Ischaemia/Reperfusion Contributes to Colonic Injury Following Experimental Aortic Surgery


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Objectives: ischaemia of the colon is an important complication of abdominal aortic aneurysm (AAA) repair. The aim of this animal study was to investigate the effect of sequential ischaemia and reperfusion on sigmoid mucosal pO2 and its association with local ET-1 release.

Material and Methods: twelve pigs underwent colonic ischaemia followed by complete reperfusion. Six other animals were sham controls. A Clark-type microcatheter was used for continuous mucosal pO2 measurements. Serial systemic and inferior mesenteric vein blood samples were obtained for determination of ET-1 concentration. Neutrophil extravasation was assessed by tissue myeloperoxidase (MPO) activity.

Results: arterial occlusion was associated with a gradual decrease of mucosal pO2 and local release of ET-1. After restoration of blood flow, mucosal pO2 returned to near baseline values, whereas ET-1 reached its maximum concentration during the reperfusion period. MPO activity was significantly increased.

Conclusions: colonic ischaemia and reperfusion causes neutrophil extravasation and local ET-1.

Key Words: Experimental; Aortic surgery; Ischaemia/Reperfusion; Colonic injury.

Introduction

Ischaemia of the colon is an important and often a fatal complication in abdominal aortic aneurysm (AAA) repair.1-5 Patency of the inferior mesenteric artery (IMA) and hypogastric collateral flow may reduce colonic ischaemia in elective AAA repair, but not in those operated for rupture where hypotension and intraoperative blood loss strongly correlate with subsequent colonic ischaemia.6-9 The aim of our study was to investigate the effect of sequential ischaemia and reperfusion on sigmoid mucosal pO2 and subsequent ET-1 concentration in an animal model.

Material and Methods

Experiments were performed in accordance with the institutional guidelines for the care and use of laboratory animals and were approved by the local Animal Ethics Committee.

Anaesthesia

Eighteen fasted young pigs of either sex, weighing 25 to 35 kg, were anaesthetised with ketamine at 10 mg/kg body weight intramuscularly followed by methomidate at 5 mg/kg body weight and azaperone at 2 mg/kg body weight intravenously for endotracheal intubation. Anaesthesia was maintained with 1%-2% isoflurane. The lungs were ventilated with a mixture of room air and oxygen to give 40% oxygen in the breathing air by aid of a volume-controlled ventilator with a positive end-expiratory pressure of 3-4 cm of H2O (Tiberius 19; Drägerwerk, Lübeck, Germany). Tidal volume was maintained at 10 ml/kg body weight, and ventilatory frequency was adjusted (13-18 beats per minute) to maintain PaCO2 at 4.5-5.5 kPa. Intravenous catheters were introduced through the right cephalic vein for central venous pressure measurements, withdrawal of blood samples and thermodilution fluid injections during measurements of cardiac output (CO). A Swan-Ganz catheter was positioned in the pulmonary artery through the right external jugular vein for measurement of CO which was performed using the thermodilution method, using 5 ml bolus injections of saline solution at room
applied to its origin. After stabilisation of the pO₂ values, the aorta and iliac arteries were occluded with vascular clamps. At this moment, arterial circulation of the distal colonic segment is limited to the vascular arcades originating from the superior mesenteric artery (Arc of Riolan, marginal artery of Drummond). To produce near complete ischaemia of the distal colonic segment, the marginal artery was finally occluded by an additional bulldog clamp. After 30 min ischaemia of the sigmoid colon and stable pO₂ values, the sigmoid colon was subjected to reperfusion. After 60 min of reperfusion, the animals were sacrificed by administration of potassium chloride. Full thickness biopsy specimens of the distal colonic segments were obtained before and after the experiments in both animal groups.

**Evaluation of clinicopathologic parameters**

Mean arterial pressure (MAP) and cardiac output (CO) MAP and CO were continuously monitored and maintained between 60 and 70 mmHg, respectively, 4.5 and 5.5 l/min.

**Surgical procedure and experimental design**

The animals were anaesthetised as described above and placed in the supine position. A midline incision from the xiphisternum to the pubis was performed. The porcine colon is adherent to itself in the form of a double-spiral, but the distal part is free and accessible. Accordingly, the distal colonic segment was separated from the mesocolon and the inferior mesenteric artery (IMA) was isolated at its origin from the aorta. Subsequently, the abdominal aorta, iliac arteries, and the marginal artery were dissected carefully to avoid vasospasms. The inferior mesenteric vein was dissected and cannulated with a 16 Ga × 12″ (30 cm) polyurethane indwelling catheter with side holes (Arrow International Inc., Reading, PA) for withdrawal of local blood samples (Fig. 1). Finally, the pO₂ and temperature probe were inserted in the submucosa of the sigmoid colon by means of a special needle and fixed in this position with 5-0 polydiaxonone sutures. Administration of 2000 U of heparin was performed and systemic and local baseline blood samples withdrawn. Subsequent local and systemic blood sampling was performed at 30 min intervals. All blood samples were immediately centrifuged, and the supernatant plasma frozen at −85 °C for later analysis. Six control animals were left with intact blood flow, but dissection was identical. In the 12 animals undergoing ischaemia, the IMA was occluded with a small bulldog clamp applied to its origin. After stabilisation of the pO₂ values, the aorta and iliac arteries were occluded with vascular clamps. At this moment, arterial circulation of the distal colonic segment is limited to the vascular arcades originating from the superior mesenteric artery (Arc of Riolan, marginal artery of Drummond). To produce near complete ischaemia of the distal colonic segment, the marginal artery was finally occluded by an additional bulldog clamp. After 30 min ischaemia of the sigmoid colon and stable pO₂ values, the sigmoid colon was subjected to reperfusion. After 60 min of reperfusion, the animals were sacrificed by administration of potassium chloride. Full thickness biopsy specimens of the distal colonic segments were obtained before and after the experiments in both animal groups.

**ET-1 assays**

Systemic and local ET-1 plasma levels were measured with a porcine specific enzyme-linked immuno-absorbent assay (ELISA) kit (ET-1 ELISA assay kit, Sodiag SA, Losone, Switzerland).

Biopsy sections were evaluated for the presence or absence of ischaemia by a pathologist blinded to the clinical circumstances from which the tissue was obtained. A grading scale was developed on the basis of previous histologic studies describing intestinal pathologic findings as follows: grade 0, normal mucosa; grade 1, subepithelial oedema; grade 2, desquamated superficial epithelium; grade 3, damage of the upper half of glands; grade 4, disappearance of glands. Tissue-associated MPO activity was determined by a modification of the method of Krawisz et al. previously described in detail.

**Statistical analysis**

All values are reported as means±SE. Comparisons between groups were made with the Mann–Whitney
**Results**

**Histopathologic study**

The histological grading of the colon specimen from the ischaemic animals differed significantly from the control animals \( (p<0.005) \). The changes ranged from subepithelial oedema with vascular dilatation to damage of the upper half of glands (grades 1 to 3) and were associated with a marked neutrophil infiltration (Figs 2 and 3). MPO activity after I/R was significantly increased compared to baseline and the control group (Fig 4).

**Local endothelin-1 concentration**

Mean local basal ET-1 concentrations were similar between groups at baseline and not significantly different after occlusion of the IMA (Fig. 5). Subsequent aortic cross-clamping produced a significant increase in local ET-1 concentration compared to baseline and control values \( (p<0.05) \) without further increase after temporary occlusion of the marginal artery. The reperfusion period was characterised by a maximum increase in ET-1 concentration \( (p<0.05) \) only after 30 min, decreasing subsequently after 60 min to near baseline values.

**Systemic endothelin-1 concentration**

Mean systemic ET-1 concentration in the I/R group of animals did not increase significantly compared to the
periods, preoperative shock, and extrinsic mesenteric compression from a large retroperitoneal haematoma are mainly involved in the high incidence of colon ischaemia following ruptured AAAs.\textsuperscript{4,6,14}

While improper ligation of the IMA is probably an important causative factor for colonic ischaemia in patients with elective AAA operations, it plays only a minor role in case of AAA rupture.\textsuperscript{4,6} Moreover, necrosis of the distal colon has been reported to occur despite high retrograde IMA stump pressure.\textsuperscript{15}

Successful resuscitation from haemorrhage shock, increasing cardiac output and finally releasing the Reperf.

![Fig. 6. Mean systemic plasma concentrations of endothelin-1 (ET-1) after sequential I/R and in control animals. Abbreviations: IMA = inferior mesenteric artery, MA = marginal artery.](image)

controls and baseline except in the first 30 min of reperfusion ($p<0.05$) (Fig. 6).

**Tissue pO$_2$ concentration**

Mean basal tissue pO$_2$ concentrations were similar between groups at baseline. Tissue pO$_2$ concentration decreased significantly compared to the controls and baseline after sequential occlusion of the IMA, aorta and marginal artery ($p<0.05$). Release of the blood flow produced a rapid increase in tissue pO$_2$ concentration to near baseline values after 30 and 60 min, respectively (Fig. 7).

**Discussion**

There is growing evidence that multiple pathophysiological factors, such as long hypotensive periods, preoperative shock, and extrinsic mesenteric compression from a large retroperitoneal haematoma are mainly involved in the high incidence of colon ischaemia following ruptured AAAs.\textsuperscript{4,6,14}

While improper ligation of the IMA is probably an important causative factor for colonic ischaemia in patients with elective AAA operations, it plays only a minor role in case of AAA rupture.\textsuperscript{4,6} Moreover, necrosis of the distal colon has been reported to occur despite high retrograde IMA stump pressure.\textsuperscript{15}

Successful resuscitation from haemorrhage shock, increasing cardiac output and finally releasing the aortic cross-clamping will induce reperfusion of the ischaemic colon, thus mimicking an ischaemia/reperfusion (I/R) injury.

To investigate some of the effects of I/R injury on the colon, we adopted an experimental porcine model. Different experimental porcine models of colonic ischaemia have been described previously.\textsuperscript{10,16,17} However, in none of these studies has reperfusion injury been investigated. It is well established that the tissue injury that follows I/R involves early interactions between the endothelial cells of the microvasculature and circulating leukocytes.\textsuperscript{13,18,19} Cell adhesion molecules, such as ICAM-1 and the $\beta_2$ integrin complex CD11 and CD18 on both neutrophils and vascular endothelial cells are upregulated, causing neutrophil adhesion and extravasation into the extravascular space, where they cause considerable tissue damage through the release of proteases including enzymes from the cascade system.\textsuperscript{18}

In the current study, MPO activity in the sigmoid colon was significantly more elevated compared to controls and baseline except in the first 30 min of reperfusion ($p<0.05$) (Fig. 6).

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of ET-1, micro-circulation was maintained and the tissue from I/R injury protected. Accordingly, we found that local plasma ET-1 concentration continuously increased with ongoing ischaemia to reach its maximum concentration 30 min after begin of the reperfusion.

A variety of methods to assess intestinal viability at operation have been described, including Doppler ultrasonography, fluorescein fluorescence patterns with ultraviolet light and pH measurement in the wall of the sigmoid colon (intramural pH). So far, the clinical value of these methods to detect ischaemia is limited. Postoperative identification of mesenteric ischaemia usually requires a high index of suspicion by the clinician. Measurement of intramural pH to detect colon ischaemia is a relatively reliable predictor of colon ischaemia. Sigmodioscopy has been also recommended.

In conclusion, experimental porcine colon ischaemia followed by a reperfusion period caused significant neutrophil activation and extravasation as well as a significant local ET-1 release. Ischaemia and reperfusion of the colon can be reliably and accurately assessed by measuring tissue-pO_2 concentration with a Clark type indwelling electrode.

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References


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