Impaired Organ Perfusion

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The aim of this work was to investigate the efficiency of organ perfusion during acute organ support, as performed during extracorporeal mechanical blood circulation in cardiac patients, donor management, organ procurement and organ preservation prior to transplantation. The investigations were conducted in clinical studies, animal studies and in-vitro experimental settings. The efforts were concentrated on testing the diagnostic value of new, specific and sensitive biomarkers for organ injury, in order to help an early and effective therapeutic strategy.

Chapter 1 introduces the reader into the subject by offering basic theoretical knowledge concerning cardiopulmonary bypass, organ preservation and organ donation prior to transplantation.

The study presented in Chapter 2 was designed to document the effects of dexamethasone on cytokine release and perioperative myocardial, pulmonary, renal, intestinal and hepatic damage, as assessed by specific and sensitive (bio)markers. A prospective, double-blind, placebo-controlled, randomized trial for dexamethasone was conducted in 20 patients, receiving either dexamethasone (1 mg/kg before anesthesia induction and 0.5 mg/kg after 8 hours) or placebo. Different markers were used to assess the inflammatory response: Interleukin-6, Interleukin-8, Interleukin-10, C-reactive protein, tryptase. Organ damage was investigated using plasma heart-type fatty acid binding protein, Troponin I, and Creatine kinase-MB to assess myocardial injury, urine N-acetyl-glucosaminidase and microalbuminuria to investigate renal injury, intestinal/liver type fatty acid binding protein to assess the small intestine injury, and α-Glutathione S-transferase for the hepatic injury. Dexamethasone, as administered in this study, effectively inhibited the release of pro-inflammatory interleukins and increased plasma concentration of anti-inflammatory
interleukins. However, dexamethasone treatment offered no protection against transient, perioperative renal, intestinal and hepatic injury in patients undergoing on-pump coronary artery bypass grafting. Dexamethasone treatment resulted in more pronounced postoperative pulmonary dysfunction, prolonged time to tracheal extubation and initiated postoperative hyperglycaemia. The high blood glucose levels were found strong significant predictors for renal and intestinal tissue injury.

In Chapter 3 we described the experimental infrastructure and clinical application of a comprehensive operative strategy that aimed to limit postoperative myocardial, renal and intestinal tissue injury in patients undergoing heart–lung machine assisted coronary artery bypass grafting. A prospective, pseudo–double blind, randomized clinical trial, investigating the clinical benefits of the new experimental intraoperative protocol was performed in 40 patients. The experimental operative protocol was developed to meet multiple objectives: (1) homogeneous cooling of the myocardium by combining cold crystalloid cardioplegia technique with intracavitary cooling of the heart; (2) prevention of excessive hemodilution by autologous priming of the extracorporeal circuit and partial recovery of the cardioplegic fluid; (3) corporeal normothermia, possible on the account of a more efficient topical cooling of the heart. Clinical outcome and transient postoperative injury of the myocardium, kidneys, and small intestine were investigated. Postoperative myocardial damage, as quantified by plasma levels of creatine kinase MB, was significantly lower in the patients in the experimental group. Transient proximal tubules injury was significantly attenuated in the patients benefiting from the experimental operative protocol, as shown by the urine concentrations of N–acetyl–beta–D glucosaminidase. Transient intestinal damage, as quantified by the urinary excretion of intestinal–type fatty acid binding protein, was significantly decreased in patients undergoing on pump coronary artery bypass grafting according the experimental protocol.

With the aim set on investigating variation in blood rheology during cardiopulmonary bypass and subsequent effects on (micro)circulation, we focused in Chapter 4 on red blood cell aggregation and endothelial activation. The present study addresses two different hydroxyethyl starch (HES) solutions commonly used in the clinical practice as priming solutions for the heart–lung machine and as plasma expanders. Red blood cell aggregation was measured by means of Laser–assisted Optical Rotation Cell Analyzer, in an in vitro study designed to mimic the human blood–material interactions during extracorporeal circulation. A clinical study investigating endothelial activation was conducted in 20 patients undergoing elective coronary bypass surgery, randomly assigned for cardiopulmonary bypass using either HAES–steril 6% (HES 200/0.5) or Voluven 6% (HES 130/0.4). The property of red blood cells to form aggregates at low shear rates was profoundly altered in our in vitro model mimicking the human blood–material interactions during
extracorporeal circulation. In parallel with the decrease in red blood cell aggregation, blood viscosity declined also. A functional and/or structural alteration of vascular endothelial cells during extracorporeal circulation was documented by elevated plasma concentrations of von Willebrand Factor, thrombomodulin, tissue plasminogen activator and E-Selectine. Differences between HES groups were evident post-bypass. While the markers of endothelial activation recovered in HES 200/0.5 group, HES 130/0.4 was associated on the first postoperative day with further increase of vWF and tPA. In parallel with the decrease in red blood cell aggregation, blood viscosity declined also. A functional and/or structural alteration of vascular endothelial cells during extracorporeal circulation was documented by elevated plasma concentrations of von Willebrand Factor, thrombomodulin, tissue plasminogen activator and E-Selectine. Differences between HES groups were evident post-bypass. While the markers of endothelial activation recovered in HES 200/0.5 group, HES 130/0.4 was associated on the first postoperative day with further increase of vWF and tPA.

The observations made in vitro on red blood cell aggregability coupled to the observation made in vivo on endothelial cell activation suggest a hypothetical new pathophysiological mechanism implicated in the post-cardiopulmonary bypass syndrome. We hypothesized that the drop in red blood cell aggregation added to plasma viscosity reduction and non-physiologic flow conditions during extracorporeal circulation, are important factors contributing to variation of shear stress at the vascular wall, leading to endothelial activation.

In Chapter 5, the hypothesis emerged in the previous chapter, concerning a possible interconnection between red blood cell aggregation and endothelial function, is verified in an experimental animal model of isovolemic hemodilution. We induced acute isovolemic hemodilution (30 ml/kg exchange transfusion with colloid solutions) in an “aggregating species” (pigs), and investigated the hypoxic oxidative stress (plasma Malondialdehyde, ex-vivo oxygen radicals production in heart, lung, kidney, liver, ileum tissue-biopsies), erythrocyte aggregation (LORCA), and endothelial activation (Real Time Quantitative Taqman RT-PCR on von Willebrand Factor (vWF), E- and P-Selectins, and endothelial nitric oxide synthase gene-expression in tissue biopsies). The production of superoxide and hydroxyl radicals, measured as $H_2O_2$ generation, was similar at all times in sham-operated and hemodiluted animals, which indicates that a similar hypoxic oxidative stress is present, and oxygen delivery to the tissue during acute hemodilution is maintained. Acute isovolemic hemodilution was followed by a dramatic drop in erythrocyte aggregation and immediate pro-thrombotic (significant vWF mRNA up-regulation in heart, lungs, kidney, liver, ileum) and pro-inflammatory (significant E- and P-Selectins mRNA up-regulation in lungs and ileum) endothelial activation. Low erythrocyte aggregations were significantly associated with increased mRNA-expressions of vWF (heart, liver, ileum) and P-Selectin (lungs, ileum and heart). In this way, we were able to demonstrate that
Summary

Erythrocyte aggregation can actively modulate thrombogenicity and inflammation by inducing release of endothelium-dependent pro-thrombotic factors and expression of pro-inflammatory adhesion molecules.

Approaching the subject of organ perfusion in transplantation, the study presented in Chapter 6 we investigated the time sequence for the progression of pro-inflammatory and pro-coagulatory endothelial activation, oxidative stress and organ injury in brain dead rat donors. The brain death model used in this study was a slow model, simulating the clinical condition of brain death due to intracranial hemorrhage. Brain death was induced by slowly inflating a balloon-catheter inserted in the extradural space. To assess time-dependent changes due to brain death, rats were sacrificed half hour, one hour, two hours, and four hours after brain death induction and compared to sham-operated controls.

The mRNA expression of the adhesion molecules E- and P-Selectins, known to promote inflammation by mediating rolling and extravasation of leukocytes, were up-regulated shortly (half hour) after brain death induction. Additionally, platelet trapping, most probably due to platelet adhesion to the vascular wall, was visualized as early as half hour after inducing the brain death. A significant increase in plasma levels of von Willebrand factor, and increased mRNA expression of Aα and Bβ fibrinogen, were observed.

Oxidative stress started to increase after induction of brain death, and became significant only after two hours of brain death. Brain death related donor kidney damage was diagnosed as early as half hour in renal tubules, with enhanced loss of viability when the state of brain death was prolonged.

When assessing the efficiency of organ procurement, the study in Chapter 7 aimed to determine the effect of HES and University of Wisconsin solution on the extent and kinetics of human red blood cell aggregation, and to morphologically characterize these aggregates. Human red blood cell aggregability and deformability were investigated in vitro, at 4°C, with a Laser-assisted Optical Rotation Cell Analyzer. The study of red blood cell aggregation in a binary HES-HES system gave an indication about the nature of HES-red blood cells interactions. Bright field microscopy and atomic force microscopy were used to morphologically characterize the aggregates size and form.

High molecular weight HES and University of Wisconsin solution had a potent hyper-aggregating effect. red blood cell aggregates were of large size and their resistance to dissociation by flow induced shear stress was high. These data suggest that gravity-induced hydrostatic perfusion pressures presently used in procurement can not easily dissociate the abnormal red blood cell aggregates. In addition, the small vessel diameter in marginal areas compared to the aggregates size make these vessel category prone to mechanical obstruction during organ procurement. Low molecular weight
HES treatment of blood yielded a decline of blood viscosity values, decreased the red blood cell aggregability and slowed the process in time.

**Perspectives**

Development and validation of new extracorporeal assist devices are highly desirable when performing artificial organ support. Pulsatile perfusion remains a challenging therapeutic choice. Either used as bridge to transplantation, bridge to recovery, or during coronary artery bypass grafting with cardiac arrest, an effective pulsatile perfusion might improve clinical outcomes.

In the same line of research, hypothermic machine perfusion providing a pulsatile blood flow is known to offer better protection against cold ischemic injury when compared with cold storage in marginal donor organs. Special effort has to be invested in testing in both experimental and clinical settings the benefits on graft viability when perfused with this newly available hypothermic, pulsatile machine preservation systems.

Last but not least, special scientific attention has to address the pathophysiology of disease and placing it in a clinical relevant context. In this respect, our efforts in documenting new mechanisms of endothelial activation related to variation in blood rheology parameters, the potential consequences of red blood cell aggregation on (micro)circulation might prove to be valuable in managing complications in both cardiac and transplant patients.