Functional heterogeneity of oxygen supply with blood and hemoglobin-based oxygen carriers in porcine models of hemorrhagic shock
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Resuscitation from hemorrhage is ultimately aimed at restoring oxygen delivery to the organs by improving microcirculatory perfusion and oxygenation. In current clinical practice, guidelines for resuscitation primarily provide for the monitoring of macrocirculatory parameters, such as arterial blood pressure, heart rate, and cardiac output; and of systemic metabolic and oxygenation parameters, such as plasma lactate and venous oxygen saturation. Ideally, the effectiveness of resuscitation would be monitored by measuring regional oxygenation because it is known from sepsis that macro- and microvascular parameters are not naturally related to each other.118

In this thesis, the heterogeneity of oxygenation during hemorrhage and resuscitation was investigated to reveal the relationships between micro- and macrovascular parameters and furthermore the relationships between and within organs. We focused on oxygenation of the heart as a vital organ and the small intestine as a non-vital organ. Regional oxygenation was measured using the quenching of Pd-porphyrin phosphorescence technique. This measurement technique, however, is not possible to use in humans; thus, our experiments were performed in anesthetized pigs. Hemorrhage, in all experiments, was created by a controlled withdrawal of a fixed volume of circulating blood. Therefore, the severity of shock was not determined by either macro- or microcirculatory parameters. Resuscitation was performed after a short period of induced hemorrhage. The first part of the thesis described the effects of resuscitation with autologous blood (chapters 1-3). In the second part (chapters 4-7), resuscitation with hemoglobin-based oxygen carriers (HBOCs) was investigated. As alternative solutions for blood in the treatment of hemorrhage, these compounds not only provide volume and oxygen, but they also directly or indirectly affect vascular tone. We investigated how the combination of these effects translated into the transport and distribution of oxygen to the various tissue beds during and after resuscitation from hemorrhage.

The aim of Chapter 1 was to investigate the relationship between the microvascular and venous oxygen pressures in the pig intestine during hemorrhagic shock and resuscitation. Microvascular PO\textsubscript{2} (µPO\textsubscript{2}), as measured by the quenching of Pd-porphyrin phosphorescence technique, was related to values of mesenteric venous blood gasses, blood flow, ilial CO\textsubscript{2} production and global hemodynamic parameters. In one group, moderate shock was induced by withdrawal of 25 ml/kg (30%) of the circulating blood volume. Seven of these animals were resuscitated with a crystalloid solution and four with the withdrawn autologous blood. In a second group, a more severe shock was induced by withdrawal of 40 ml/kg (50%) of the circulating blood volume; these animals were not resuscitated. The baseline mesenteric venous
PO$_2$ and μPO$_2$ values were similar (60 ± 9 and 60 ± 11 mmHg, respectively). During moderate shock, μPO$_2$ dropped significantly below mesenteric venous PO$_2$ (26 ± 10 vs 35 ± 8 mmHg). After resuscitation with crystalloid solution, μPO$_2$ and mesenteric venous PO$_2$ rose to 44 ± 9 and 44 ± 6 mmHg, respectively. In the group that received the withdrawn blood, the values were 41 ± 9 and 53 ± 12 mmHg, respectively. Severe shock resulted in a drop in mesenteric venous PO$_2$ to a value similar to that seen in the moderate shock group, but gut μPO$_2$ dropped to a much lower value than that of the moderate shock group (15 ± 5 vs 26 ± 10 mmHg). The results indicated that the oxygenation of the microcirculation of the gut can fall lower than the venous PO$_2$ under the conditions of hemorrhagic shock.

In Chapter 2, the regional heterogeneity of the oxygen supply-consumption ratio within the heart was discussed. This heterogeneity is an important functional parameter because it determines whether regions within the heart are normoxic or dysoxic. Although the heterogeneity of the supply side of oxygen has primarily been described using flow heterogeneity, the diffusion component of the oxygen supply should not be ignored. Such oxygen diffusion does not seem to originate from arterioles or venules within the heart, but it appears to occur between capillaries, in contrast to data recently obtained from other tissues. Oxygen diffusion might even become the primary determinant of oxygen supply during obstructed flow conditions. Studies aimed at the modeling of regional blood flow and oxygen consumption have demonstrated marked regional heterogeneity of oxygen consumption matched by flow heterogeneity. Direct, non-invasive indicators of the balance between oxygen supply and consumption include NADH videofluorimetry (mitochondrial energy state) and microvascular PO$_2$ measurement using the Pd-porphyrin phosphorescence technique. These indicators have shown a relatively homogeneous distribution during physiological conditions supporting the notion of the regional matching of oxygen supply with oxygen consumption. NADH videofluorimetry, however, has demonstrated large increases in the functional heterogeneity of this ratio in compromised hearts (ischemia, hypoxia, hypertrophy and endotoxemia), with specific areas, referred to as microcirculatory weak units, predisposed to showing the first signs of dysoxia. It has been suggested that these weak units show the largest relative reduction in flow (independent of absolute flow levels) during compromised conditions, with dysoxia initially developing at the venous ends of the capillaries.

Because disparity between the macro- and microcirculation is thought to occur as a result of (micro)vascular dysfunction in some types of shock, in Chapter 3, we investigated both macro- and microcirculatory parameters in the heart and the gut. We hypothesized that the microcirculation in the gut would follow the macrocirculation in the acute phase of hemorrhagic shock and isovolemic autologous whole
blood resuscitation but that the microcirculation in the heart would be preserved, even under conditions of macrocirculatory depression. The pigs were anesthetized and subjected to subsequent (20 and 10 ml/kg) controlled hemorrhagic shock and isovolemic (30 ml/kg) resuscitation with autologous blood. Quantitative measurement of microvascular oxygen pressure (μPO₂) was performed by phosphorimetry on the gut and heart simultaneously. Measurements of systemic hemodynamic and regional oxygen-derived parameters, as well as μPO₂, were performed at baseline, after the first and second phases of hemorrhage, and after resuscitation. Half of the pigs responded to resuscitation, whereas the other half died spontaneously within 20-30 min after reinfusion of the withdrawn blood, without significant differences in macro- or microcirculatory parameters at baseline or after hemorrhage. Correlation analysis showed that the microvascular PO₂ in the heart and the gut was closely related to macrocirculatory parameters (cardiac index, mean arterial pressure, and oxygen delivery) during hemorrhage and resuscitation. This study demonstrated that the microcirculation in the gut (which is a non-vital organ) and heart (which is a vital organ) follow the macrocirculation in the acute phase of hemorrhagic shock and in isovolemic autologous whole blood resuscitation.

As an alternative to allogeneic blood, hemoglobin-based oxygen carriers (HBOCs) have been developed. In Chapter 4, we reviewed the mechanisms of oxygen delivery by different HBOCs, the modifications made to the hemoglobin molecules to reduce the adverse effects associated with HBOCs, and their effects on (micro) vascular autoregulation and on blood rheology. We furthermore described the effects of HBOCs at the microcirculatory level in several organs.

In Chapter 5, we investigated the efficacy of resuscitation with polyHbXl (a polymerized cross-linked hemoglobin solution) in pigs. We investigated whether vasconstriction, caused by modified hemoglobin solutions, changed regional tissue perfusion and oxygenation and affected cardiac function. To this end, twelve pigs (27±2 kg) were anesthetized and subjected to a controlled hemorrhagic shock model (25 mL/kg). Next, they were resuscitated with either an isovolemic volume of autologous blood (n=6) or polyHbXl (n=6). At baseline, after shock, and during a 150-minute observation period following resuscitation, systemic hemodynamic parameters and systemic and cardiac oxygenation parameters were determined. In addition, catecholamines, high cardiac energy phosphates, and regional blood flow were measured, the latter with radioactive microspheres. We found that resuscitation with polyHbXl resulted in pulmonary hypertension but not in systemic hypertension, although both the pulmonary and systemic vascular resistances were increased. This finding was the result of a lower stroke volume. Due to lower arterial oxygen content after resuscitation with polyHbXl, systemic oxygen delivery was lower compared
with autologous blood resuscitation and it resulted in a higher oxygen extraction ratio. However, because of a greater blood flow to the left ventricle, regional oxygen delivery to the heart and cardiac function were maintained, similar to the autologous group. Hence, this study showed that resuscitation with polyHbXl after hemorrhagic shock in pigs restored hemodynamics and catecholamines at least as well as autologous blood and resulted in pulmonary hypertension. Cardiac oxygen delivery and cardiac function were well preserved.

In Chapter 6, we investigated the influence of resuscitation from hemorrhage with a low volume of DCLHb (a diaspirin cross-linked hemoglobin solution) on gut microvascular oxygen pressure (μPO\textsubscript{2}) in anesthetized pigs using palladium-porphyrin quenching of phosphorescence. Values of gut μPO\textsubscript{2} were studied in relation to regional intestinal parameters, as well as global metabolic and circulatory parameters. Controlled hemorrhagic shock (blood withdrawal of 40 mL/kg) was followed by resuscitation with either a combination of lactated Ringer’s solution and modified gelatin (lactR/Gel) or a low dose (5 ml/kg) of 10% DCLHb. After resuscitation, gut μPO\textsubscript{2} was similarly improved in the lactR/Gel group (from 25 ± 10 mm Hg to 53 ± 8 mm Hg) and in the DCLHb group (from 23 ± 9 mm Hg to 46 ± 6 mm Hg), a finding that was associated with increased gut oxygen delivery. However, the improvement after resuscitation with DCLHb was sustained for a longer period of time (75 vs. 30 min). Mesenteric venous PO\textsubscript{2} was increased after resuscitation with lactated Ringer’s solution and modified gelatin but not after resuscitation with DCLHb, the latter of which was associated with an increase in gut oxygen consumption in that group. We concluded that the measurement of μPO\textsubscript{2} by the palladium-porphyrin phosphorescence technique revealed DCLHb to be an effective carrier of oxygen to the microcirculation of the gut. Additionally, this effect can be achieved with a lower volume than is currently used in resuscitation procedures.

According to the results from the experiments in Chapter 6, we used different doses of DCLHb for resuscitation from hemorrhage in Chapter 7. Furthermore, we investigated the oxygenation of the heart, as this organ can be compromised with increased dosage of DCLHb. We tested the hypothesis that resuscitation with HBOCs affects the oxygenation of the microcirculation differently between and within organs. In addition, we tested the influence of the volume of DCLHb on the microcirculatory oxygenation of the heart and of the gut serosa and mucosa in a porcine model of hemorrhage. The pigs underwent controlled hemorrhage (30 mL/kg over 1 hour), which was followed by resuscitation with 10, 20, or 30 mL/kg DCLHb or by isovolemic resuscitation with 30 mL/kg of a 6% hydroxyethyl starch solution (HAES). The measurements included systemic and regional hemodynamic and oxygenation parameters. The microvascular oxygen pressures (μPO\textsubscript{2}) of the epicardium
and of the serosa and mucosa of the ileum were again measured simultaneously using the palladium-porphyrin phosphorescence technique. The measurements were obtained for up to 120 minutes after resuscitation. After hemorrhage, a low volume of DCLHb restored both cardiac and intestinal μPO$_2$. Resuscitation of gut μPO$_2$ with a low volume of DCLHb was as effective as isovolemic resuscitation with HAES. Higher volumes of DCLHb did not restore cardiac μPO$_2$, as isovolemic resuscitation with HAES did, but they did increase gut μPO$_2$ to hyperoxic values in dose-dependent manner. The effects were similar for the serosal and mucosal μPO$_2$. In contrast to the sustained hypertensive effect after resuscitation with DCLHb, the effects of DCLHb on regional oxygenation and on hemodynamics were transient. This study showed that a low volume of DCLHb was effective in the resuscitation of the microcirculatory oxygenation of the heart and gut. Increasing the volume of DCLHb however, did not cause an additional increase in heart μPO$_2$ but caused hyperoxic microvascular values in the gut to be attained. We concluded that microcirculatory monitoring in this manner elucidates the regional behavior of oxygen transport to the tissues by HBOCs, whereas the systemic variables were ineffective in describing their response.

In conclusion, our experiments showed that during hemorrhage and resuscitation with blood, the heterogeneity of regional cardiac and intestinal oxygenation is limited in relation to systemic hemodynamics. Heterogeneity after resuscitation with HBOCs, however, is significant. Therefore, to investigate the efficacy of resuscitation with HBOCs for hemorrhage, it is necessary to examine the microcirculatory levels of different organs. It was demonstrated that although it had remarkable effects on the vascular system, resuscitation with DCLHb and polyHbXl after hemorrhage resulted in enhanced regional oxygenation of the heart and gut. We also observed an increased metabolic need in the gut after the administration of DCLHb, which was balanced by improved oxygen supply.

As of now, no HBOCs have been approved by the FDA. While the effectiveness by HBOCs on oxygen transport has been shown in our study and in many other studies, attenuation of all clinical studies has mainly been due to the side effects induced by these solutions. A meta-analysis of 16 clinical trials (performed between 1996 till 2008) with five different HBOCs showed a significantly increased risk of death (relative risk of 1.3) and of myocardial infarction (relative risk of 2.7).²⁵⁹ It is assumed that these adverse effects are due to scavenging of NO by free hemoglobin, which may result in systemic vasoconstriction, impaired blood flow, release of pro-inflammatory mediators and conditions of vascular thrombosis in the heart due to a loss of platelet inactivation.²⁶⁰ Extravasation of HBOCs into the vasculature induces the scavenging of NO. Indeed, during our experiment using polyHbXl, described in
Chapter 5, we also observed extravasation of non-cross-linked Hb molecules, which were visible in all of the exposed organs. In this experiment, however, we observed no systemic hypertension and only pulmonary hypertension without impaired cardiac function. The development of polyHbXI was terminated after the experience of hemorrhagic lesions in the small intestines during experiments in rats and baboons. Additionally, clinical trials with DCLHb were terminated because of increased mortality in the HBOC group among trauma patients. It was assumed that the restoration and stabilization of mean arterial pressure by even low volumes of DCLHb, as described also in our experiment in Chapter 6, masked the insufficient oxygenation of different organs and resulted in limited and insufficient fluid resuscitation. However, there is still discussion about the relevance of hypertension as the cause of increased mortality. As the need remains real for an alternative to blood as a resuscitation fluid is, new artificial oxygen carriers are in development. The development of these HBOCs remains focused on avoiding vasoactivity and on improving their oxygen loading and unloading characteristics. No products for clinical use are currently available.

It is obvious that before new HBOCs can be clinically evaluated, these products must be investigated with regard to their efficacy on systemic hemodynamics and on the microvascular oxygenation of different organs. Although there is currently no FDA-approved HBOC, the development of HBOCs has definitely increased our knowledge about the behavior of the microcirculation under compromised circulatory conditions. Furthermore, this research has also driven the development of clinically applicable techniques for studying the microcirculation, such as SDF imaging.