WINNER OF THE ESVS PRIZE FOR BEST EXPERIMENTAL PAPER 1994

Perioperative Vascular Flushing Perfusion in Acute Mesenteric Artery Occlusion*

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Aim: To evaluate in animals and patients a perioperative vascular flushing perfusion with antioxidants in order to improve postischaemic condition of the bowel and hence, to prolong the warm ischaemia tolerance time.

Materials: 40 rats and 10 patients with acute mesenteric artery occlusion.

Methods: Intestinal ischaemia was induced in 40 rats by clipping the superior mesenteric artery for 1 h. Thirty animals received a vascular flushing perfusion with oxygen radical scavengers (ascorbate, tocopherol or oxypurinol). Histology, ATP, ADP levels were examined in tissue biopsies and malondialdehyde, lactate dehydrogenase, alkaline phosphatase levels were measured in blood during ischaemia and 60 min after reperfusion.

Results: ATP restoration was particularly improved in the oxypurinol group (ATP/ADP-ratio = 2 vs. 1.2 in the control group). Malondialdehyde increase observed after reperfusion as a marker of radical induced lipid destruction was significantly attenuated (control group 160% of base level vs. 127% in the ascorbate group, 133% in the tocopherol group, 121% in the oxypurinol group). Histological alterations during ischaemia/reperfusion were markedly less extensive in the perfusion groups than in control.

The patients treated perioperatively with arterial flushing perfusion had an overall mortality of 10%.

Conclusion: Vascular flushing perfusion with oxygen radical scavengers prevents radical induced ischaemic damage and may prolong the warm ischaemic tolerance time of the intestine.

Key Words: Vascular surgery; Intestine; Ischaemia; Reperfusion; Free radicals; Ascorbate; Tocopherol; Oxypurinol; Animal; Clinical study.

Introduction

Despite modern surgical therapy and intensive care the outcome of patients with acute mesenteric artery occlusion is still unsatisfactory (mortality up to 80%). 1 The short intestinal warm ischaemia tolerance time limits surgeon's time for vascular reconstruction. The aim of the present study was to see whether perioperative vascular flushing perfusion could improve the postischaemic condition of the bowel, leading to prolongation of the warm ischaemia tolerance time.

The reperfusion period of the intestine is associated with rapid changes of nucleotide levels and with the formation of oxygen free radicals. 2 Therefore, in the animal experiments adenine nucleotides and malondialdehyde (marker of radical induced lipid peroxidation) were measured and the radical scavenger ascorbate, tocopherol or oxypurinol were added to the perfusate in order to prevent cell injury due to lipid peroxidation.

*Presented at the 8th annual meeting of the European Society for Vascular Surgery, Berlin, Germany (September 1994).

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Material and Methods

Animal experiments

The studies were carried out in accordance with the declaration of Helsinki and with the Guide for the Care and Use of Laboratory Animals as adopted by the National Institutes of Health.

Male Lewis rats (300 +/- 30 g body weight) were used in all experiments. After overnight fasting animals were anesthetised with hexobarbital 100 mg/kg b.w. given intraperitoneally and placed on a heating pad. Following median laparotomy the superior mesenteric artery was dissected and occluded with a microvascular clip for 1 h.

Group 1 (control, n = 10) received no treatment during ischaemia. In groups 2-4 (n = 30) we performed intraoperative vascular flushing perfusion. The perfusate consisted of physiologic electrolyte solution containing 30 000 IE/l heparin and 40 mval/l sodium bicarbonate (pH = 7.45). In Groups 2, 3 and 4, 1 g/l ascorbate (Ascorvit®, Jenapharm, Jena, Germany), 20 mg/l tocopherol (Alpha tocopheryl acetate, Serva, Heidelberg, Germany) or 150 mg/l oxypurinol (allopurinol metabolite, Henning, Berlin, Germany) respectively were added to the perfusate. The perfusate was given continuously into the superior mesenteric artery by a tube via a vascular incision distal to the clip. Perfusion flow (2 ml/h) and pressure (max. 30 mmHg) were limited by a programmable syringe pump. At the end of the ischaemic period the vessel incision was sutured and recirculation established. After 60 min of reperfusion the animals were killed by hexobarbital overdose.

Tissue biopsies were carried out before and at the end of the ischaemia as well as 10 and 60 min after reperfusion. Samples for the determination of adenine nucleotides were transferred immediately into fluid nitrogen (-196 °C). Nucleotides were assessed by high performance liquid-chromatography (HPLC) as described previously.3 Tissue samples were fixed in a solution of 10% formaldehyde for histological evaluation. Samples were embedded in paraffin and coloured with haematoxylin-eosin. A semiquantitative scoring system was used for the evaluation of biopsies (Table 1).

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Findings</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Villus</td>
<td>normal</td>
<td>1</td>
</tr>
<tr>
<td>Villus tips</td>
<td>cytolysis</td>
<td>2</td>
</tr>
<tr>
<td>Lateral region</td>
<td>lysis &lt;30%</td>
<td>3</td>
</tr>
<tr>
<td>Lateral region</td>
<td>lysis &gt;30%</td>
<td>4</td>
</tr>
<tr>
<td>Crypts</td>
<td>destroyed</td>
<td>5</td>
</tr>
</tbody>
</table>

Clinical study

The study included patients (n = 10; four male and six female) undergoing treatment for acute mesenteric artery occlusion at the Department of Vascular Surgery (Städtische Krankenanstalten Esslingen a.N., Germany) in January 1993-July 1994. The mean age of the patients was 70.5 years (maximum 86 years, minimum 49 years).

Vascular flushing perfusion started after angiographic diagnosis of mesenteric artery occlusion via the angiography catheter. The exclusion criterium for primary perfusion treatment was proximal occlusion of a main mesenteric artery. In these cases perfusion started intraoperatively after thrombectomy. Initial perfusate consisted of 500 ml physiological electrolyte solution containing 30 mg papaverine (Paveron®, Karlspharma, Karlsruhe, Germany), 10 mg tolazolin (Priscol®, Dispersa, Germering, Germany) and 20 µg alprostadil (Prostavasin®, Schwarz Pharma, Monheim, Germany). Further perfusion treatment with 50 ml/h lactated Ringer’s solution included 2500 IE/l heparin, 2 × 30 mg papaverin, 3 × 10 mg tolazolin and 2 × 20 µg alprostadil within 24 h. Additional treatments included antibiotics and total parenteral nutrition. Perfusion was performed under clinical observation and haemoglobin, haematocrit, lactate levels and leucocyte counts were monitored. Control angiography was done via the perfusion catheter. Perfusion was continued for 3–5 days postoperatively.

Results

Animal experiments

Histology After 1 h of ischaemia we found mucosal alterations scoring 2 (Fig. 1). We observed mosaic-like pictures with altered regions of mucosa close to unaffected sections. In the control group after 1 h of reperfusion further alterations with apical and lateral desquamation of epithelial cells accompanied by
villus adhesion and atrophy were observed (score 4; Fig. 2). Antioxidative treatment protected the intestinal mucosa in the early reperfusion period (score 2; Fig. 3).

Biochemical parameters During ischaemia the tissue ATP/ADP-ratio decreased in all experimental groups. The recovery of the ATP/ADP-ratio in the control group was incomplete after 60 min of reperfusion (Fig.

Fig. 1. Histological picture after 60 min of intestinal ischaemia. Slight apical epithelial desquamation (score 2). Haematoxylin-eosin stain, magnification × 50.

Fig. 2. Control group; after 60 min of ischaemia and 60 min of reperfusion. Lateral cytolysis > 30% (score 4). Haematoxylin-eosin stain, magnification × 50.
4). Reoxygenation led to rapid nucleotide recovery particularly in animals with intraoperative oxypurinol perfusion.

The results of the malondialdehyde (MDA) analysis are to be seen in Fig. 5. In the control group we found significant elevated plasma MDA levels after reperfusion compared to the groups with vascular flushing perfusion. The postischaemic increase could be observed up to 15 min of recirculation. In ascorbate and oxypurinol treated animals MDA peak levels were found after 2 min of reperfusion, in the toco-pherol group after 5 min of reperfusion. The ascorbate group reached normal values after 15 min of recirculation. Oxypurinol and tocopherol treated animals showed normal MDA values after 60 min, whereas in the control group the MDA levels were still significant elevated at the end of the observation period.

Analysis of serum enzymes revealed significantly decreased postischaemic release of alkaline phosphatase in animals treated with radical scavengers (Fig. 6). Release of lactate dehydrogenase was similar in all groups (Fig. 7). The lowest activities were found in the ascorbate and oxypurinol groups.

Clinical study

Table 2 shows the clinical data, treatment and outcome of patients. In six patients we found an occlusion of branches of the superior mesenteric artery. Four patients also had partial occlusion of the coeliac trunk. Revascularisation was performed in five cases with thrombectomy and direct suture. In two cases a venous patch was inserted. In three cases no revascularisation was carried out. Intestinal resection was needed in five cases. The mean resection length was 106 cm (maximum 150 cm, minimum 50 cm). In one case conservative perfusion treatment without revascularisation or intestinal resection led to recovery.

The operative mortality (within 30 days) was 0%. One patient died after 33 days because of multiple organ failure. The other nine patients recovered and were discharged home. The overall mortality was 10%.

Discussion

Histological changes in ischaemia/reperfusion

It is known that the extent of structural damages during ischaemia is moderate compared to changes after reperfusion. Parks et al. and Younes et al. showed in hypotension models of intestinal ischaemia that the development of mucosal damages starts during ischaemia, but is significantly enhanced

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Fig. 3. Intraoperative vascular perfusion with heparinised physiologic electrolyte solution and ascorbate; after 60 min ischaemia and 15 min reperfusion. Only slight changes (score 2). Haematoxylin-eosin stain, magnification × 50.
shortly after reperfusion. These results implicate reactions which are initiated by the return of oxygenated blood to the ischaemic tissue as the cause of reperfusion-induced injury.

Indeed, it has been shown that anoxic reperfusion (reperfusion without oxygen) leads to only little damage. It is generally accepted that the reactions initiated after reperfusion involve the formation of cytotoxic oxidants derived from molecular oxygen. Our histological examinations confirmed the findings described above. The highest damage scores occurred after 60 min of reperfusion. Park et al. demonstrated analogous results after 5 h of cold ischaemia using a similar grading scale from 0 to 8. At the end of

![Graph 1](image1.png)

**Fig. 4.** ATP/ADP-ratio (mean ± SEM) of intestinal tissue after 60 min ischaemia and 60 min reperfusion. *p < 0.05 compared to control group.

![Graph 2](image2.png)

**Fig. 5.** Plasma malondialdehyde levels during 60 min of reperfusion (●) control group; (○) ascorbate group; (■) tocopherol group; (△) oxypurinol group. Results expressed as % of endischaemic level (mean ± SEM). *p < 0.05 compared to antioxidant groups.

![Graph 3](image3.png)

**Fig. 6.** Alkaline phosphatase activity (mean ± SEM) after 60 min intestinal ischaemia and 60 min reperfusion. *p < 0.05; **p < 0.01 compared to control group.

![Graph 4](image4.png)

**Fig. 7.** Lactate dehydrogenase activity (mean ± SEM) after 60 min intestinal ischaemia and 60 min reperfusion.
Table 2. Results of the clinical study on 10 patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Occlusion</th>
<th>Revascularisation</th>
<th>Resection</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. M.K., 55 y., m.</td>
<td>A.m.s.</td>
<td>Direct suture</td>
<td>130 cm intestine</td>
<td>Recovery</td>
</tr>
<tr>
<td>2. K.A., 71 y., f.</td>
<td>A.m.s.</td>
<td>Direct suture</td>
<td>No</td>
<td>Recovery</td>
</tr>
<tr>
<td>3. B.R., 74 y., m.</td>
<td>A.m.s.</td>
<td>Venous patch</td>
<td>100 cm intestine</td>
<td>MOF 33 d.p.o.</td>
</tr>
<tr>
<td>4. K.E., 78 y., f.</td>
<td>A.m.s.</td>
<td>Direct suture</td>
<td>No</td>
<td>Recovery</td>
</tr>
<tr>
<td>5. S.H., 49 y., f.</td>
<td>A.m.s. + Tr. coel.</td>
<td>Venous patch</td>
<td>100 cm intestine</td>
<td>Recovery</td>
</tr>
<tr>
<td>6. C.R., 81 y., f.</td>
<td>A.m.s. + Tr. coel.</td>
<td>Direct suture</td>
<td>No</td>
<td>Recovery</td>
</tr>
<tr>
<td>7. K.M., 79 y., f.</td>
<td>A.m.s. + Tr. coel.</td>
<td>No</td>
<td>No</td>
<td>Recovery</td>
</tr>
<tr>
<td>8. S.H., 62 y., m.</td>
<td>A.m.s.</td>
<td>No</td>
<td>50 cm intestine</td>
<td>Recovery</td>
</tr>
<tr>
<td>9. N.N., 70 y., m.</td>
<td>A.m.s.</td>
<td>Direct suture</td>
<td>No</td>
<td>Recovery</td>
</tr>
<tr>
<td>10. W.R., 86 y., f.</td>
<td>A.m.s. + Tr. coel.</td>
<td>No</td>
<td>150 cm intestine</td>
<td>Recovery</td>
</tr>
</tbody>
</table>

y=years; m=male; f=female; A.m.s.=arterial mesenterica superior; Tr. coel.=truncus coeliacus; MOF=death because of multiple organ failure.

ischaemia moderate villous injury (median grade 3), exacerbating to grade 4 following reperfusion, was observed.

The mucosal lesions observed in the early recirculation period decreased followed intraoperative perfusion treatment particularly with ascorbate containing solution.

Biochemical changes in ischaemia/reperfusion

Although no single process can be identified as the critical event in reperfusion injury, most studies indicate that depletion of cellular energy stores and accumulation of toxic metabolites contribute to cell death.2,8,10,11

Restoration of blood flow is fundamental in rescuing ischaemic organs, as this permits both regeneration of cell charge and washout of toxic metabolites. However, reoxygenation paradoxically leads to deleterious alterations too. Therefore, the combination of flushing procedure to minimise metabolite accumulation and the application of radical scavengers to reduce postischaemic alterations seemed to be a possible method for in situ-protectio of ischaemic intestine. Because the flushing solution is incorporated completely into the circulation (in contrast to organ preservation in transplantation), only physiological solutions could be used. Therefore, we administered heparinised electrolyte solution buffered with sodium bicarbonate. The slight hyperosmolarity of the perfusate appeared to be useful to prevent cellular oedema.

During ischaemia the equilibrium between consumption and formation of ATP is disturbed. The diminished ATP formation — especially on the basis of the decrease of mitochondrial oxidative phosphorylation — can only be compensated insufficiently by an increased metabolic flux through the glycolytic pathway.7 After recirculation the mitochondrial ATP formation is dominant in comparison with the glycolytic energy production. From the beneficial effect of antioxidants on ATP/ADP-ratio after reperfusion one may conclude that there is a radical induced disturbance of the mitochondrial respiratory chain during reoxygenation of the intestine. In the present study oxypurinol was particularly effective. It acts directly on nucleotide metabolism (inhibition of xanthine oxidase). In previous studies we have also demonstrated protective effects on intestinal nucleotide levels by the administration of superoxide dismutase.12,13

Malondialdehyde represents a marker of lipid peroxidation. The evidence of malondialdehyde increase in the postischaemic plasma of the control group confirms that lipid membrane injury is indeed caused by the action of oxygen free radicals. The experimental groups treated with antioxidants showed significantly lower values as compared to the control group. Kolvenbach et al.10 observed a similar postischaemic malondialdehyde increase with a peak in the initial reperfusion period after cross-clamping of the aorta in human (study without administration of antioxidants).

Determination of lactate dehydrogenase revealed similar values after 1 h of reperfusion but we observed significant differences between the alkaline phosphatase levels of all antioxidants groups and the control group. Lowest release was found in the tocopherol group. Analysis of the intestinal AP-isoenzymes is in progress to confirm this finding.

The incomplete recovery of some parameters after 1 h of reperfusion may imply additional generation of radicals in a later period of reperfusion or delayed recovery processes. During the late stages of reperfusion the activation of polymorphonuclear leukocytes leads to further release of oxygen free radicals.14 The
discrepancy between biochemical normalisation and morphological recovery has been described previously.\textsuperscript{15}

**Clinical discussion**

The final success of vascular surgery on mesenteric arteries depends on the duration of pre- and intraoperative intestinal ischaemia. Clinical experiences suggest a maximum warm ischaemia tolerance time of the intestine between 30 to 60 min. Preoperative existing ischaemia (mesenteric infarction) leads in the most cases to extensive bowel resection. Testart \textit{et al.}\textsuperscript{16} reported that of nine emergency mesenteric artery reconstructions, only one early direct aortic reimplantation was successful. The other cases were bypasses and their failures were due either to shock at revascularisation, thrombosis of the by-pass, or persistent ischaemic lesions. Harju \textit{et al.}\textsuperscript{1} described 43 cases with massive resection of the small bowel due to thromboembolism of the superior artery. The mortality was 60%.

Most authors agree with the opinion that the intestinal injury is to be seen in relation to the role of oxygen free radicals.\textsuperscript{2,10,11} Experimental studies indicated that the administration of radical scavengers may attenuate reperfusion injury.\textsuperscript{7,11} Efforts to protect the intestine against the consequences of ischaemia-reperfusion in clinical practice are still at an early stage. The present clinical study demonstrates that perioperative vascular flushing perfusion seems to be effective, but the number of patients was too low for conclusive results. The addition of radical scavengers to the perfusate may be useful for prolongation of warm ischaemia tolerance time and reduction of reperfusion injury.

**References**