Kinetics of inflammatory parameters after intestinal ischemia reperfusion injury

F. Braun, M. Hosseini, S. Laabs, B. Sattler**, P. Bothur, K. Elias, B. Ringe

Klinik für Transplantationschirurgie and Zentrum Pathologie**, Georg-August-Universität, Göttingen, Germany.

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Introduction

Little is known about the early response after intestinal ischemia-reperfusion injury due to the complex pathophysiology that could be characterized as an exaggerated inflammatory response at the microcirculatory level. The underlying mechanisms involve reactive oxygen metabolites, expression and upregulation of adhesion molecules, leukocyte-endothelial cell adhesion, and a reduced bioavailability of nitric oxide (Banda, 1996 ; Boros, 1995 ; Cicalese, 1996 ; Park, 1991 ; Parks, 1982). The consequences of this inflammatory process are cellular necrosis, organ dysfunction, and breakdown of the mucosal barrier.

Graft loss or bacterial translocation with septicemia are the consequences in the immunosuppressed small bowel graft recipient (de Bruin, 1994 ; Frezza, 1996 ; Grant, 1991 ; Tzakis, 1994).

However, the mechanisms that underlie ischemia-reperfusion injury have not been precisely defined yet. Therefore, we developed an ex vivo small bowel perfusion model for studying ischemia-reperfusion injury. As a first part of the project, we investigated the time course of inflammatory parameters during intestinal ischemia-reperfusion injury.

Methods

Small bowels of four pigs (25-30 kg) were flushed with cold HTK-solution, explanted, and stored for 2 h at 4°C. Intestines were reperfused with porcine blood in an ex vivo closed circuit perfusion chamber. Blood flow was achieved to 200-400 ml/min (Braun, 1998).

Venous blood samples and intestinal biopsies from the distal ileum were drawn at and every 30 min after reperfusion. Mucosal damage was classified according to the grading of Chiu *et al.* (Chiu, 1970) : (grade 0) normal mucosa, (grade 1) development of subepithelial space at the tip of the villus, (grade 2) extension of the space with epithelial lifting, (grade 3) massive epithelial lifting with a few denuded villi (grade 4) denuded villi with exposed capillaries, and (grade 5) disintegration of the lamina propria, ulceration, and hemorrhage.

Interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), transforming growth factor-beta (TGF-ß), and endothelin-1 (ET-1) were measured by enzyme linked immunosorbent assays (ELISAs) (human IL-6, human TNF-α, human TGF-ß1, human Endothelin-1, all from R&D Systems Europe, Abingdon, United Kingdom). All ELISA's were performed strictly according to the instructions of the manufacturer. E-Selectin was detected by immunohistochemistry (APAAP) on cryo-preserved sections using an anti-CD62E IgG1 antibody (NatuTec GmbH, Frankfurt a.M., Germany).

Results

The mean (± SD) perfusion time was 560 ± 28 minutes. The mean (± SD) arterial pressure was measured from 50.3 ± 19.2 mmHg at start of reperfusion, 77.5 ± 8.5 mmHg at 1 h, 89.0 ± 6.7 mmHg at 7 h, 129.7 ± 20.5 mmHg at 8 h, and 181.3 ± 55.5 mmHg at 9 h after reperfusion. The mean (± SD) flow was 216.7 ± 23.6 ml/min at start of reperfusion, 283.3 ± 23.6 ml/min at 1 h, 350.0 ± 70.7 ml/min at 3 h, and 366.7 ± 47.1 ml/min at 9 h after reperfusion.

Histologically, normal mucosa was present at the end of the cold ischemia time (grade 0). Subepithelial spaces at the apex of the villus were present at 2 h after reperfusion (grade 1). The upper villus halfes showed subepithelial spaces and apex denudation beginning at 5 to 7 h after reperfusion (grade 2). Denudation of the upper villus and subepitehlial spaces in the lower villus halfes were present beginning 8 h after reperfusion (grade 3).

Immunohistochemically, endothelial cells of the arterioles stained positive for anti-CD62E starting 2 h after reperfusion. The venous endothelial cells stained positive for anti-CD62E starting after 4-6 h (Figure 1).

After reperfusion, mean (± SD) TNF-α concentrations showed a peak 166.1 ± 99.6 pg/ml at 1½ h and 171.1 ± 146.0 pg/ml 6 h after reperfusion. Mean (± SD) concentrations of TGF-ß were measured 619.0 ± 393.8 pg/ml at reperfusion, 498.5 ± 345.3 pg/ml at 1 h, and 845.8 ± 652.7 at 9 h after reperfusion. Mean (± SD) concentrations of ET-1 increased from 5.4 ± 2.4 pg/ml at time of reperfusion to 24.3 ± 9.9 pg/ml 9 h

Address : Prof. Dr. med. Burckhardt Ringe, Klinik für Transplantationschirurgie, Georg-August-Universität, Robert-Koch-Strasse 40, Göttingen, Germany.
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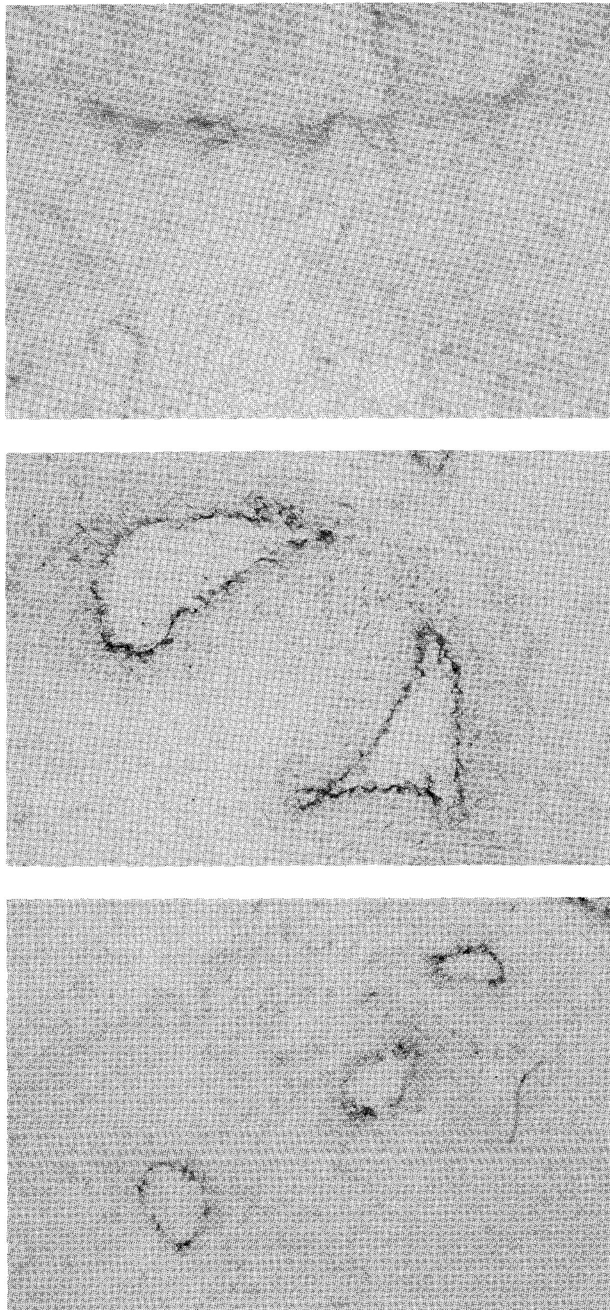

Fig. 1. — Immunohistochemical (APAAP) determination of E-selctin-expression after 2 h (top), 5 h (middle), and 7½ h (bottom) of reperfusion.

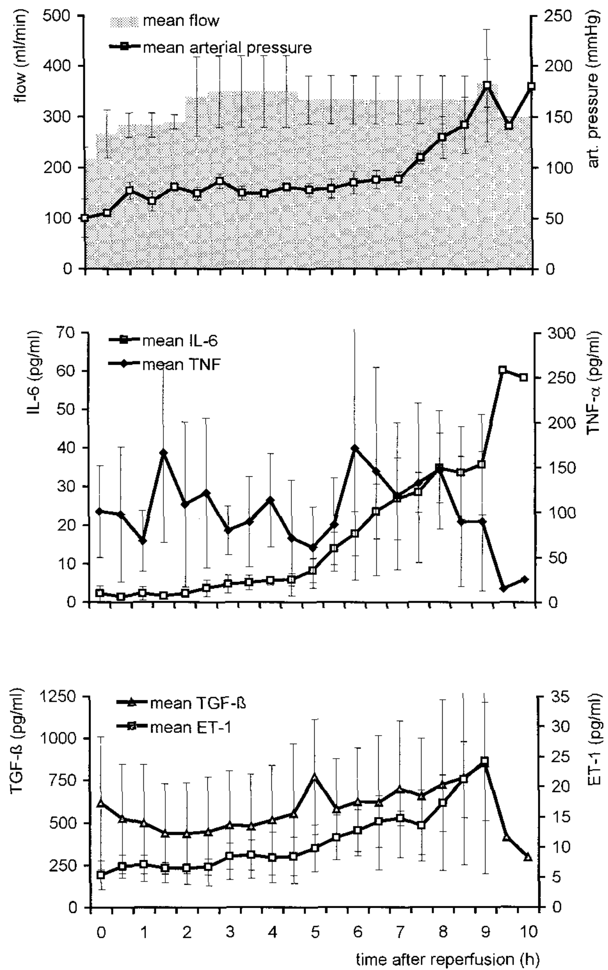

Fig. 2. — The arterial pressure and the flow rate during reperfusion are drawn on top of the figure. The time courses of IL-6 and TNF-α are depicted in the middle and TGF-ß and ET-1 in the bottom figure.

after reperfusion. Mean (± SD) concentrations of IL-6 were measured 2.4 ± 2.0 pg/ml at start of reperfusion, 5.8 ± 1.2 pg/ml at 4 h, 17.9 ± 5.7 pg/ml at 6 h, and 35.9 ± 13.1 pg/ml 9 h after reperfusion (figure 2).

Discussion

Each organ transplant sustains injury during the time it is removed from the donor, stored in a preservation solution, and transplanted into the recipient. Until reperfusion, the graft is exposed to cold and warm ischemia periods leading to a loss of mitochondrial respiration and consequently ATP depletion (Jaeschke, 1996). Intestinal ischemia-reperfusion injury results through both tissue hypoxia and reperfusion phenomena mediated by neutrophils (Wyble, 1996). A series of tissue reactions is involved during that process.

TNF-α and IL-6 are proinflammatory cytokines preferentially produced by monocyte-macrophages. However, cytokine production in the inflamed intestine is not limited to immune cells, and epithelial, muscle cells, and fibroblasts synthesis and respond to cytokines. IL-6 is also produced by epithelial cells and is invariably elevated in all types of inflammation, while TNF-α is not consistently elevated according to disease severity, but based on the inflammatory and cytotoxic activity involvement in gut inflammation is expected. In knock out animals with colitis, interferon-γ and TNF-α appear to be the most important mediators of tissue damage. Furthermore, the use of TNF-α monoclonal antibodies decreases intestinal inflammation (Fiocchio, 1996). IL-6 is one of the major mediators of inflammation, and IL-6 gene activation has been implicated in the pathogenesis of ischemia-reperfusion injury. *In vitro*, hy-

poxia, but not reoxygenation, induced the activation of NF-κB through the degradation of Iκ Bα and resulted in the κB-dependent transcriptional activation of the IL-6 gene (Muraoka, 1997).

Recently, ET-1 was found to induce leukocyte rolling and adherence through a predominantly ETA receptor-mediated mechanism in the submucosal venules of the intestinal microcirculation in a rat model studying ischemia-reperfusion injury by intravital fluorescence videomicroscopy (Boros, 1998). Also, ET-1 can release proinflammatory cytokines *in vitro* and *in vivo*. The generation of TNF-α caused by ET-1 involves the activation of ETA-receptors, activation of tyrosine kinase resulting in the phosphorylation of intracellular proteins, and activation of, hitherto, unknown transcription factors, finally resulting in transcription and translation of the TNF-α gene (Ruetten, 1997). Vise versa, a study on cultured vascular endothelial cells showed that IL-6, TNF-α, and IL-1 stimulated ET mRNA expression (Kahaleh, 1997). In an *in vitro* model, the binding of ET-1 to the isopeptide selective ETA receptor was reduced by preexposure to TGF-ß, while IL-6 and TNF-α did not effect ET-1 binding (Cristiani, 1994). TGF-ß plays a role in IgA production, promoting the isotype switch from IgM to IgA, in conjunction with generalized suppression, and is involved in mucosal healing (Mayer, 1996 ; Fiocchi, 1996).

E-selectin mediates the adhesion and transmigration of neutrophils in the microcirculation. The expression of E-selectin is influenced by cytokines. In a human intestinal perfusion model, E-selectin expression was greater with TNF-α and IL-1 combined than with either cytokine alone. Upregulation of E-selectin was demonstrated as early as 2 hours with maximum effects at 4 hours (Wyble, 1996).

In our perfusion model ET-1 increased in parallel with the arterial pressure. The unchanged flow rate during the rise in arterial pressure implies the presence of shunts at that time. The elevation of IL-6 concentrations was correlated to the degree of mucosal damage in biopsies. Especially, ET-1 and IL-6 appear to be useful parameters for monitoring the ischemia-reperfusion injury in this model. The time course of E-selectin reported by Wyble *et al.* is in accordance with our immunohistochemical findings (Wyble, 1996).

The complexity of the inflammatory cascade and their interactions during ischemia-reperfusion needs further investigation. Further study of inflammatory parameters in this model might allow the development of intervention strategies.

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