Therapeutic interventions in shock
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Chapter 1

Introduction and Aim of the thesis
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1.1 General introduction

Circulatory shock (hereafter referred to as shock), or acute circulatory failure, is defined as systemic reduction of tissue perfusion or (micro) circulatory arrest [1]. Shock is one of the most frequently diagnosed, but still poorly understood clinical conditions in critically ill patients. Shock is classically defined in clinical subcategories. Hemorrhagic shock (HS) because of massive bleeding is a form of hypovolemic shock and a major contributor to early mortality in surgical and trauma patients. Septic shock is a form of distributive shock and the host response to a systemic infection, and associated with the loss of peripheral resistance and maldistribution of blood flow [2].

Hemorrhagic shock and septic shock-associated multiple organ dysfunction syndrome (MODS) is still a major cause of morbidity and mortality in critical ill patients in Intensive Care Units (ICU) [1]. Multiple mechanisms may contribute to shock associated MODS, and include hemodynamic disturbances, impaired tissue oxygen delivery, systemic inflammation, and cross-talk between endothelial cells and leukocytes [3]. The systemic inflammatory cascade, which can be triggered by infections or a variety of non-infectious insults such as traumatic hemorrhage, ischemia, and burns, is considered as the leading cause for the development of MODS. The pathophysiology of systemic inflammation is characterized by initial release of pro-inflammatory and vasoactive mediators into the systemic circulation, which activate intracellular signaling pathways. These will cause amongst others, microvascular dysfunction and/or mitochondrial dysregulation, resulting in tissue hypoperfusion and biochemical and biophysical alterations, finally leading to MODS [3, 4].

To restore the circulating volume and limit tissue hypoxia, resuscitation by intravenous fluids and administration of vasopressors is frequently used as the initial therapeutic intervention in hemorrhagic shock and septic shock patients [5]. However, the optimal resuscitation strategy is controversial and the type and dose of fluid, the control of bleeding and blood pressure, and the prevention of traumatic coagulopathy are not without debate [6-8].

Being highly responsive to local blood flow and disturbances in blood composition, endothelial cells are both active participants and mediators in the pathogenesis of shock-induced MODS [9]. The
clinical hallmarks of shock - hypotension, vascular leakage, systemic inflammation responses, and leukocyte recruitment - are all regulated at the level of endothelial cells in the microvasculature. Thus endothelial cells are potential targets for therapeutic interventions in the treatment of shock-associated MODS.

1.1.1 Hemorrhagic shock and sepsis preclinical animal models

Two clinically frequently encountered forms of shock are associated with hemorrhage and sepsis.

Hemorrhagic shock (HS) is defined as a clinical syndrome resulting from decreased blood perfusion in vital organs due to a loss of intravascular blood volume. Upon hemorrhage, the initial compensatory mechanisms can result in arterial vasoconstriction and increased heart rate, which are able to maintain blood pressure and redistribute the cardiac output in favor of vital organs [10]. However, these endogenous mechanisms have limited capacity, and early and effective therapies are needed to counteract the occurrence of cellular dysfunction and organ damage when counteractive mechanisms do not suffice anymore [11]. Besides contributing to the development of MODS as described above, HS is associated with the risk of secondary infections, such as pneumonia and sepsis.

To study the effects of HS on organ function and investigate effects of potential treatment strategies, several experimental animal models of HS have been developed, and include (1) fixed-volume hemorrhage, (2) fixed-pressure hemorrhage, and (3) uncontrolled hemorrhage [10]. In the animal model of fixed-volume hemorrhage, a predetermined percentage of the calculated total blood volume is removed over a dedicated time period. The advantage of such a model is that the physiological hemodynamic responses and the natural compensatory mechanisms following acute blood loss of a specific volume can be studied. The disadvantage is that the degree of hypotension is poorly defined, which may be associated with uncertain severity of the disease. In the fixed-pressure hemorrhage, animals are subjected to bleeding until the mean arterial pressure (MAP) reaches a predetermined level. Additional blood withdrawal or restitution of small volumes of blood is performed to maintain MAP at this level during the shock period. In this type of model, the degree and duration of hypotension are accurately controllable by monitoring the blood pressure, which contributes to the
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Experimental reproducibility and standardization. On the other hand, this model eliminates the effects of the compensatory mechanisms, which does not reflect the clinical situation. In an uncontrolled hemorrhage model, hemorrhage is induced by a standardized vascular trauma. This model might be the most clinically relevant rodent model, and would be the best model for preclinical testing of various therapies, though the reproducibility of the results is limited. In this thesis, taking model-related pathophysiology, reproducibility and reliability into account, the pressure-fixed hemorrhage model is used to evaluate microvascular endothelial behavior in major organs and MODS as well as the effects of pharmacological interventions.

Sepsis is characterized by the host response to (proven or suspected) overwhelming infection induced by bacteria or other pathogens [12]. Despite early diagnosis, removal of the septic focus by surgery or drainage, or the early application of antibiotics, sepsis remains the leading cause of death in critically ill patients. The pathophysiological complexity of sepsis makes the development of therapeutic drugs to counteract its consequences difficult [5, 13]. Clinically, sepsis is recognized as a disease continuum with progressively increased mortality rates from sepsis to severe sepsis when sepsis is accompanied by acute organ failure, and to septic shock when organ failure is accompanied by refractory hypotension [5]. In the development of sepsis, the host immune response is characterized by an initial pro-inflammatory response (systemic inflammatory response syndrome, SIRS) followed by immune paralysis (compensatory anti-inflammatory response syndrome, CARS) which is responsible for secondary infections. The excessive host response will induce microcirculatory alterations, tissue edema by capillary leakage, and leukocyte recruitment which will lead to tissue damage and MODS [3, 14].

Several animal models of sepsis have been developed to study sepsis pathogenesis and to test potential therapeutic agents [15]. These models include (1) endogenous bacterial product or bacterial toxin injection (amongst others the injection of lipopolysaccharide (LPS) which is a part of the outer membrane of Gram-negative bacteria), (2) disruption of an endogenous protective barrier (e.g., cecal ligation and puncture, CLP), and (3) intravenous or intrapulmonary infusion of exogenous bacteria. Recently the role of rodent models in predicting human pathology for systemic inflammatory response syndrome has been extensively
debated [16, 17]. Two drawbacks of all sepsis models with respect to clinical relevance are the differences in disease progress kinetics and the lack of clinically used supportive therapeutic interventions. The onset and development of sepsis to organ damage occurs in hours to days in most animal models, while in patients it may take a longer time. Moreover, modern ICU mechanical ventilation and other supportive measures are applied in the course of septic shock in patients, and these interventions cannot be easily mimicked in rodent animal models [18]. Despite these disadvantages, the animal sepsis models have contributed significantly to our understanding of the pathogenesis of sepsis [13]. In this thesis, the endotoxemia model induced by systemic LPS administration is employed because of its high reproducibility in pathophysiological reactions and its reliability to mimic some of the initial clinical features of sepsis in patients, including vasodilatation, release of inflammatory mediators, and increased vascular leakage [13]. Moreover, the intravenous LPS injection can be performed in human volunteers, which could be helpful for the translation of mouse data to human species [19].

1.1.2 Inflammatory responses in hemorrhagic shock and sepsis

The process of inflammation starts with a response of the innate immune system to an injurious stimulus. Pattern recognition receptors (PRR) on immune cells (e.g., macrophages, monocytes, or dendritic cells) recognize the pathogen-associated molecular patterns (PAMPs) associated with infections and damage-associated molecular patterns (DAMPs) associated with tissue injury, leading to the release of cytokines by the immune cells and initiation of the inflammatory responses. HS is conceived as a global ischemic insult that is frequently associated with a systemic inflammatory response triggered by cell disruption/tissue damage-induced endogenous DAMPs, which renders shock patients to develop MODS and secondary infections [20]. Conventional resuscitation fluids can restore circulatory volume and tissue perfusion, but they can also trigger even more severe inflammatory responses [21].

The dysregulation of the immune system is also a notable feature of severe sepsis. During sepsis, the binding of infection-associated PAMPs to PRR, e.g., toll-like receptor (TLR)-4 on several cell types, induces the production of cytokines and chemokines, resulting in the activation of the immune system and endothelium. The severity of the sepsis-associated
initial pro-inflammatory response affects the intensity of multiple organ dysfunction and shock [22]. Following the initial inflammatory response, a hypo-inflammatory state occurs to limit the excessive production of cytokines and organ damage [23]. This immunodeficiency state can lead to secondary infections that contribute to the failure of recovery of sepsis patients [22].

The release of cytokines and chemokines is an important factor in the development of SIRS. Upon infectious or non-infectious injury, TNFα, interleukin (IL)-1β, IL-6, and IL-8 are released excessively in a sequential manner, resulting in a cytokine storm [24]. TNFα and IL-1β, which are most rapidly produced, are considered the main inducers of most of the initial pathophysiological disturbances, such as the production of nitric oxide, the activation of cyclooxygenase enzymes, the expression and release of adhesion molecules, the increase of endothelial cell permeability, and the release of other cytokines and chemokines such as IL-6 and IL-8 [25, 26]. The serum levels of IL-6 have been reported to correlate with the degree of tissue injury, indicating that IL-6 is a clinically relevant and useful parameter to estimate the clinical course and outcome of the severe trauma and/or sepsis [27, 28]. IL-8 is a potent chemoattractant secreted by multiple cell types in response to inflammatory stimuli. Its production following injury leads to the recruitment and activation of leukocytes at the site of injury, therefore playing an important role in the pathophysiology of inflammation [29]. Anti-IL-8 antibody dramatically suppressed accumulation of neutrophils in the alveolar space and prevented LPS challenge-induced acute lethality [30].

1.1.3 Endothelial cells in shock

The vasculature plays a pivotal role in the pathogenesis of MODS in shock, and as mentioned above, is involved in the processes underlying tissue hypoperfusion, increased vascular permeability, and leukocyte influx into organs. Throughout the body, the vasculature consists of large arteries and veins, small arterioles and venules, and capillaries. The vascular segments differ in their architectures and functions and play distinct roles in health and diseases [31]. The inner surface of all blood vessels is covered by endothelial cells. In arteries and veins, the endothelium forms a continuous uninterrupted layer of endothelial cells, held together by tight junctions, while the endothelium in
capillaries and post-capillary venules - which have no muscle cell layer - may be continuous or discontinuous, and forms a barrier for selective permeability based on the needs of the underlying tissue [32]. Endothelial cells are highly active in sensing and responding to the stresses induced by alterations of the local extracellular microenvironment, as for instance experienced during shock, thereby modulating a variety of vascular functions [9].

Upon inflammatory insults, the transmigration of leukocytes into underlying tissues in organs mainly occurs in the capillaries and post-capillary venules to induce a local immune response [33]. This process is facilitated by a sequential and multi-step adhesion cascade between leukocytes and endothelial cells [34], as indicated in Figure 1. The initial step of leukocyte recruitment is the tethering and/or rolling of leukocytes on the endothelium mediated by the interactions between amongst others endothelial and leukocyte selectins (P-selectin, E-selectin, L-selectin) and their counter ligands such as P-selectin glycoprotein ligand-1 (PSGL-1) [34]. Following rolling, leukocytes are activated by signaling molecules recognized by G-protein-coupled receptors (e.g., inflammatory lipids, chemokines, chemoattractants), resulting in the activation of leukocyte integrin receptors such as lymphocyte function associated antigen (LFA)-1, macrophage antigen (Mac)-1, and very late antigen (VLA)-4. The activation of integrin receptors will increase the affinity of leukocytes for their endothelial ligands that belong to the immunoglobulin gene superfamily (IgSF), including vascular cell adhesion molecule (VCAM)-1 and intercellular adhesion molecule (ICAM)-1, which facilitate the firm adhesion of leukocytes to the endothelium [35]. Eventually, adherent leukocytes will transmigrate through the vessel wall to inflammatory sites mediated by integrins and endothelial intercellular tight junction molecules such as Platelet-endothelial adhesion molecule-1 (PECAM-1) [35]. Other players that have been implicated in the process of leukocyte-endothelial transmigration have been reviewed by Muller [36].

1.1.4 Microcirculatory alterations in shock

Local blood flow causes mechanical stress in the blood vessel wall. This mechanical stress can be divided into two categories: a) circumferential stress due to pulse pressure variation inside the vessel, and b) shear stress due to blood flow [37]. Circumferential stresses due to blood pressure
Figure 1. The multistep interaction of leukocyte and endothelial cells. Under flow conditions, activated endothelial cells are triggered to express selectins (P-selectin and E-selectin) on their surfaces, which will interact with their ligands such as P-selectin glycoprotein ligand-1 (PSGL-1), mediating the capture and rolling of fast-moving leukocytes. The interactions of L-selectin on leukocytes with ligands on inflamed endothelial cells and other leukocytes also mediate the capture and rolling process [34]. Thereafter, chemokines and other pro-inflammatory molecules activate leukocyte integrin receptors and their endothelial ligands of the immunoglobulin gene superfamily (IgSF) including vascular cell adhesion molecule (VCAM)-1 and intercellular adhesion molecule (ICAM)-1. Integrin-IgSF interactions are involved in the firm adhesion and arrest of leukocytes on the endothelium. The migration of leukocytes into underlying tissues is mediated by integrin binding to extracellular matrix ligands and endothelial tight junction adhesion molecules [35]. Capture, rolling, firm adhesion, and transmigration all happen in parallel, involving different leukocytes in the same microvessels.
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are transferred to all vessel wall layers (intima, media and adventitia) while shear stress is applied mainly to the endothelium being directly in contact with the blood. Hemodynamic forces are heterogeneous due to the differences in blood vessel geometries and the different regions of the vasculature. Wall shear stresses due to blood flow at different locations in the circulation have been estimated [38, 39]. Blood flow in vivo is pulsatile in arterioles while it tends to be laminar in vessels larger than 0.5mm in diameter. Mean shear stress is the lowest in large veins, such as vena cava where it is often lower than 1 dyne/cm², while small arterioles tend to face the highest shear stress which can reach 60 to 80 dyne/cm², and blood flow in small venules is also high (20 to 40 dyne/cm²) [38]. It has been estimated using a computer modeling approach that shear stress in glomerular capillaries ranges from approximately 1 to 95 dyne/cm² [40]. Shear stress-induced mechanotransduction, which represents the cellular responses to physical alterations in the local environment, is an important property of endothelial cells, and regulates endothelial behavior, including cell proliferation/survival, metabolism, cytoskeletal reorganization, and cell morphology [41].

Mechanosensors of endothelial cells include membrane proteins, integrins, ion channels, and intercellular junction proteins, as well as local membrane structures, such as caveolae, cytoskeleton, membrane lipids, and glycocalyx [42]. Upon initial application of laminar shear stress, mechanosensors respond through adaptor molecules to trigger the activation of a cascade of signaling pathways, such as phosphatidylinositol-3-kinase (PI3K)/Akt, and mitogen-activated protein kinases (MAPKs) [43-45]. Thereafter, transcription factors and cofactors, e.g., Kruppel-like factor (KLF)-2, nuclear factor (NF) -κB, and activator protein (AP)-1, are activated to regulate the expression of several functional genes, such as endothelial nitric oxide synthase (eNOS), VCAM-1, monocyte chemoattractant protein (MCP)-1, and Angiopoietin (Ang)-2 [46, 47]. Shear stress with different patterns distinctly influences endothelial gene expression and induces different functional consequences via complex mechanisms, which will here not be further discussed as most of the studies in this respect have been focused on flow profiles observed in arteriosclerosis occurring in arteries. Yet, since endothelial cells can adapt themselves to a sudden increase or decrease of shear stress from normal levels due to changes in blood flow, investigation of the underlying processes may contribute
to a better understanding of their roles in specific pathophysiological conditions in the microvasculature, as encountered in hemorrhagic shock and septic shock-associated organ hypoperfusion.

In hemorrhagic shock, acute bleeding leads to a decrease in the circulating blood volume, which leads to macrovascular hemodynamic abnormalities, e.g., decreased cardiac output and systemic hypotension, and alterations in the microcirculation. Both the macro- and microcirculation respond rapidly to compensate for the loss of circulating volume and to limit tissue hypoxia. The macrovascular response involves the autonomic nervous system-modulated increase in heart rate and arterial vasoconstriction, which will redistribute the remaining blood volume from the splanchnic, musculocutaneous, and renal circulation to vital organs such as the brain and the heart [48, 49]. The microcirculation distributes blood flow throughout individual organs to provide adequate oxygen delivery for every cell in the organs. Upon HS, in addition to the macrovascular redistribution, the blood is also redistributed within the microvascular networks of each organ according to oxygen demand as well as arteriolar and capillary resistances [49]. However, these adaptive mechanisms are limited. Upon severe hemorrhage, alterations of microcirculatory blood flow will contribute to the development of organ dysfunction despite the microvascular compensatory mechanisms. Persistent decrease in cardiac output and oxygen delivery induces a progressive decrease in capillary blood flow and functional capillary density with increased perfusion heterogeneity [50].

In septic shock, marked alterations in microcirculation have been observed in multiple experimental and clinical trials [14, 51, 52]. While a dense network of capillaries exists under normal conditions, patients with severe sepsis have a decrease in functional capillary density and microvascular blood flow as well as an increase in heterogeneity of capillary perfusion due to the presence of intermittently or non-perfused capillaries in close proximity to well-perfused capillaries [1, 14], as shown by the sublingual microcirculation under sepsis condition [51]. These microvascular changes are more pronounced in non-survivors than in survivors [52]. Microcirculatory alterations in sepsis may be explained by multiple mechanisms, involving endothelial activation, impaired endothelial cell sensitivity to vasoconstricting and vasodilating agents, glycocalyx degradation, increased leukocyte
adhesion on the endothelium, enhanced microvascular permeability with capillary leakage [14, 53]. Furthermore, the diffusion distance for oxygen is changed due to the decreased capillary density and increased heterogeneous microvascular blood flow, leading to alterations in oxygen extraction and tissue oxygenation even in the well-perfused organ [54, 55]. Although fluid resuscitation and vasoactive reagents may reverse the systemic hemodynamic alterations, the significant microvascular alterations may persist and contribute to the development of MODS [52]. Several trials have demonstrated the association between the severity of microvascular dysfunction and the development of organ dysfunction and mortality [56, 57]. Thus the crucial issue is to understand the contribution of the underlying molecular processes that lead to these microvascular alterations, and whether they can be improved with therapy.

The scope of the thesis encompasses shear stress and inflammatory activation related endothelial cell responses and the pharmacological intervention aimed at microvascular endothelial responses in hemorrhagic shock and sepsis.

1.1.5 Therapeutic interventions in the treatment of shock

In both HS and sepsis, the overwhelming inflammatory responses can be more threatening than the initial insults. Therefore, efficient anti-inflammatory strategies would potentially provide therapeutic benefits for both HS and septic patients, yet until now no clinical proven therapies are available [58]. IkappaB kinase (IKK)/NF-κB signaling is crucially involved in regulating the expression of many inflammatory mediators. Inappropriate and prolonged activation of NF-κB has been linked to several inflammation-associated diseases [59, 60]. It is tempting to speculate that IKK/NF-κB signaling can thus be considered an important therapeutic target for reducing tissue injury in shock patients [61]. Several strategies exist to block the activation of NF-κB, including NF-κB-specific oligodeoxynucleotide (ODN) decoys which can inhibit the DNA binding activity of NF-κB [62, 63], the mutation of the functional part of IkB, a primary endogenous inhibitor of NF-κB, to block its phosphorylation-dependent degradation while keeping its NF-κB binding activity, or direct overexpression of intracellular levels of IkB to retain NF-κB inactivated in the cytoplasm. In addition, NF-κB activation can be selectively inhibited by several small chemical pharmacological
inhibitors at different activation steps of this signaling pathway [61]. BAY11-7082, a selective IKK inhibitor, can effectively inhibit the activation of IKK, thereby inhibiting the phosphorylation of IκB and in turn attenuating the nuclear translocation of NF-κB, leading to the inhibition of the transcription of a variety of pro-inflammatory genes including cytokines, chemokines, and endothelial adhesion molecules.

Furthermore, the transcription of genes is partly modulated by posttranslational modifications. Amongst others lysine (de)acetylation affects a large number of histone and non-histone proteins. The significance of histone acetylation lies in the modification of chromatin structure and dynamics, and thereby gene transcription regulation. The acetylation of non-histone proteins plays an important role in regulating mRNA stability, protein localization, degradation, and protein-protein and protein-DNA interactions [64]. Lysine acetylation which is associated with transcriptional activation is mediated by histone acetyltransferases (HATs) that transfer acetyl groups from acetyl-coenzyme A to the amino group of lysines. The activity of HATs is counterbalanced by the activity of histone deacetylases (HDACs), which mediate the removal of acetyl group from the lysine residues. Both hemorrhage and sepsis are associated with an imbalance in the activities of HATs and HDACs in favor of deacetylation of proteins, and HDAC inhibitors can induce protein acetylation and restore this balance [65]. The reported pro-survival and anti-inflammatory effects of HDAC inhibitors indicate the emerging roles of HDAC inhibitors in therapeutic interventions of HS and septic shock [65]. The short chain fatty acid valproic acid (VPA) besides being frequently used in the long-term therapy of epilepsy and bipolar disorder, has been reported to have direct inhibitory effect on the catalytic activity of HDACs [66]. VPA modulates protein acetylation via reversing HDAC-mediated transcriptional repression, thereby mediating various cell functions such as cell differentiation and cell apoptosis, which can be used as a strategy for treatment of cancers [67-69]. More recently, VPA as HDAC inhibitor has been shown to have anti-inflammatory effects [70, 71], and proven beneficial against HS-associated apoptosis and organ injury [72, 73].

1.1.6 Endothelial targeted delivery of drugs in shock

The aim of drug delivery is to target drugs into selected cell populations to increase drug efficacy while concomitantly reducing toxicity or
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Disadvantageous pharmacokinetic behavior of the drug. Endothelial cells are easily accessible for intravenously administered substances and their heterogeneity allows for organ and/or disease specific drug delivery, which makes them an attractive target for selective drug delivery approaches, also in the context of therapeutic intervention in shock [74, 75].

Drug delivery systems such as lipid based liposomal formulations are often based on passive targeting of liposomes to sites with increased vascular permeability including sites of inflammation [76]. However, upon inflammatory stimulation, organs also display a vascular bed specific pattern of expression of adhesion molecule such as E-selectin and VCAM-1 [21, 77], providing opportunities to specifically deliver compounds to (micro)vascular endothelial subsets [78, 79].

Glucocorticoids inhibit many of the initial events in an inflammatory response and are extensively used for the treatment of both acute and chronic inflammatory diseases, such as ischemia, sepsis, asthma, and rheumatoid arthritis. Glucocorticoids exert their effects by binding to glucocorticoid receptors (GRs) in the cytoplasm which then dimerize and translocate into the nucleus where they can bind to glucocorticoid responsive elements on glucocorticoid-responsive genes, resulting in increased transcription of, e.g., anti-inflammatory genes. The transrepression effect of glucocorticoids on the expression of multiple inflammatory genes is considered as indirect genomic effects due the inhibition, by activated GRs, of activated transcription factors, such as NF-κB and activator protein (AP)-1, which in turn regulate the transcription of inflammatory genes [80]. The molecular mechanisms of glucocorticoids fit the pathophysiological mechanisms of sepsis and are appropriate to counteract the uncontrolled inflammation and to restore organ functions during sepsis [81]. However, systemic administration of glucocorticoids, especially at high doses or over a long period of time, has adverse effects. For instance, glucocorticoids can lead to hyperglycemia as they reduce peripheral use of glucose (due to reduced insulin sensitivity) and increase glucose production and induce immunodeficiency, resulting in higher susceptibility of the host to infections [82]. Moreover, it has been demonstrated that the administration of glucocorticoids fails to improve survival in patients with septic shock [83]. Thus, to minimize undesired side effects and to improve therapeutic effectiveness of glucocorticoids, endothelial cell
targeted delivery of glucocorticoids to endothelial cells by selective drug carriers can be a promising strategy. Using endothelial cells specific selective drug carrier, we have previously demonstrated in a murine model of glomerulonephritis that endothelial specific delivery of the liposome-encapsulated dexamethasone locally inhibits the expression of pro-inflammatory genes without affecting blood glucose levels [84].

1.2 Aim of the thesis

Despite substantial knowledge about the pathophysiology of shock, designing an effective strategy for the treatment of this condition is still a challenge. We and others previously showed that hemorrhage and sepsis-related shock induce pro-inflammatory activation of microvascular endothelial cells in an organ and microvascular segment specific manner [21, 85]. Creating a better understanding of the underlying molecular control of endothelial behavior during shock to assist in identifying potential targets for therapeutic intervention is the overall aim of this thesis.

The occurrence of blood flow disturbances in the vascular system is a hallmark of circulatory shock. Little insight is available on the effects of flow cessation and its later recovery on endothelial activation as appear in hemorrhagic shock and subsequent resuscitation. In order to study flow alterations separately from shock-associated cytokine production and leukocyte activation as well as tissue hypoxia as occur in vivo, we chose in Chapter 2 an in vitro approach to dissect blood flow changes from the other co-existing factors. We investigated the role of shear stress and shear stress alterations per se on endothelial activation as well as their effects on endothelial responses to concomitant cytokine challenge.

A role of epigenetic mechanisms, more specifically of histone (de)acetylation, in regulating endothelial gene expression and function in response to shear stress has recently emerged [86]. Furthermore, IKK/NF-κB signaling is known to play a central role in controlling pro-inflammatory activation of cells. To better understand these molecular mechanisms in microvascular endothelial activation during these processes as occur during circulatory shock and resuscitation, we investigated in Chapter 3 endothelial responses to the pre-incubation
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of histone deacetylase inhibitor valproic acid and IKK inhibitor BAY11-7082 during flow cessation and subsequent reflow in the absence and presence of cytokines in the in vitro cell culture model.

Considering the limitations of the in vitro loss of flow/restoration of flow model as it cannot completely mimic the microenvironment of endothelial cells in tissues, we studied in Chapter 4 the effects of the histone deacetylase inhibitor valproic acid and the IKK inhibitor BAY11-7082 on microvascular endothelial behavior in a mouse model of pressure-controlled hemorrhagic shock and resuscitation.

Besides hemorrhage induced flow disturbances, bacterial infection-induced sepsis and septic shock are also common causes of death in hospitalized patients. Glucocorticoids have been frequently recommended in the treatment of inflammatory diseases as they might blunt the overwhelming immune response. However, the systemic administration of glucocorticoids is associated with side effects such as immunodeficiency [82] and is lack of proven clinical benefit in the treatment of sepsis [83]. In Chapter 5, using an lipopolysaccharide (LPS)-induced endotoxemia mouse model, we aimed to selectively deliver dexamethasone, a clinically applied glucocorticoid, to activated endothelial cells by endothelial targeted immunoliposomes, and to determine its pharmacological effects on endothelial pro-inflammatory responses induced by LPS.

Finally, in Chapter 6, the results of the studies presented in this thesis are summarized and discussed in the context of our current knowledge, and new insights and possible implications of our findings are put in perspective for future investigation.
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