

GUT AND VESSEL ALTERATIONS INDUCED BY MESENTERIC ISCHAEMIA/REPERFUSION IN RATS

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SUMMARY

A rat model of transient occlusion of the superior mesenteric artery was used to study the intestinal and vascular injury induced by ischaemia/reperfusion (I/R). A pronounced intestinal injury was observed, ranging from hyperaemia to severe haemorrhagic necrosis and bleeding. The length of the damaged intestinal segments reached 58.6% of the small intestine with a decrease of the wet weight in the I/R group. Sham operation resulted in 100% survival, I/R decreased survival to 40% after 24 h. Following I/R a significant increase of vascular permeability was observed in the small intestine. γ -glutamyl transpeptidase activity decreased aborally in sham operated rats and I/R reduced it further in all parts of the small intestine. I/R resulted in damaged endothelium-dependent relaxation of mesenteric artery rings. This was manifested by decreased maximal responses of arterial preparations to acetylcholine as well as decreased pD_2 values. The results confirmed and specified the presumed effect of I/R on the small intestine and on vascular functions.

Key words: ischaemia, reperfusion, superior mesenteric artery, reactive oxygen species, vascular permeability, vessel reactivity, γ -glutamyl transpeptidase

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INTRODUCTION

Reperfusion injury after preceding ischaemia is likely to play a major role in many clinical conditions, including shock, trauma, surgery, coronary artery occlusion, stroke, vascular reconstruction and organ transplantation. Restoration of blood flow results in reactive oxygen species (ROS) formation (1), leading to various biochemical and morphological alterations. The endothelium is the main source of the superoxide radical and also an important source of nitric oxide (NO). Superoxide reacts with NO to form peroxynitrite, resulting in depletion of endogenous vascular NO, endothelial dysfunction and neutrophil-mediated tissue injury (2).

ROS formation in the intestine may trigger injury also in other distant organs, e.g. the heart or lungs and affect overall vascular functions (3). The aim of this study was to characterise the intestinal as well as vascular injury induced by I/R of the superior mesenteric artery (SMA) in rats.

METHODS

Male Wistar rats weighing 230–280 g were used. The animals were fed a pelleted standard rodent diet, with access to tap water and diet ad libitum. They were acclimatised for one week before experiments and then randomly assigned into experimental groups. All procedures received approval from the local Commission for Ethics.

In rats anaesthetised by 50 mg/kg thiopental i.p., ischaemia was induced by occluding SMA for 90 min. Reperfusion was started by removal of the clamp. The duration of I/R was chosen based on the results of previous experiments, to be suitable for both intestinal and vascular injuries. Sham-operated animals were used as controls. The rats were sacrificed by decapitation after 90 min of reperfusion, except survival experiments in which the rats were observed for 2, 6, and 24 h after ischaemia. After decapitation and exsanguination, the aorta with SMA and small intestine were removed. The extent of haemorrhagic injury was assessed macroscopically and the damage was expressed as percentage of the intestinal length. Wet weight of the intestines was recorded. SMA was cleaned of adherent tissue and cut into rings, approx. 2 mm long. The rings were mounted between two L-shaped hooks in water-jacketed (37 ± 0.5 °C) chambers containing Krebs-Henseleit solution bubbled with a mixture of 95% O₂ and 5% CO₂ at pH 7.4, and stretched to the initial tension of 15 mN. After the 60-minute stabilisation period isometric contractions were recorded. The rings were contracted by 1 μ mol/l phenylephrine (PE). After the contraction plateau had been reached, acetylcholine (AC) was added (10^{-8} – 10^{-5} mol/l). The rings were washed to reach the initial tension value and afterwards they were preincubated with indomethacin (10^{-5} mol/l) and ω -nitro L-arginine (L-NA, 10^{-4} mol/l) for 30 minutes to block prostacycline and NO syntheses. Then the contraction was evoked by PE and at the plateau of contraction the response to AC was recorded again.

The extravasation of Evans blue (EB) dye into the tissue was used as an index of increased vascular permeability (4). In separate

experiments γ -glutamyl transpeptidase (GGTP) activity was assessed according to Orlowski and Meister (5) in three consecutive samples of the small intestine, in the liver, lungs and heart.

The values are expressed as mean \pm SEM. P values less than 0.05 were considered significant. Group differences were assessed by ANOVA followed by Tukey's test.

RESULTS

Following mesenteric I/R (90 min/90 min), we observed pronounced intestinal injury of various intensity, ranging from hyperaemia to severe haemorrhagic necrosis and bleeding into the lumen, with maximal changes occurring in the terminal ileum. The intestinal wall with early edematous changes became thin and distended. The wet weight of the small intestine decreased significantly from the control value 5.1 ± 0.2 g to 3.4 ± 0.2 g in the I/R group. The length of the damaged intestinal segments reached 58.6% of the whole small intestine. The survival of rats was observed for 2, 6, and 24 h after mesenteric occlusion. Sham operation

resulted in 100% survival, I/R decreased survival to 40% after 24 h. A significant increase of vascular permeability was observed in the small intestine after mesenteric I/R. EB control (sham) values 18.3 ± 2.0 increased to 40.4 ± 4.0 μ g/g ww (Fig. 1). We observed also vascular I/R-induced injury, manifested as decreased endothelium-dependent relaxation of SMA. Whereas arterial rings taken from sham-operated animals responded to AC with maximum relaxation reaching $93.9 \pm 1.7\%$, after I/R maximal relaxation was $75.8 \pm 3.6\%$ and pD_2 values were 7.2 and 6.6, respectively. After the blockade of NOS and prostaglandin synthesis by L-NA and indomethacin, the responses of arteries from I/R rats to AC were not further inhibited (Fig. 2).

GGTP activity in the control rats decreased aborally. Mesenteric I/R induced a significant reduction of GGTP activity in all parts of the small intestine tested. A similar reduction was observed in the lungs while there was an increase of GGTP (nonsignificant) in the liver and heart (Figs. 3, 4). The observed GGTP changes persisted in the intestine for 24 h after reperfusion in spite of the fact that macroscopically no intestinal damage was present at that time. However, in the other organs studied, i.e. the liver,

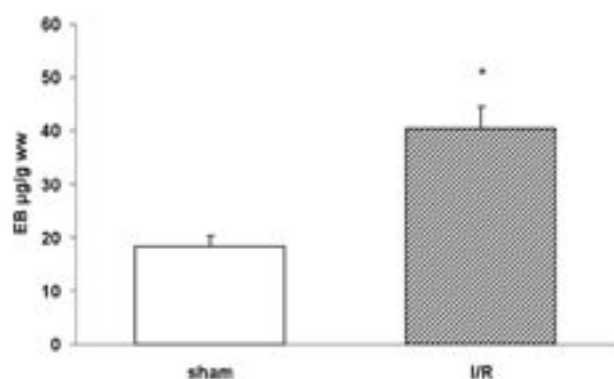


Fig. 1. Vascular permeability changes of the small intestine induced by ischaemia/reperfusion of the superior mesenteric artery, expressed as μ g Evans blue/g wet weight. Values are mean \pm SEM, $n = 6$. * $p < 0.05$, I/R vs sham-operated rats.

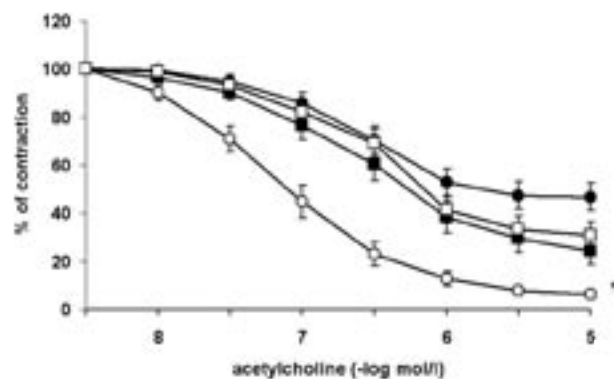


Fig. 2. Effect of ischaemia/reperfusion of the superior mesenteric artery on endothelial-dependent relaxation of SMA, expressed as % of phenylephrine-induced contraction. Values are mean \pm SEM, $n = 10-12$. (○ - sham, ● - sham+L-NA+indomethacin, □ - I/R, ■ - I/R+L-NA+indomethacin). * $p < 0.05$, sham-operated rats vs other groups.

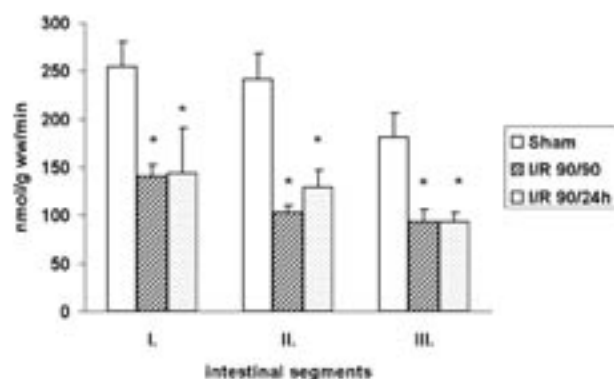


Fig. 3. γ -Glutamyl transpeptidase activity changes of the small intestine induced by ischaemia/reperfusion of the superior mesenteric artery, expressed as nmol/gww/min. Values are mean \pm SEM, $n = 5$. * $p < 0.05$, I/R vs sham operated rats.

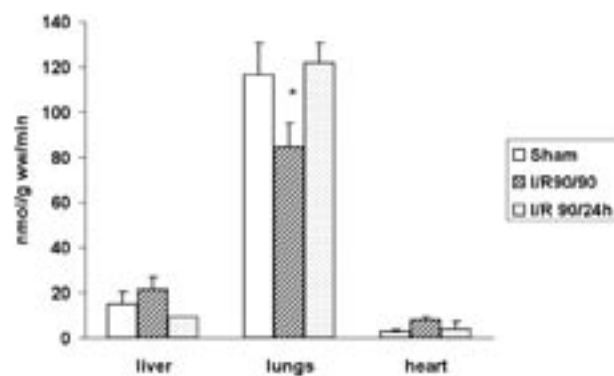


Fig. 4. γ -Glutamyl transpeptidase activity changes of the lungs, liver and heart induced by ischaemia/reperfusion of the superior mesenteric artery, expressed as nmol/gww/min. Values are mean \pm SEM, $n = 5$. * $p < 0.05$, I/R vs sham operated rats.

lungs and heart, there was a return to control GGTP values 24 h after ischaemia.

DISCUSSION

The obtained results showed pronounced intestinal injury induced by mesenteric I/R, characterised by haemorrhagic changes of the intestinal wall and extending to more than half of the length of the small intestine. The ileum was the most vulnerable part of the intestine. An increase of vascular permeability accompanied these changes. Denudation of the intestinal epithelium may be responsible for the observed decrease of the intestinal wet weight after I/R.

Endothelium-dependent relaxation of mesenteric arteries taken from I/R animals was decreased compared to that of arterial preparations taken from sham-operated animals, indicating endothelial injury. Both NO and endothelium derived hyperpolarising factor (EDHF) systems contribute to endothelium-dependent relaxation of the mesenteric artery, of which the latter can be identified after blockade of NOS. In our experimental conditions, I/R seems to have impaired NO-mediated vasorelaxation, as responses to AC before and after the inhibition of NOS were the same.

GGTP is a cell surface enzyme that cleaves γ -glutamyl amide bonds. GGTP plays an important role in the turnover of glutathione and in amino-acid transport and protein biosynthesis (6). GGTP was found to be involved also in cell repair and growth and in neoplastic processes. Since mesenteric I/R injury is associated with the reduction of endogenous glutathione level (7) and with pronounced structural changes, such as denudation of epithelial lining of the intestine, changes of GGTP activity were to be expected. Our experiments showed that GGTP alterations might be

found not only in various inflammatory situations, as described by Singh et al (8), but also in I/R-induced injury.

The results confirmed and specified the presumed effect of I/R on the small intestine, expressed mainly as increased vascular permeability with protein leakage and subsequent haemorrhagic injury of the intestine as well as its effect on endothelium-dependent relaxation affecting vessel functions.

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