Hemorrhagic Shock-induced Endothelial Cell Activation in a Spontaneous Breathing and a Mechanical Ventilation Hemorrhagic Shock Model Is Induced by a Proinflammatory Response and Not by Hypoxia

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ABSTRACT

Introduction: The interaction between neutrophils and activated endothelium is essential for the development of multiple organ dysfunction in patients with hemorrhagic shock (HS). Mechanical ventilation frequently is used in patients with HS. The authors sought to investigate the consequences of mechanical ventilation of mice subjected to HS on microvascular endothelial activation in the lung and kidney.

Methods: Anesthetized wild type C57BL/6 male mice were subjected to controlled hemorrhage; subgroups of mice were mechanically ventilated during the HS insult. To study the effect of acute hypoxia on the mice, the animals were housed in hypoxic cages. Gene expression levels was assessed by quantitative real-time polymerase chain reaction. Protein expression was assessed by immunohistochemistry and enzyme-linked immunosorbent assay.

Results: Ninety minutes after the shock induction, a vascular endothelial activation in the lung and kidney. No differences in adhesion molecules between the spontaneously breathing and mechanically ventilated mice were found. Concentrations of the proinflammatory cytokines chemokine (C-X-C motif) ligand 1 (11.0-fold) and interleukin-6 (21.7-fold) were increased after 90 min of HS. Two hours of 6% oxygen did not induce the expression of E-selectin, vascular cell adhesion molecule 1, and intercellular adhesion molecule 1 expression represented by E-selectin, vascular cell adhesion molecule 1, and intercellular adhesion molecule 1 in the kidneys and the lung.

Conclusions: Hemorrhagic shock leads to an early and reversible proinflammatory endothelial activation in kidney and lung. HS-induced endothelial activation is not changed by mechanical ventilation during the shock phase. Hypoxia alone does not lead to endothelial activation. The observed proinflammatory endothelial activation is mostly ischemia-reperfusion-dependent and not related to hypoxia.

MAJOR improvements in emergency medicine have led to decreased early deaths from hemorrhagic shock (HS). However, this renders patients vulnerable for second-
ary complications. A feared complication that can be caused by HS is multiorgan dysfunction syndrome. This syndrome causes severe morbidity and mortality and is a major challenge in critical care medicine. A systemic inflammatory response is considered the leading cause for the development of multiorgan dysfunction syndrome. Two failing organs are the lung and the kidney. Lung failure, the so-called acute respiratory distress syndrome, and acute kidney injury are strongly associated with patient morbidity and mortality.

For symptoms of acute respiratory distress syndrome and acute kidney injury, patients are treated with mechanical ventilation and renal replacement therapy. The precise mechanisms leading to multiorgan dysfunction syndrome after HS remain largely unknown. One of the proposed mechanisms is infiltration of neutrophils into the tissues, leading to major organ damage through release of proteases and oxygen-derived radicals. The interaction between neutrophils and endothelium is crucial for the migration of neutrophils into tissues. This neutrophil migration is regulated by the induction of adhesion molecules on the endothelium, including E-selectin, vascular cell adhesion molecule 1 (VCAM-1), and intercellular adhesion molecule 1 (ICAM-1). We recently have shown that HS leads to early and organ-specific proinflammatory microvascular endothelial activation. This organ and microvascular bed-specific endothelial activation also is seen in animal models subjected to septic shock, where it coincides with increased vascular leakage.

Hemorrhagic shock occurs frequently in the operating theater as a result of difficult-to-control surgical bleeding. Patients with HS attributable to other causes, including trauma or gastrointestinal bleeding, require resuscitation and procedures to control the bleeding. Therefore, patients with HS often are intubated and mechanically ventilated to allow intervention procedures and warrant a patent airway and gas exchange. Mechanical ventilation (MV) during conditions of HS can act as a double-edged sword. Intubation and MV may protect organs from hypoxia and hypercapnia but also may initiate an inflammatory reaction and induce proinflammatory activation in the lung and distant organs.

To follow up on our previous observation of HS-induced microvascular endothelial priming during the shock phase, our aim was to investigate the beneficial, neutral, or harmful consequences of MV in mice subjected to HS on microvascular endothelial activation in the lung and kidney. MV is started to prevent hypoxia and threatened oxygen delivery. HS by definition decreases oxygen delivery, yet the role of decreased oxygen availability in endothelial activation is not clear. To study the effect of a decreased oxygen delivery in vivo on endothelial cell (EC) activation we also evaluated endothelial proinflammatory adhesion molecule expression in hypoxic mice.

Materials and Methods

Animals

Eight- to 12-week-old C57BL/6 male mice (20–30 g) were obtained from Harlan Nederland (Horst, The Netherlands). Mice were maintained on mouse chow and tap water ad libitum in a temperature-controlled chamber at 24°C with a 12:12-h light-dark cycle. All procedures were approved by the local committee for care and use of laboratory animals (DEC, University Medical Center Groningen, Groningen, The Netherlands) and were performed according to national and international guidelines on animal experimentation.

Mouse Shock Model

The mouse HS model has been documented extensively elsewhere. In short, mice were anesthetized with isoflurane inspiratory 3.0% induction in N2O (2 l/min) and O2 (1 l/min); after-anesthesia was continued with isoflurane (inspiratory, 1.4%), N2O (66%), and O2 (33%). The left femoral artery was cannulated for monitoring mean arterial pressure (MAP), blood withdrawal, and resuscitation. HS was achieved by blood withdrawal until a reduction of the MAP to 30 mmHg. Additional blood withdrawal or restitution of small volumes of blood was performed to maintain a pressure-constant HS model with an MAP at 30 mmHg during this period. A subset of mice was resuscitated after 90 min of HS with hydroxyethyl starch 130/0.4 (6%; Voluven; Fresenius-Kabi, Bad Homburg, Germany) at two times the volume of blood withdrawn and killed 24 h after HS induction. During sacrifice, blood was withdrawn via aortic puncture under isoflurane anesthesia, and the kidneys and lung were excised, snap-frozen in metal cups on liquid nitrogen, and stored at −80°C until analysis.

Mouse MV Model

The MV model used has been described previously. In short, mice were anesthetized with isoflurane inspiratory 3.0% induction in N2O (2 l/min) and O2 (1 l/min). Animals were orally intubated under direct vision with an endotracheal tube (0.82 mm ID, 1.1 mm OD, length 25 mm). Endotracheal tube position was confirmed by bilateral chest excursions. Subsequently, animals were connected to the ventilator (MiniVent®; Hugo Sachs Elektronik-Harvard Apparatus, March-Hugstetten, Germany). Tidal volume was set at 180 µl; frequency was set at 150/min. All animals received 4 cm H2O positive end-expiratory pressure. After intubation, anesthesia was continued with isoflurane (inspiratory, 1.4%), N2O (66%), and O2 (33%). The anesthetic regimen between the spontaneously breathing and mechanically ventilated mice was the same. The investigators were not blinded to the treatment.

Arterial Blood Gas Analysis

Blood for blood gas analysis was withdrawn at three time points directly after shock induction via the arterial line, 90 min after shock induction just before sacrifice, and 24 h after shock induction via aortic puncture during anesthesia. Samples were analyzed immediately after collection on an ABL800 blood gas analyzer (Radiometer, Zoetermeer, The Netherlands).
Mouse Hypoxia Model
To examine the role of acute hypoxia on endothelial proinflammatory and hypoxia driven genes, a subset of mice was housed for 2 h in respiratory cages to manipulate oxygen concentration. Oxygen concentration was set at 21%, 10%, and 6%, respectively. Hypoxia-exposed mice and unexposed control mice were killed during isoflurane anesthesia, after which blood was withdrawn and kidney and lung were harvested and handled as described in the mouse shock model section of Materials and Methods.

Gene Expression Analysis by Quantitative Real-time Polymerase Chain Reaction
RNA was extracted from 20 × 5-μm cryosections from kidney and mouse lung and isolated using the RNeasy Mini Plus Kit (Qiagen; Westburg, Leusden, The Netherlands) according to the manufacturer's instructions. Integrity of RNA was determined by gel electrophoresis. RNA yield and purity were measured by an NanoDrop® ND-1000 ultraviolet-visual spectroscopy spectrophotometer (NanoDrop Technologies, Rockland, DE). One microgram of RNA was reverse-transcribed using SuperScript® III reverse transcriptase (Invitrogen, Carlsbad, CA; CXCL-1: ELISA-Kit; BioSource, Camarillo, CA; CXCL-1: ELISA-Kit; R&D Systems, Minneapolis, MN). Lower detection limits were 32 pg/ml for TNF-α, 28 pg/ml for IL-6, and 6%, respectively. Hypoxia-exposed mice and unexposed control mice were killed during isoflurane anesthesia, after which blood was withdrawn and kidney and lung were harvested and handled as described in the mouse shock model section of Materials and Methods.

Cytokine Analysis
TNF-α, interleukin (IL)-6, and chemokine (C-X-C motif) ligand 1 (CXCL-1) concentrations in plasma were analyzed by enzyme-linked immunosorbent assay (TNF-α, IL-6: Cytoset; BioSource, Camarillo, CA; CXCL-1: ELISA-Kit; R&D Systems, Minneapolis, MN). Lower detection limits were 32 pg/ml for TNF-α; 160 pg/ml for IL-6; and 160 pg/ml for CXCL-1.

Localization of Adhesion Molecule Expression Using Immunohistochemistry
Localization of CD31, E-selectin, VCAM-1, and ICAM-1 expression was determined in kidney by immunohistochemistry. Snap-frozen organs were cryostat cut at 5 μm, mounted onto glass slides, and fixed with acetone for 10 min. After sections were dried, they were incubated for 45 min at room temperature with primary rat antimouse antibodies recognizing CD31 (clone MECA13.3; BD Biosciences Pharmingen, Alphen aan den Rijn, The Netherlands), E-selectin (MES-1, provided by Dr. Derek Brown, Ph.D., scientist, UCB Celltech, Brussels, Belgium), and ICAM-1 (clone YN1/1.7; American Type Culture Collection) in the presence of fetal calf serum, 5%. After the sections were washed, endogenous peroxidase was blocked by incubation with H2O2, 0.1%, in phosphate buffered saline for 20 min. This was followed by incubation for 30 min at room temperature with horseradish peroxidase conjugated secondary antibodies (rabbit antirat IgG, DAKO, Glostrup, Denmark). Conjugates were diluted 1:50 in phosphate buffered saline supplemented with normal mouse serum, 2%. Sections with isotype-matched controls and E-selectin antibodies were further incubated for 30 min at room temperature with horseradish peroxidase-conjugated goat antirabbit antibody (Southern Biotech Association, Birmingham, AL) diluted 1:100 in phosphate buffered saline. Between incubations with antibodies, sections were washed extensively with phosphate buffered saline. Peroxidase activity was detected with 3-amin-9-ethylcarbazole (Sigma–Aldrich Chemical, St. Louis, MO), and sections were counterstained with Mayer's hematoxylin (Klinipath, Duiven, The Netherlands).

Statistical Analysis
Statistical significance of differences was studied by means of unpaired independent Student t test or one-way ANOVA with post hoc comparison using Dunnett correction. First 90-min time points and 24-h time points HS were compared with HS combined with MV. When there were no differences, HS and MV at the same time points were pooled for increased statistical power. All statistical analyses were performed using GraphPad Prism (GraphPad Software, San Diego, CA). We used two-tailed hypothesis testing with all tests. Several of the variables under study may violate the assumptions of parametric tests, so the results of the statistical tests were confirmed using nonparametric equivalents. We used a two-way repeated measures ANOVA (group × time) to compare MAP during the shock phase between the spontaneously breathing and mechanically ventilated mice. Differences were considered to be significant when P < 0.05.

Results
During anesthesia, mice were intubated and mechanically ventilated or allowed to breathe spontaneously while blood was withdrawn to reach an MAP of 30 mmHg (table 1). No statistically significant differences in blood pressure were ob-
studied between the groups during the shock phase ($P = 0.99$) (fig. 1). The initial blood pressure after induction in the spontaneous breathing group (mean 82 mmHg, SD 16.2) was not significantly different statistically from the blood pressure in the MV group (mean 71 mmHg, SD 13.8). Mice in all the groups remained normoxic and did not become hypercapnic (table 2). Mechanically ventilated mice had increased metabolic acidosis at 90 min of HS compared with the spontaneously breathing mice with a statistically significant lower pH and lower base excess at 90 min after shock induction. This metabolic acidosis was resolved 24 h after the HS insult (table 2).

Ninety minutes after shock induction, an endothelial proinflammatory activation was observed, as reflected by increased E-selectin, VCAM-1, and ICAM-1 mRNA expression (fig. 2). After 24 h, the proinflammatory endothelial activation genes in both the MV and the spontaneously breathing group were back to baseline (fig. 2). No differences between the spontaneously breathing and mechanically ventilated mice were found for E-selectin, VCAM-1, and ICAM-1 at 90 min or 24 h after shock induction. The lung and the kidney have different endothelial response patterns during HS. VCAM-1 mRNA expression levels were up-regulated in the lung after shock (fig. 2D) but unchanged in the kidney (fig. 2C). In the kidney and the lung, mRNA expression levels of both E-selectin and ICAM-1 were up-regulated after 90 min of HS (fig. 2, A, B, E, and F).

To further extend our knowledge on microvascular bed-specific differences of endothelial activation in HS, we examined these endothelial proinflammatory activation differences within the kidney by immunohistochemistry of different renal microvascular beds. The pan endothelial marker protein CD31 was expressed in all microvascular beds, including arterioles, glomeruli, the peritubular vascular capillaries and venules in control mice (fig. 3A) and did not change after shock induction with or without MV (fig. 3, B and C). In the kidney, E-selectin was absent in the control kidney but strongly up-regulated 90 min after shock induction in glomerular capillaries both with and without MV (fig. 3, D, E, and F). In contrast, in peritubular and arteriolar vascular beds, E-selectin could not be detected after shock induction (fig. 3, E and F). In control kidneys, constitutive ICAM-1 expression was observed in all vascular beds. In HS kidneys, increased ICAM-1 expression was observed most prominently in the peritubular capillaries (fig. 3, G, H, and I). Overall, no visible differences were found in the expression of adhesion molecules in the kidney related to MV.

Endothelial proinflammatory activation can be caused by proinflammatory cytokines and hypoxia. First, we investigated the mRNA expression levels in lungs and kidneys of the proinflammatory cytokines TNF-α, IL-6, and MCP-1 in time (fig. 4). No differences between the spontaneously breathing and mechanically ventilated HS mice were found for TNF-α, IL-6, and MCP-1 90 min after shock induction (fig. 4, A, B, C, D, E, and F). In the kidney and lung, 90-min HS induced a small increase in mRNA for TNF-α (fig. 4, A and B) and IL-6 (fig. 4, C and D), whereas MCP-1 mRNA levels were unchanged (fig. 4, E and F). To investigate whether the proinflammatory cytokines TNF-α, CXCL-1, and IL-6 were produced in remote organs, we measured soluble cytokine proteins in plasma. Because of insufficient blood sample, we were not able to perform cytokine enzyme-linked immunosorbent assay in one mouse in the 90-min HS group. Although TNF-α protein in plasma was not changed at 90 min (fig. 5A), concentrations of the proinflammatory cytokines CXCL-1 (11.0-fold) and IL-6 (21.7-fold) were statistically significantly increased after 90 min (fig. 5, B and C). MV during 90 min of HS did not affect these HS-induced changes in IL-6 and CXCL-1 in the systemic circulation (fig. 5). At 24 h after the shock period, all proinflammatory cytokine concentrations in plasma had returned to baseline (fig. 5).

Hypoxia can induce HIF-1α via transcriptional control and posttranslational processes affecting the protein concentration, with an increase in VEGF-A as one of the downstream consequences. Thus, we investigated whether shock-induced changes in cellular oxygen concentrations influenced HIF-1α and VEGF-A in our model. During the shock period, HIF-1α mRNA was not induced in the kidney, whereas in

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HS = hemorrhagic shock; MV = mechanical ventilation.
the lung, it led to HIF-1α mRNA up-regulation, with no differences between HS alone and HS combined with MV (fig. 4). VEGF-A mRNA showed a small but statistically significant up-regulation in the 90 min with MV compared with HS alone in lung and kidney compared with the spontaneously breathing mice (fig. 4, I and J).

The up-regulation of HIF-1α in the lung and the up-regulation of VEGF-A in the MV group implies that a hypoxic condition may have occurred during the shock period in the absence and presence of MV. To determine the potential contribution of tissue hypoxia to the proinflammatory endothelial activation observed, we studied endothelial proinflammatory activation in response to short-term exposure to severe hypoxia only (table 1). To expose the mice to hypoxia during a similar period of time as the HS mice, including the time needed for instrumentation and anesthesia, mice were housed for 120 min in hypoxic cages with three different oxygen concentrations (fig. 6). Neither 120 min of oxygen, 6%, nor oxygen (10%; data not shown) induced the expression of the proinflammatory genes E-selectin, VCAM-1, and ICAM-1 in the kidney and lung of mice (fig. 7). Surprisingly, this acute and severe hypoxia also did not increase the mRNA expression of HIF-1α and VEGF-A in lung and kidney (fig. 7, M, N, O, and P). However, the severe hypoxia did induce a proinflammatory response in the lung; 2 h of hypoxia, 6%, led to the up-regulation of proinflammatory cytokines IL-6 and MCP-1 mRNA, whereas TNF-α mRNA was unchanged (fig. 7). In the kidney, no differences in mRNA for IL-6, MCP-1, and TNF-α were observed between normal oxygen concentrations and severe hypoxia (fig. 7, G, H, I, J, and K).

Discussion

The interaction between neutrophils and activated endothelium is crucial for the development of multiple organ dysfunction in patients with HS, but the effects of MV and whole body hypoxia on this endothelial activation are not known. In this study of MV during HS in mice, we demonstrated that the proinflammatory EC activation in the lung and the kidney was not prevented or augmented by MV. In addition, we found that the organ-specific endothelial activation in HS is not induced by an impaired oxygen delivery but merely by a systemic proinflammatory response induced by HS.

To our knowledge, we are the first to report the effect of MV during HS and its effects on EC activation in mice. MV alone is able to induce an inflammatory response in mice and...
man. In mice, MV induced TNF-α, IL-6, and IL-1β in mice lung 60 min after MV initiation, whereas plasma protein concentrations followed some time thereafter. In addition, MV in mice led to endothelial activation in lung and distant organs within 120 min after MV initiation. The data in our studies show that, compared with the HS insult, no additional detrimental effects on endothelial inflammation are induced when MV is performed in animals with HS. This implies that the insult given is too mild or too short to add additional detrimental effects.

Our results on the early proinflammatory cytokine release during HS corroborate several other animal studies. First, previous work from our own group shows that mRNA for TNF-α is up-regulated in kidney and heart 90 min after the initiation of the HS. Liu and Dubick showed in rats with HS to a MAP of 50 mmHg during 60 min that the mRNA for TNF-α was up-regulated in kidney and liver. In a mouse model, TNF-α measured in serum was increased 30 min after shock onset.

Decreased tissue oxygen delivery, leading to cellular hypoxia, is considered to be an important mediator of systemic inflammation after massive hemorrhage. During hypoxic conditions, HIF-1α accumulates in the cell, translocates to the nucleus, and forms a stable heterodimer, after which it induces gene transcription. HIF-1α can also be regulated at the transcriptional level, as demonstrated in tumor models. Vascular endothelial growth factor A is a downstream target gene of HIF-1α that is primarily regulated at the transcriptional level and a major controller of vascular permeability in shock states. Under hypoxic conditions, up-regulation of VEGF-A by HIF-1α occurs within minutes. In cellular experiments using the human umbilical vein cell line EA.hy926, incubation for 16 h in oxygen, 1%, induced HIF-1 nuclear translocation. This was accompanied by ICAM-1 and E-selectin mRNA up-regulation compared with cells that were incubated in normoxia. However, in human aortic endothelial cells incubated for 8 h in oxygen (4%) hypoxia, no up-regulation of E-selectin, VCAM-1, or ICAM-1 could be observed by Illumina gene microarray analysis. We did not see any statistically significant induction of mRNA of HIF-1α or VEGF-A during HS alone, but MV combined with HS led to a small increase in HIF-1α and VEGF-A mRNA. In addition, HIF-1α and VEGF-A were not affected during our acute hypoxia experiments in mice without shock. Our data are contrary to the findings of Koury et al., who showed that in an HS model in the rat, HIF-1α concentrations as measured by Western blotting were increased in the ileac mucosa after 90 min of HS. Hierholzer et al. observed an increase in HIF-1α nuclear activity in the lung after a 40-mmHg MAP shock period for 2.5 h. No increased HIF-1α activation in livers of animals subjected to 40 mmHg MAP HS for 60 min was found.
From these studies it becomes clear that there are large organ-, insult-, and time-frame–dependent differences that regulate HIF-1α/HIF-2α responsiveness in HS models. Thus, our data and those reported by others suggest that severe whole-body hypoxia is not the driving factor for EC activation in HS in kidney and lung but that large interorgan differences may exist. Our animal model of HS with or without MV has several limitations. First, in our pressure-constant HS model, increased metabolic acidosis was observed in the mechanically ventilated mice at 90 min of HS compared with the spontaneously breathing mice. This suggests the occurrence of decreased organ perfusion in vital organs in the MV group. This decreased organ hypoperfusion could lead to deleterious outcomes later. In addition, we used a short shock period to mimic the short and severe nonresuscitated HS seen in the clinic, but the described proinflammatory effects of MV may be more pronounced after longer periods of MV. It is possible that, after the shock insult has resolved, ongoing MV may lead to additional organ damage. However, this clinically important question cannot be tested in this small ro-

**Fig. 4.** Kinetics of expression of proinflammatory- and hypoxia-related genes in kidney and lung during hemorrhagic shock (HS) in the presence or absence of mechanical ventilation (MV). Gene expression levels of the proinflammatory cytokines tumor necrosis factor-α (TNF-α) (A, B), interleukin-6 (IL-6) (C, D), and monocyte chemotactic protein-1 (MCP-1) (E, F) and the hypoxia-related molecules hypoxia-inducible factor-1α (HIF-1α) (G, H) and vascular endothelial growth factor-A (VEGF-A) (I, J) in kidney (A, C, E, G, I) and lung (B, D, F, H, J) analyzed by quantitative real-time polymerase chain reaction using glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as housekeeping gene. Data are expressed as mean ± SEM. P < 0.05 values are marked with an asterisk (*). mRNA = messenger ribonucleic acid.

**Fig. 5.** Proinflammatory cytokines in plasma during hemorrhagic shock (HS) and resuscitation in the presence or absence of mechanical ventilation (MV). Concentrations of tumor necrosis factor-α (TNF-α) (A), chemokine (C-X-C motif) ligand 1 (CXCL-1) (B), and interleukin 6 (IL-6) in control (C), HS during 90 min with and without MV, and 24 after the HS insult with or without MV. Data are expressed as mean. * P < 0.05 compared with control mice. 

**Fig. 6.** Inspiratory oxygen concentration of mice housed in hypoxic cages for 120 min. Concentrations of inspiratory oxygen were controlled during a 120-min stay in hypoxic cages. Inspiratory oxygen concentrations were maintained at 21% (triangles), 10% (circles), and 6% (squares). Mice were killed after 120 min.
dent model because of instabilities of this model after prolonged MV. The absence of evidence of differences in endothelial activation in a pressure-constant mouse HS model induced by MV at 90 min of HS is not evidence of the absence of the effects of MV during longer periods or after the resolution of HS on endothelial activation in vital organs. A larger animal model with more physiologic monitoring might in the future provide more insight regarding the consequences of longer MV exposure after the shock insult on EC activation. In addition, in our hypoxic cage experiments, we did not measure blood gases or oxygen delivery capacity. However, in man it has been shown that 12% normobaric hypoxia is sufficient to cause hyperlactatemia as an indicator of insufficient oxygen transport. Thus, we hypothesized that oxygen, 6%, is sufficient to induce an impaired oxygen delivery. This oxygen concentration leads to an estimated P O₂ of 4–5 kPa and an oxygen saturation of 50–60%. In our model, the severe hypoxia induced a proinflammatory response in the lung; 2 h of 6% hypoxia led to the up-regulation of IL-6 and MCP-1 mRNA. Finally, the translation of our rodent results to patients with HS is difficult. In our experiments, we used young male, otherwise healthy mice, which do not model older patients with multiple comorbidities. The effects of aging and comorbidity on the systemic and microvascular responsiveness to HS and MV are now being investigated.

The early endothelial activation found in this study suggests that the therapeutic window to attenuate EC activation takes place early during the shock insult. This EC activation is induced at least in part by a proinflammatory response induced by proinflammatory cytokines, whereas in the mouse, whole-body cellular hypoxia does not induce endothelial adhesion molecules in the same time frame. In our model of HS, MV per se does not add to the proinflammatory endothelial activation seen in lung and kidneys. The observed proinflammatory endothelial activation is mostly ischemia- or reperfusion-dependent and not related to hypoxia. Additional studies will investigate potential therapeutic strategies to diminish proinflammatory endothelial activation in HS on endothelial dysfunction.

Fig. 7. The kinetics of messenger RNA (mRNA) expression of endothelial cell adhesion molecules, proinflammatory cytokines, and hypoxia-related genes during acute hypoxia. Gene expression of E-selectin (A, B), vascular cell adhesion molecule 1 (VCAM-1) (C, D), and intercellular adhesion molecule 1 (ICAM-1) (E, F), the proinflammatory cytokines tumor necrosis factor-α (TNF-α) (G, H), interleukin-6 (IL-6) (I, J), and monocyte chemotactic protein-1 (MCP-1) (K, L), the hypoxia-related molecules hypoxia-inducible factor-1α (HIF-1α) (M, N), and vascular endothelial growth factor-A (VEGF-A) (O, P) in kidney (A, C, E, G, I, K, M, O) and lung (B, D, F, H, J, L, N, P). Gene expression was analyzed by quantitative real-time polymerase chain reaction using glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as housekeeping gene, after 120 min of acute hypoxia, during normoxia (21% oxygen), and hypoxia (6% oxygen). Data are expressed as mean ± SEM. * P < 0.05 compared with normoxic mice (21% oxygen). ND = not detectable.
Leiden University Medical Center, Leiden, The Netherlands), for his expertise in performing some of the animal experiments; Jack J. Lijtenberg, M.D., Ph.D., Intensivist (Department of Critical Care, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands), for fruitful discussions and critical reading of the manuscript; and Douglas J. Eleved, Ph.D., Researcher (Department of Anesthesiology, University Medical Center Groningen), for statistical advice.

References


Endothelial Activation in Hemorrhagic Shock