric mucosal lesions. Ranitidine and cimetidine do not have direct influence on the oxygen-derived free radicals, therefore the pathophysiology of this phenomenon involves other routes and needs other explanations.

The oxygen-derived free radicals have an important role in the pathogenesis of gastric mucosal lesions in hypotension-reperfusion model and these free radicals can cause histamine release in vitro system (44,53,54,86). Local histamine release was found after intestinal ischaemia and reperfusion, and antioxidant pre-treatment was effective against this histamine release in canines (16). The plasma histamine level of rats is high (1,15) because of the low activity of histamine-methyltransferase and histamine-diamine-oxidase enzymes of microvascular endothelial cells. In human the normal level of histamine is 0.2-1.4 ng/ml, but the level is 17 ng/ml in rats (1,15).

The role of histamine and the regulation of histamine release (80) has been studied for many years but further investigation is clearly necessary. It was published that there is histamine-induced cytoprotection against gastric lesions induced by HCl in rats (76). The mucosal protective action of histamine may be mediated by endogenous prostaglandins through stimulation of H_2-receptors.

Histamine release has been demonstrated in haemorrhagic shock (60) in the canine circulation. The shape of the curve of the change in histamine concentration was similar in our experiment. Significant histamine release was shown at the end of shock period. The re-infusion of shed blood was not followed by a further histamine release as it could have been anticipated when the oxygen-derived free radicals damage membranes. Allopurinol and SOD pre-treatment were effective against the formation of gastric lesions, but they did not modify

significantly the histamine curve. The oxygen-derived free radicals were thus not found as endogenous histamine releasers in this setting.

Ranitidine pre-treatment 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 mg/kg and 2 mg/kg were ineffective. Ranitidine, the H_2-receptor antagonist given intra-peritoneally as a bolus (8 mg/kg) caused a histamine release immediately after the injection. This phenomenon is well known and was published earlier (26). 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 the mortality rate of rats in the ranitidine pre-treated groups were higher compared to the other groups. We observed a similar fact in the ranitidine group.

2.5. Summary

In our first experimental study we examined whether oxygen-derived free radicals have a role in the pathogenesis of gastric mucosal lesions produced by haemorrhagic shock-reperfusion model. We also examined the possible protective effect of the superoxide scavenger compound, MTDQ-DA.

We proved that haemorrhagic shock and reperfusion caused severe gastric mucosal lesions in rats. Allopurinol, MTDQ-DA and high dose cimetidine pre-treatment significantly reduced the formation of these lesions. An increased endogenous peroxidation was present in the damaged tissue as shown histochemically in the shock group. Both allopurinol and MTDQ-DA were found effective against the increased peroxidation and permeability of gastric mucosa. These results suggest that oxygen-derived free radicals may play an important role in the pathogenesis of gastric mucosal lesions produced by ischaemia.

It was the first occasion when MTDQ-DA was applied in haemorrhagic shock model to protect the gastric mucosa. MTDQ-DA was effective against the increased peroxidation and the formation of mucosal lesions.

In our second experimental study allopurinol, SOD and high dose ranitidine pre-treatment had protective effect against the formation of gastric mucosal lesions.

Histamine levels were determined in the shock group; in the allopurinol, SOD and ranitidine (8mg/kg) pre-treated groups. In the shock group the histamine level did not change

significantly during the preparative surgery, but there was a significant increase in histamine level by the end of shock period. The histamine concentration in the plasma after the re-infusion of the shed blood remained essentially at the same level for five minutes, and later it decreased dramatically. Allopurinol and SOD pre-treatment did not influence significantly the values of histamine. In these groups the plasma histamine levels were slightly smaller compared to the shock group but the difference was not significant. After the re-infusion of the shed blood no histamine release was detected in these groups. In the ranitidine pre-treated group the plasma histamine values were different as compared to the shock group. The ranitidine bolus injection caused significant histamine release immediately and histamine level remained significantly higher in this group as compared to the shock group except for the final value which was lower. After the initial histamine release another increase of histamine level could be observed by the end of shock period. After the re-infusion of shed blood a small increase of histamine level was found, but it was statistically not significant. We concluded that oxygen-derived free radicals were not endogenous histamine releasers in this model.

3. Enhancing the endogenous defence system of gastric mucosa by exogenous betaine-palmitate (experimental study)

3.1. Introduction

Acetylsalicylic acid (ASA) is extensively used 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 (22). 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 destabilising phosphatidylcholine within the mucus layer (30). Similarly, it has been observed that non-steroid anti-inflammatory drug (NSAID)-induced mucosal injury could be prevented when the drugs were chemically associated with phosphatidylcholine before administration (51). 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 (57). After ingestion, ASA is hydrolysed rapidly to salicylate, a potent uncoupler of mitochondrial oxidative phosphorylation (57). 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 ischemia-reoxygenation injury has recently been demonstrated (11, 82).

3.1.1. The goal of the study

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

3.2. Materials and methods

3.2.1. Induction of subacute gastritis

The experiments were performed on 101 male Wistar rats (average weight 250 g). 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. The animals were randomly allotted into 5 groups. The animals in group 1, which served as vehicle-treated control, received 15 ml/kg buffered 0.11 M potassium hydroxide-(KOH) via a flexible esophageal tube under light inhalation anesthesia. The procedure was repeated 3 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.

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 anesthesia exactly 2 h after the last treatment, and the stomach was removed, cut along the greater 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.

3.2.2. Groups of studies

The different drugs were given into the stomach of animals at the same time when ASA was injected through the gastric tube.

The numbers of animals used for each series of studies are shown below:

1. Untreated group: 15 ml/kg volume 0,11 M (vehicle) KOH
2. ASA-treated group: 10 ml/kg volume 200 mg/kg ASA + 5 ml/kg KOH
3. ASA + betaine: 10 ml/kg 200 mg/kg ASA + 5 ml/kg (37,5 mg/kg) betaine
4. ASA+betaine-palmitate: 10 ml/kg 200 mg/kg ASA+5 ml/kg (100 mg/kg) betaine-palmitate
5. ASA + palmitic acid: 10 ml/kg 200 mg/kg ASA + 5 ml/kg (62,5 mg/kg) palmitic acid

3.2.3. Measurements after the experimental procedures

3.2.3.1. Measurements of gastric lesions (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 then taken. The pictures were digitised, 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.

3.2.3.2. 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 anesthetized, the external jugular vein was cannulated and Evans blue (a 10 mg/kg bolus in 1 ml/kg saline) was injected iv. Thirty min 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 placed into 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 an UV-1601 spectrophotometer (Shimadzu, Japan). The protein contents of the samples were determined by the procedure of Lowry et al. (52). 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]

3.2.3.3. ATP measurement of gastric wall

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 and Trautschold (50). 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.

3.2.3.4. Histological grading

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 hematoxylin-eosin. Mucosal damage was assessed on the scale of Arvidsson et al. (4), with the following criteria:

Grade 0 = normal mucosa;

Grade I = edema 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 the gastric crypts;

Grade IV = disappearance of the glands.

The gastric mucosal damage index was calculated as the average of the damage in three different areas of the fundus.

3.2.4. 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.E.M. values are given. P values less than 0.05 were considered significant.

3.3. Results

3.3.1. Gastric morphological changes

S-VHS video images of the gastric mucosa were taken. The effects of oral ASA administration for 3 days on the gastric morphology were similar that we presented in Figure 2.1.

The Figure 3.1 shows that the ASA treatment induced severe macroscopic mucosal damage (without protective pre-treatment), the area of the gastric erosions reaching more than 30% of the total surface of the stomach. When betaine-palmitate was administered in combination with ASA, the area of macroscopic damage was significantly reduced ($p < 0.01$; Figure 3.1). 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 extensive injury, but the difference in extent of macroscopic mucosal damage from that for the vehicle-treated control group was statistically not significant.

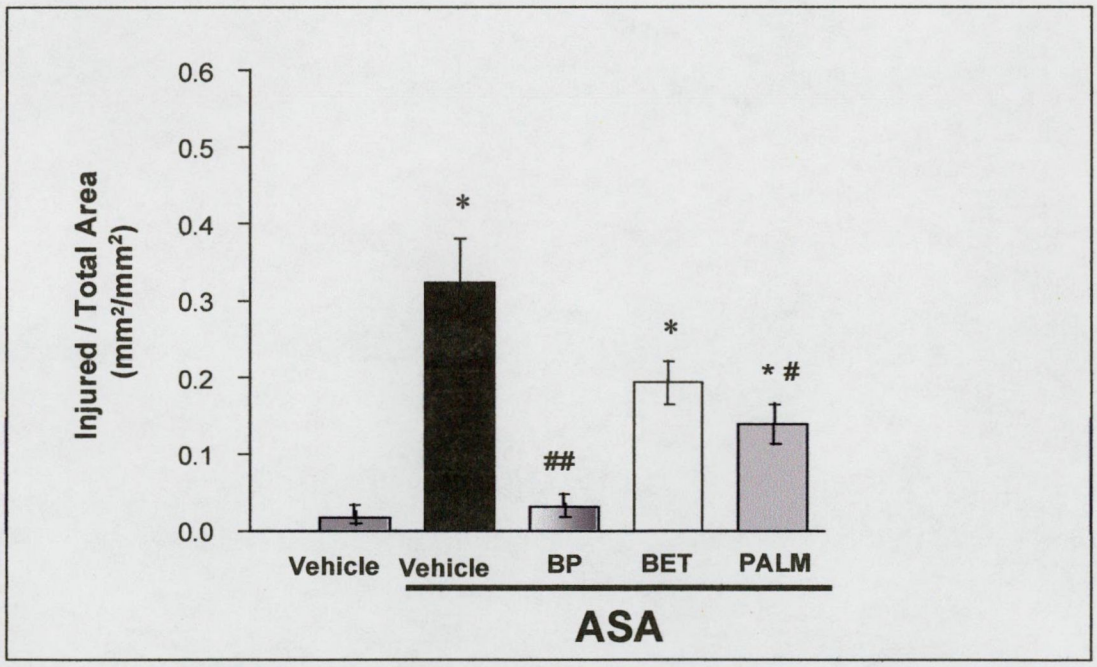

Figure 3.1 Effects of betaine (Bet), betaine-palmitate (BP) and palmitic acid (PA) on macroscopic damage in the gastric mucosa.

Data are means ± S.E.M. * $P < 0.05$ vs. control, ## $P<0.01$ vs. vehicle +ASA

3.3.2. Histological grading (gastric mucosal damage index)

Figure 3.2 shows the result of applying gastric mucosal damage index. The biopsy samples from the vehicle-treated control group exhibited a damaged index of approximately 1 and the 3-day long ASA administration (without protective pre-treatment) induced severe microscopic tissue damage and resulted in an injury damage index of 7. 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 tissue damage index was reduced, a value statistically not significantly different from that for the ASA-treated group.

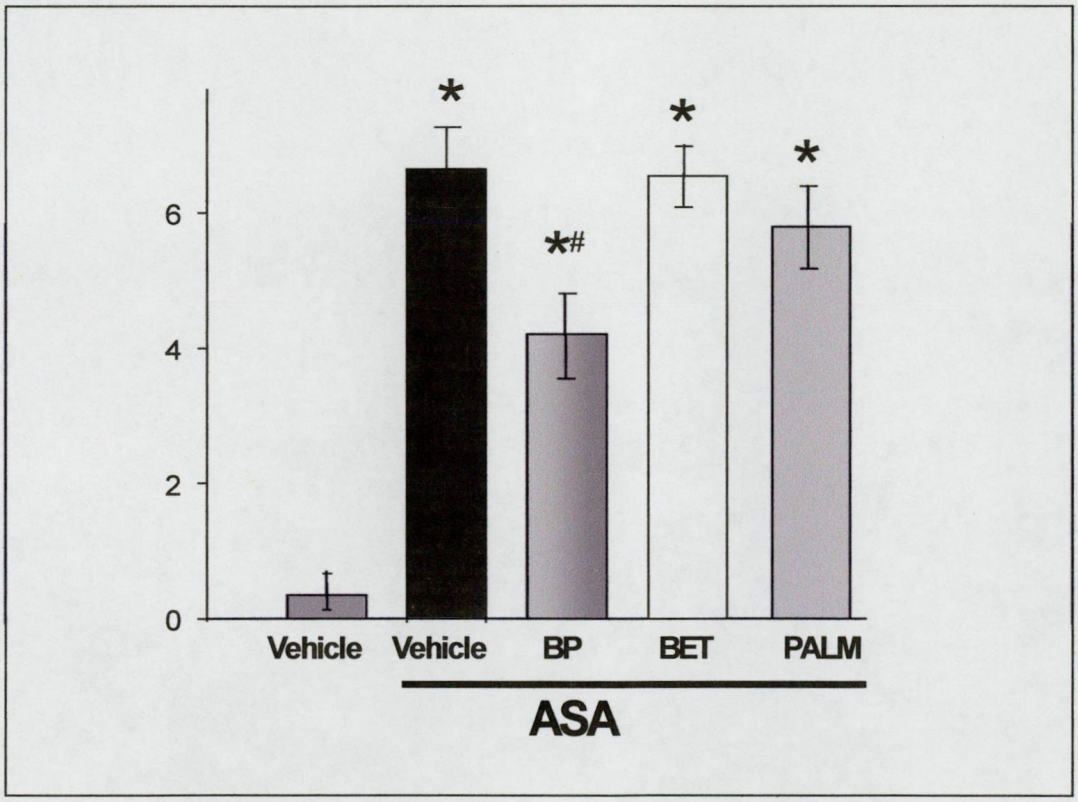

Figure 3.2 Changes of gastric mucosal damaged index. It was calculated as the sum of the maximum damage in three fundic area (histological grading of gastric mucosal lesions). Data are presented as means ± S.E.M. *P<0.01 vs. control group; #P<0.05 vs. Vehicle+ASA

3.3.3. Permeability changes

ASA induced a significant increase in microvascular permeability as compared to the control group. PI, 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 (Figure 3.3). Betaine administration alone was ineffective in reducing the degree of ASA-induced PI increase.

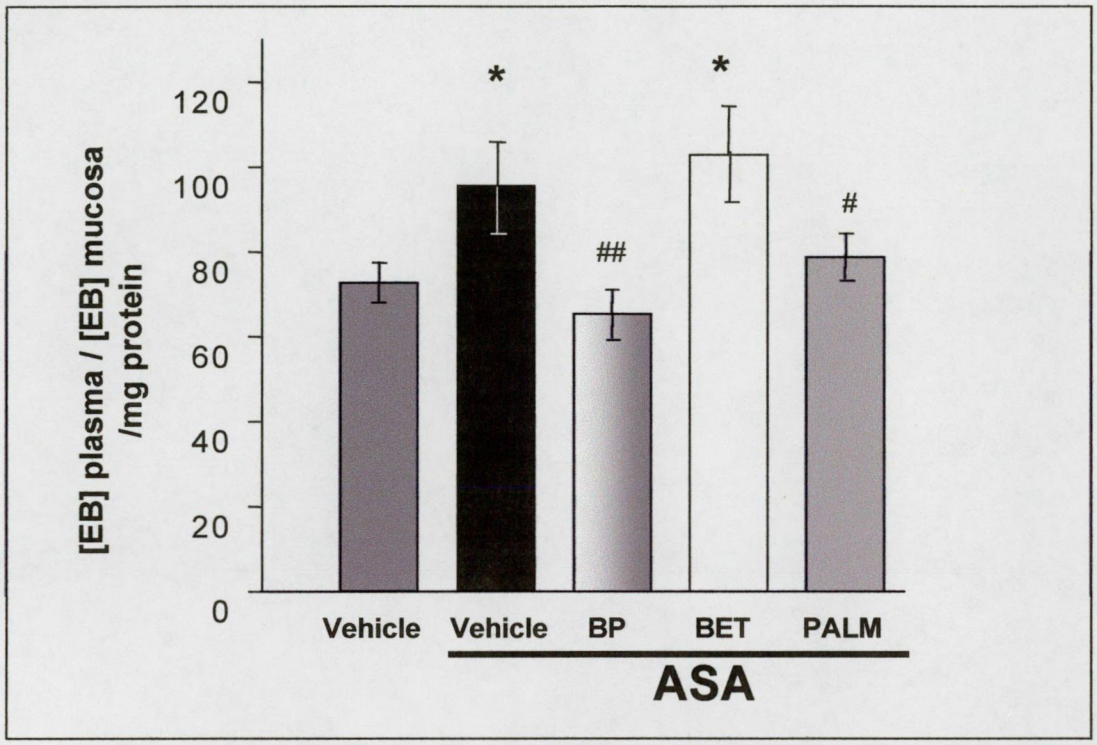

Figure 3.3 Effects of betaine (BET), betaine-palmitate (BP) and palmitic acid (PA) on the microvascular permeability index (PI) of the gastric mucosa. Data are presented as means ± S.E.M. * $P < 0.05$ vs. control, # $P < 0.05$ vs. vehicle+ASA-treated group.

3.3.4. 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 (Figure 3.4). 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.

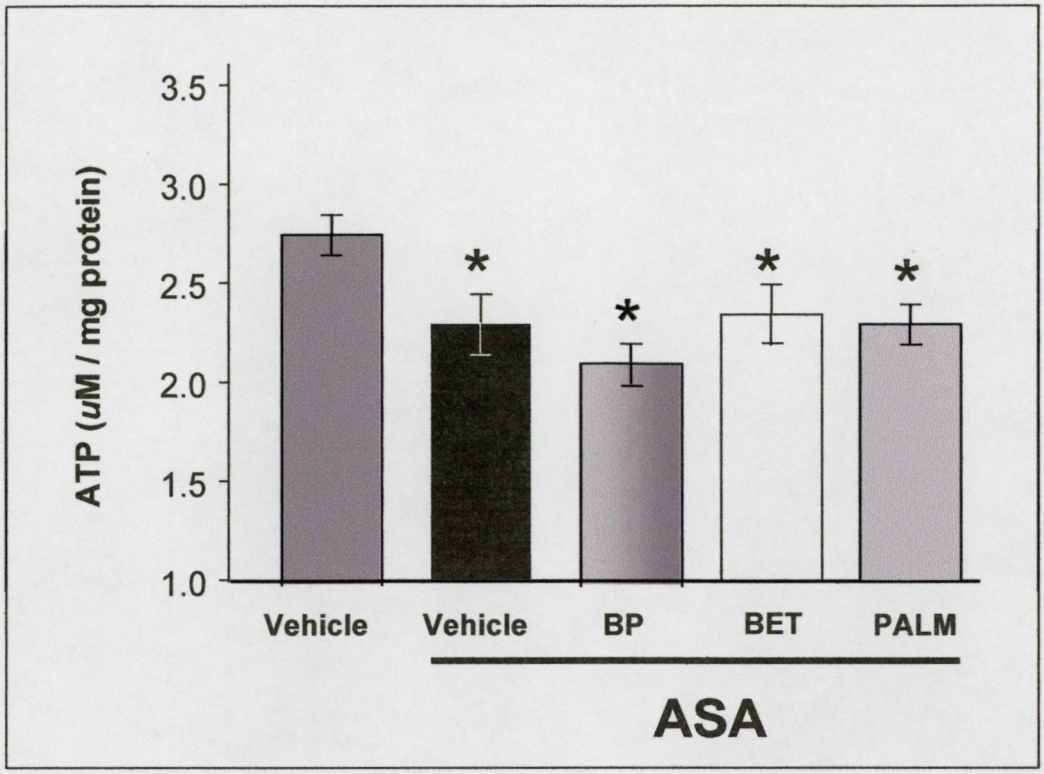

Figure 3.4 betaine (BET), betaine-palmitate (BP) and palmitic acid (PALM) on the ATP content of the gastric mucosa during subacute gastritis.

Data are presented as means ± S.E.M. * $P < 0.01$ vs. control group.

3.4. 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 (23). The hypothesis that ATP depletion is primarily responsible for mucosal injury has received much support over the past few years (29, 38). After ingestion, ASA is hydrolysed rapidly to salicylate, a potent uncoupler of mitochondrial oxidative phosphorylation (57,69). 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 (37), and the literature data suggest that gastric mucosal microvessels are among the major targets for aspirin-induced injury (77). Tarnawski et al. (77) 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 hemostasis 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 (3). 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 (18).

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 protect cell components in adverse conditions (11,12,13,41,56). 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 (45).

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 (46). Betaine synthesised 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 (55). 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 (70). 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 (68). The sinusoidal endothelial cells release osmolytes upon vasopressin and glucagon stimulation (83). 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. (24) 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.

3.5. Summary

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.

Betaine-palmitate may be an effective drug in the clinical practice for protection and of treatment different ulceration of stomach.

4. Final summary

1 Clinical Studies

1.1 The results of elective radical rectal operations were similar to international standards: low early mortality and complications rate occured during the examined period (1985-1997) at the Department of Surgery of the Medical University of Szeged.

1.2 Due to the circular staplers and the double stapling technique the rate of sphincter-saving anterior resection increased from 33 % to 72 % in the middle part tumours of rectum. At the same time the postoperative morbidity rate and the number of re-operations decreased.

1.3 The preventive treatment of peri-operative gastrointestinal bleeding were successful, because the earlier 14% decreased to 3%. However the 3% rate did not change in spite of the better pre-operative bowel cleaning, shorter operative time and novel, effective antibiotic profilaxis.

2. Protection of gastric mucosa by antioxidant pre-treatment (animal experiments)

2.1 We proved that the applied haemorrhagic shock-reperfusion model caused severe gastric mucosal lesions in rats.

2.2 An increased endogenous peroxidation was shown in the damaged tissue by histochemical methods.

2.3 Both allopurinol and MTDQ-DA pre-treatment were effective against the increased endogenous peroxidation of gastric mucosa. Allopurinol, SOD, MTDQ-DA and the higher dose ranitidine and cimetidine pre-treatment were effective against the gastric mucosal lesions.

2.4 These results suggest that the oxygen-derived free radicals may play an important role in the pathogenesis of gastric mucosal lesions caused by hypotensive-reperfusion condition.

2.5 MTDQ-DA pre-treatment was effective against the increased peroxidation and the formation of gastric mucosal lesions.

2.6 MTDQ-DA can be used as antioxidant pre-treatment for the protection of gastric mucosa.

2.7 The ranitidine bolus injection caused significant, immediate histamine release.

2.8. The oxygen-derived free radicals were not found to be endogenous histamine releasers in our experiments.

3. Enhancing the endogenous defence system of gastric mucosa by exogenous betaine-palmitate (experimental study)

3.1. It was demonstrated that 3 days ASA-treatment caused severe gastric mucosal lesions in rats.

3.2. ASA-treatment caused an ATP-depletion of gastric wall and increased microvascular permeability (The results of the present study are consistent with the hypothesis that ATP depletion accompanies the evolution of mucosal ulceration).

3.3 We examined the protective effect of betaine, palmitic acid and betaine palmitate. None of them was effective against the decreased mucosal ATP-content.

3.4. Betaine-palmitate administration significantly prevented ASA-induced disruption of the mucosal barrier (decreased the gastric musosal lesions and the increased microvascular permeability).

3.5. 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.

3.6. Betaine-palmitate may be an effective drug in the clinical practice for protection against the ASA-induced gastric mucosal lesions and treatment of different ulceration of stomach.

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Abbreviations

TME =	total mesorectal excision
SOD =	superoxide dismutase
MTDQ-DA =	6,6'-metilene-bis 2,2-dimethyl-4-methane szulfonic acid sodium-1,2 dihydroquinoline
ASA =	acetylsalicylic acid
ATP =	adenosine triphosphate
KOH =	potassium hydroxide
HCl =	hidrocloric acid
PI =	[Evans blue concentraction in tissue]/[Evans blue concentraction in plasma]
NSAID	non-steroidal antiinflammatory drug
EB	Evans blue
BP	betaine palmitate
BET	betaine
PALM	palmitic acid

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