PROTECTIVE EFFECTS OF THE COMPLEMENT INHIBITOR COMPSATIN CP40 IN HEMORRHAGIC SHOCK

Martijn van Grijensven,† Daniel Ricklin,‡‡ Stephanie Denk,§ Rebecca Halbgabeauer,‖ Christian K. Braun,† Anke Schultze,† Felix Hönes,§ Sofia Koutsogiannaki,† Alexandra Primikyri,† Edimara Reis, David Messerer,§ Sebastian Hafner,† Peter Radermacher,§ Ali-Reza Biglarnia, † Joel V. Tuplano,,” Benjamin Mayer,†† Kristina Nilsson,‡‡ Bo Nilsson,†† John D. Lambris,† and Markus Huber-Lang§

† Experimental Trauma Surgery, Department of Trauma Surgery, Klinikum rechts der Isar, Technical University of Munich, Munich, Germany; ‡ Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, Pennsylvania; † Department of Pharmaceutical Sciences, University of Basel, Basel, Switzerland; ‡ Institute of Clinical and Experimental Trauma-Immunology, University of Ulm, Ulm, Germany; ‖ Institute for Anaesthesiological Pathophysiology and Process Development, University of Ulm, Ulm, Germany; § Department of Transplantation, Malmö University Hospital, Lund University, Lund, Sweden; † Institute of Epidemiology and Medical Biometry, University of Ulm, Ulm, Germany; and †† Department of Immunology, Genetics and Pathology, Uppsala University, Uppsala, Sweden

ABSTRACT—Trauma-induced hemorrhagic shock (HS) plays a decisive role in the development of immune, coagulation, and organ dysfunction often resulting in a poor clinical outcome. Imbalanced complement activation is intricately associated with the molecular danger response and organ damage after HS. Thus, inhibition of the central complement component C3 as turnstile of both inflammation and coagulation is hypothesized as a rational strategy to improve the clinical course after HS. Applying intensive care conditions, anaeathetized, monitored, and protectively ventilated nonhuman primates (NHP; cynomolgus monkeys) received a pressure-controlled severe HS (60 min at mean arterial pressure 30 mmHg) with subsequent volume resuscitation. Thirty minutes after HS, animals were randomly treated with either an analog of the C3 inhibitor compstatin (i.e., Cp40) in saline (n = 4) or with saline alone (n = 4). The observation period lasted 300 min after induction of HS. We observed improved kidney function in compstatin Cp40-treated animals after HS as determined by improved urine output, reduced damage markers and a tendency of less histopathological signs of acute kidney injury. Sham-treated animals revealed classical signs of mucosal edema, especially in the ileum and colon reflected by worsened microscopic intestinal injury scores. In contrast, Cp40-treated HS animals exhibited less histopathological signs of acute kidney injury. Furthermore, early systemic inflammation and coagulation dysfunction were both ameliorated by Cp40. The data suggest that therapeutic inhibition of C3 is capable to significantly improve immune, coagulation, and organ function and to preserve organ-barrier integrity early after traumatic HS. C3-targeted complement inhibition may therefore reflect a promising therapeutic strategy in fighting fatal consequences of HS.

KEYWORDS—Complement, hemorrhagic shock, inflammation, intestine, kidney, nonhuman primate

INTRODUCTION

Hemorrhagic shock (HS) is a major pathophysiological driver of systemic inflammation, organ barrier break down,

Copyright © 2018 by the Shock Society. Unauthorized reproduction of this article is prohibited.
additional, and highly potent, inflammatory drivers, thus possibly replacing one harmful product with another. Consequently, specific complement inhibitory strategies are needed. In experimental murine HS, the absence of C3 reduced signs of HS-induced hepatic injury and systemic inflammation (10). In rat HS, C3 depletion by cobra venom factor (CVF) resulted in improved hemodynamic parameters postresuscitation (11) and vascular reactivity to norepinephrine (12). In line with these observations, blockade of C3 converts by decay accelerating factor (DAF) in a porcine HS model seemed to significantly reduce fluid requirements, intestinal, lung, and kidney damage, and of note, also early mortality (13).

Despite broad experimental evidence corroborating beneficial effects of targeted complement intervention after traumatic HS, no valid preclinical study to investigate the early effects of specific C3 blockade during HS has so far been conducted. For an effective and translational complement inhibition at the level of C3, which marks the point of convergence of all complement activation pathways and major mediator of effector and cross talk functions, the C3 inhibitor compstatin and its analogs have emerged as promising candidate. The compstatin analog Cp40, a cyclic, nonimmunogenic peptide of 14 amino acids with strong and exclusive affinity for human and NHP C3 (14), has shown strong efficacy both in NHP models of disease, e.g., in the setting of sepsis, hemodialysis, or periodontal disease (15, 16). Moreover, several compstatin derivatives are currently in clinical development and evaluation for the treatment of various diseases.

Therefore, we hypothesized that effective C3 blockade by compstatin Cp40 in a resuscitated NHP model of HS may lead to early organ protection and improved clinical outcome.

MATERIALS AND METHODS

Compstatin Cp40

The Cp40 analog of the peptidic C3 inhibitor compstatin with the sequence dTyr-Ile-[Cys Valent (Me)Tyr-Gln-Arg-Ile]-mille (with brackets marking the disulfide-bridged cycle; Sar, sarcosine; mille, N-methyl isoleucine) was produced by solid-phase peptide synthesis as described before (14). The compound was assayed a purity of more than 98.9% and was tested negative for endotoxins. For all experiments, lyophilized Cp40 was dissolved in saline shortly before administration.

Experimental design

Eight male cynomolgus monkeys (Macaca fascicularis) weighing 4.8 ± 0.4 kg, aged 4 to 5 years, were divided into two groups (treatment and vehicle control). The study was approved by the IACUC of the Simian Conservation Breeding and Research Center in Makati City, Philippines (Study SIC-120 approval 2013-02). All experiments were performed under the conditions described in the Guide for the Care and Use of Laboratory Animals as defined by the National Institutes of Health and after the ARRIVE guidelines (see http://links.lww.com/SHK/A705). The animals were quarantined for 3 months before the study. They were fasted overnight before start of the experiment, but had access to water ad libitum. Housing was performed at 26±4°C with a relative humidity of 75 ± 25% (natural humidity) and a 12-h light–dark cycle.

Anesthesia was induced by intramuscular injection of 10 mg/kg BW ketamine and 1.0 mg/kg BW Xylazine. The animals were intubated and ventilated in a volume-controlled mode using a tidal volume of 6 mL/kg. I:E = 1:2. PEEP = 5 cmH2O, and a respiratory rate of 18 to 20 breaths/min (Servo 900B, Maquet) to maintain an arterial partial pressure of CO2 at 35 to 45 mmHg. The fraction of inspired oxygen was set at 0.30 and adjusted, if necessary, according to blood gas analyses. Body temperature was kept at 37°C.

Catheters were placed in both right and left femoral arteries: one for blood pressure and heart rate measurements and one for blood withdrawal. The left femoral vein was cannulated for blood withdrawal. A central arterial catheter (Pulsion Medical Systems, Feldkirchen, Germany) was placed in the right femoral artery. Finally, a urinary catheter was inserted through the urethra. After 15-min stabilization time (Fig. 1A), HS was induced by constantly withdrawing blood from one femoral artery. This was done until a mean arterial pressure (MAP) of 30 mmHg or until 45% of the calculated total blood volume was withdrawn. The MAP of 30 mmHg was maintained for 1 h (Fig. 1A). If necessary, more blood was withdrawn within this 1-h timeframe, unless the threshold of 45% total blood volume had been reached. After 1 h of HS, the animals were resuscitated with 4 times the withdrawn blood volume as Ringer’s lactated solution within 30 min (Fig. 1A). Thereafter, intravenous infusion of ringer’s lactated solution was maintained at 10 mL/kg/h. If the MAP fell below 60 mmHg, norepinephrine was infused. If blood sugar levels were below 80 mg/dL, Ringer’s solution was replaced by D5-Ringer’s lactated solution until a glucose level between 80 and 120 mg/dL was reached.

To mimic a first therapeutic window of opportunity, e.g., in the emergency room, 30 min after induction of HS, treatment was started with either Cp40 or vehicle (Fig. 1A). An initial bolus of 5 mL 0.9% NaCl, with addition of 3 mg/kg Cp40 in the treatment group, was given. Subsequently, a continuous infusion of 4 μg/kg/min Cp40 in 0.9% NaCl was administered via an infusion pump. A total volume of 50 mL was administered until the end of the experiment, i.e., 5 h after induction of HS. As well, the same volume of 0.9% NaCl without Cp40 was infused in the same time.

Blood pressure, heart rate, international normalized ratio (INR), and activated partial thromboplastin time (aPTT) were measured every 30 min. Rotational thromboelastometry (ROTEM; TEM GmbH, Munich, Germany) was performed only at baseline and after resuscitation due to limited instrument capacity. Blood gases, lactate, glucose, electrolytes, interleukin (IL)-6, macrophage migration inhibitory factor (MIF), urine output, serum creatinine, urine neutralophil gelatinase-associated lipocalin (NGAL), sodium, and intestinal fatty acid-binding protein (iFABP) were measured every 60 min (Fig. 1A). At the end of observation period, anesthetized animals were euthanized by intracardiac injection of 150 mg/kg BW KCl. Post mortem, the small intestine and kidneys were collected for further microscopic analysis.

Inflammation

Blood was collected and centrifuged at 2,000 x g for 15 min at 4°C. Serum samples were stored at −80°C until batch sample analysis for IL-6, MIF, macrophage inflammatory protein (MIP)-1a, and iFABP concentrations using the corresponding Quantikine ELISA kits (R&D systems) according to manufacturer’s instructions. Monkey multiplex cytokine serum analyses were performed in accordance to the manufacturer’s protocol (Life Technology, Frederick, Md).

Coagulation

Citrated blood was used for the coagulation tests. Prothrombin time (PT) and aPTT were measured using standard clinical laboratory equipment. The INR was calculated as the PT ratio from the test sample to a control sample. ROTEM analysis was performed immediately after sample collection. For each animal of the treatment or vehicle control groups, two cups (EXTEM and FIBTEM) were used. EXTEM is an extrinsically activated assay using recombinant tissue factor, and FIBTEM is an extrinsically activated test using recombinant tissue factor with cytochalasin D, which blocks the platelet skeleton and thereby inhibits platelet function. Thus, this test provides information on the fibrin component of the clot. Maximum clot firmness (MCF [mm]); the peak strength of the clot, resulting from the interaction of fibrin, activated platelets and factor XIII (FXIII) was measured. The platelet component of clot strength was calculated by subtracting FIBTEM MCF from EXTEM MCF (MCFplatelet = MCF EXTEM - MCF FIBTEM).

Kidney function

Urine output was determined every 60 min (Fig. 1A) and NGAL was determined by ELISA (R&D Systems). Serum creatinine was measured using the Jaffe reaction. Serum sodium and potassium were obtained from the blood gas measurements.

Histology

Kidney and small intestinal tissue samples were fixed in 3.7% formaldehyde (Fishar) and embedded in paraffin. Four-micrometer paraffin sections were cut and subsequently stained with Gill’s hematoxylin and eosin (H&E)
(Morphisto). Slides were visualized using a Zeiss Axio Imager A1 microscope. The lens was a 10X objective and the fields evaluated were 3,150,000 μm².

Sections of small intestine were scored as described by Cho et al. (17) in a blinded fashion. At least 80 villi per section were analyzed and means were calculated for each animal. Goblet Cells (GCs) along the epithelial lining of villi were counted and length of analyzed section of epithelium was measured with Axio Vision Software (Edition 4.9; Zeiss, Germany). The number of GCs was divided by the length of epithelium in micrometer and is depicted as fraction ratio. In addition, an immunohistochemical analysis was performed on small intestine tissue sections staining for cleaved caspase-3 (Cell Signaling Technology, Germany).

In kidney sections, 30 glomeruli per specimen were analyzed for dilation of Bowman’s capsule, precipitations in the urinary space, and neutrophilic infiltration. Results are depicted as fraction of positive findings among all glomeruli evaluated.

Per sample, five fields of view (200× magnification) were scored for tubular injury and neutrophilic infiltration. Necrosis of epithelial cells and dilation of

Fig. 1. (A) Experimental design. B, blood drawing including blood gas analysis; b, blood gas analysis only; fem, femoralis; R/L, right/left; Resusc., resuscitation; RL, Ringer’s lactate; Stab, stabilization phase; U, urine sampling. After induction of hemorrhagic shock, animals received either the compstatin analogue Cp40 or vehicle. Hemodynamic monitoring during the experimental period: (B) intra-arterial blood pressure measurements, (C) heart rate, (D) volume of the total blood drawn for induction of the hemorrhagic shock, and (E) blood gas analysis: paO₂. Mean ± SEM; n = 4/group.
tubules were graded as follows: findings in less than 5%, findings in 5% to 25%, findings in 25% to 50%, findings in 50% to 75%, and findings in more than 75% of respective tubules. Neutrophilic infiltration was graded as follows: no infiltration present, single focus of marked infiltration, and multiple foci and/or widespread infiltration.

Statistical analysis

For statistical analysis, SigmaStat version 3.5 (Systat Software, Inc.) was used. All values were expressed as means ± SEM. Data were assumed to be normally distributed and were therefore analyzed by one-way ANOVA followed by Student–Newman Keuls post hoc testing. To analyze differences between Cp40 treatment and vehicle in a kinetic way, two-way ANOVA with Sidak correction for multiple comparisons was performed. Differences were always considered significant when $P \leq 0.05$.

RESULTS

Physiology

HS could be successfully induced in all animals. In none of the animals, the maximum amount of 45% total blood volume was needed to be withdrawn. There was no difference between the two groups concerning withdrawn blood volume (Fig. 1D). All animals reached an MAP of 30 mmHg within 10 min, which was stable until the beginning of the resuscitation (Fig. 1B). Concomitantly, the heart rate increased to 150 bpm (Fig. 1C) indicating HS status. This was confirmed by low base excess, low hemoglobin concentration, and low hematocrit (data not shown). Thus, at the beginning of administration of Cp40 or vehicle, the animals were similar in their physiological status. Treatment with Cp40 did not result in differences in blood pressure over the entire observation time when compared with the vehicle control group. Upon resuscitation, MAP returned to normal levels, and no differences between the two groups could be observed (Fig. 1B). Significantly different kinetics ($P < 0.0001$) were, however, measured for heart frequency. Animals receiving Cp40 treatment returned to normal frequency within 60 min (Fig. 1C). In contrast, heart rates remained high until the end of the experiment in the vehicle-treated animals (Fig. 1C). Interestingly, both the hemoglobin and hematocrit values were significantly higher during the last 2 h of the experiment when animals were treated with Cp40. Of note, their values were almost in the lower normal range (data not shown). $p_{O_2}$ values stayed normal with Cp40 treatment, whereas they decreased at the end of the observation period in the vehicle-treated animals (Fig. 1E). Finally, the kinetics of $p_{O_2}$ values was significantly different between the Cp40- and vehicle-treated animals ($P = 0.0086$).

Inflammation

The inflammatory markers IL-6 and MIF showed a continuous and significant increase until the end of the experiment in vehicle-treated animals. Although Cp40-treated animals also had an increase in these marker, the change was much more moderate. During the last 2 h of the observation period, inflammatory marker concentrations were significantly higher in vehicle control animals when compared with Cp40-treated HS animals (Fig. 2, A and B). A similar pattern was found by trend for other key inflammatory mediators such as IL-1 receptor antagonist (IL-1RA), regulated on activation normal T cell expressed and secreted (RANTES), macrophage inflammatory protein-1α (MIP-1α), monocyte chemoattractant protein-1 (MCP-1), and interferon γ (IFNγ) (Supplementary Fig. 1, http://links.lww.com/SHK/A706).

Coagulation

INR showed a slight increase of 0.3 in both groups until 60 min after beginning of resuscitation (Fig. 2C), which can be attributed to HS. Interestingly, Cp40-treated animals maintained an INR of around 1.4, whereas vehicle treatment led to a further increase of the INR by 0.43 ± 0.03 until the end of the experiment (Fig. 2C). A similar, though less pronounced, difference of kinetics between both groups was seen for the aPTT measurements (Fig. 2D). ROTEM analysis for MCF in both EXTEM and FIBTEM indicated some reduction after resuscitation (Fig. 2, E and F), with no differences observed between the two experimental groups.

Kidney

Cp40 treatment protected the kidneys from damage as seen in the vehicle-treated animals (Fig. 3). Urine output kinetics were significantly different when comparing Cp40 with vehicle treatment ($P = 0.0009$). The urine output of animals receiving Cp40 remained significantly improved ($P < 0.0001$) over vehicle-treated animals, even after 240 min ($P < 0.01$; Fig. 3A). In fact, vehicle-treated animals hardly produced any urine from this time point on. In contrast, no significant differences could be detected for creatinine concentrations in serum (Fig. 3B). They remained within the normal range in both groups. NGAL concentrations in urine displayed similar kinetics in both groups with a trend of lower concentrations in the presence of Cp40 (Fig. 3C). Concentrations increased until 2 h after induction of HS and remained stable until 180 min. Thereafter, concentrations decreased below baseline values until the end of the experiment (Fig. 3C).

Serum sodium concentrations initially dropped in both groups during HS until the beginning of resuscitation (Fig. 3D). From that time on, concentrations in Cp40-treated animals returned to baseline levels, whereas serum sodium stayed at minimum levels in vehicle control animals until the end of HS (Fig. 3D). The kinetics between the two groups were significantly different ($P < 0.01$). Moreover, from 240 min onward, the single sodium concentrations were significantly different between Cp40- and vehicle-treated animals ($P < 0.05$). Therefore, it is likely that Cp40 treatment reversed the impaired sodium retention in the kidneys as observed in the vehicle-treated animals. Potassium levels did not differ between groups and time and were in mean 4.2 ± 0.1 mmol/L.

Histopathologic features observed in the kidney are presented by Figure 3E. Overall, kidney histology of Cp40-treated animals revealed a weak amelioration of the significant morphological alterations induced by HS in vehicle-treated animals, such as dilation of the glomerular capsule and signs of beginning protein casts (Fig. 3), which were confirmed to contain albumin using immunohistochemistry (data not shown). Histological assessment of HE- and PAS-stained sections revealed a loss of tubular brush borders after HS, which was ameliorated although statistically not significant upon Cp40 treatment (Table 1).
Small intestine

Cp40 could also reduce pathological changes in the small intestine in comparison to vehicle treatment (Fig. 4). This was already macroscopically observed during the autopsy. Almost no intestinal edema nor petechial bleedings were detected when the animals had been treated with Cp40 (Fig. 4A). Animals without Cp40 presented massive swelling of the small intestine with loss of macroscopic discernibility of villi (Fig. 4A), and their tissue did show petechial bleedings.

The GC ratio (number of GCs divided by length of intestinal epithelium) was 0.6 ± 0.004 in sham animals and decreased to 0.3 ± 0.01 after HS. Upon Cp40 treatment, the GC ratio slightly but insignificantly rose to 0.4 ± 0.01 (data not displayed). Villi damage was confirmed in histological samples of vehicle-treated animals, whereas Cp40 treatment resulted in

Copyright © 2018 by the Shock Society. Unauthorized reproduction of this article is prohibited.
preservation of the villi structures including the crypts (Fig. 4B). This was confirmed by a worsened intestinal damage score (Chiu) in vehicle control animals after HS, indicating significant small intestinal damage (Fig. 4C). In contrast, the Chiu score was not significantly different in Cp40-treated HS animals when compared with sham animals (Fig. 4C). Furthermore, the Cp40 treatment reduced by trend immunohistochemical signs of apoptotic events within the small intestine (Supplementary Fig. 2, http://links.lww.com/SHK/A707).

These observations were biochemically confirmed by measuring iFABP levels (Fig. 4D), which maintained normal (close to the detection limit) in presence of Cp40 treatment. A continuous and significant increase was determined in vehicle-treated animals until the end of the observation period (Fig. 4D). Furthermore, MIP1α concentrations in peritoneal fluid after HS was significantly decreased in animals treated with Cp40 (Fig. 4E).

**DISCUSSION**

HS and the resulting early onset of complications remain a scientific and clinical challenge. Despite the emergence of
Various resuscitation protocols, a mechanistically rational therapy remains challenging. To address HS-induced early immune, coagulation, and organ dysfunction, a specific therapeutic strategy targeting central complement components could be promising. In this study, we therefore applied the compstatin analog Cp40 during HS to effectively and specifically block C3 as a hub for complement and coagulation activation, danger molecule and pathogen clearance, and microbial immune evasion (15, 18). Of note, both animal and human phase I trial data have demonstrated that Cp40 is safe for inhibiting complement (reviewed in (19)). HS was induced in NHP in a pressure-controlled manner, and Cp40 was applied after the acute shock phase with a therapeutic delay of 30 min that mimics the clinical situation (Fig. 1A). It is noteworthy that a 30-min delay of intervention does by far not cover all trauma patients, but certainly could be acutely applied in many cases at the scene or in the hospital in cases of acute bleeding such as upper gastrointestinal bleeding. Furthermore, it could be argued that the positive effects of Cp40 may be due to a slightly (although not significant) smaller blood volume withdrawn. However, despite this by tendency smaller withdrawal of blood, the MAP in the Cp40 treated group was lower during the HS period compared with control, suggesting that the Cp40 effects observed postsuscitation on the organ functions are highly relevant. In this therapeutic setting of a resuscitated HS, we observed a marked effect of complement-targeted therapy on several outcome parameters. Concerning hemodynamics, the mean MAP course was rather superimposable in the control and Cp40 group, whereas the heart rate returned to normal values after the shock phase in the presence of Cp40, but remained pronounced, although not significantly, at each time point in the Cp40 group. In accordance, earlier studies of renal ischemia/reperfusion injury in pigs showed that creatinine levels only

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Ctrl Mean ± SEM</th>
<th>HS + NaCl Mean ± SEM</th>
<th>HS + CP40 Mean ± SEM</th>
<th>Significance NaCl vs. CP40</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glomeruli</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dilation</td>
<td>0.11 ± 0.06</td>
<td>0.73 ± 0.03*</td>
<td>0.65 ± 0.07*</td>
<td>n.s.</td>
</tr>
<tr>
<td>Precipitation</td>
<td>0.11 ± 0.08</td>
<td>0.16 ± 0.10</td>
<td>0.05 ± 0.03</td>
<td>n.s.</td>
</tr>
<tr>
<td>Infiltration</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>n.s.</td>
</tr>
<tr>
<td>Proximal tubulus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apoptosis</td>
<td>1.07 ± 0.07</td>
<td>1.40 ± 0.20</td>
<td>1.15 ± 0.05</td>
<td>n.s.</td>
</tr>
<tr>
<td>Dilation</td>
<td>1.53 ± 0.24</td>
<td>3.73 ± 0.37*</td>
<td>3.75 ± 0.38*</td>
<td>n.s.</td>
</tr>
<tr>
<td>Distal tubulus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apoptosis</td>
<td>1.07 ± 0.07</td>
<td>1.13 ± 0.07</td>
<td>1.20 ± 0.12</td>
<td>n.s.</td>
</tr>
<tr>
<td>Dilation</td>
<td>1.13 ± 0.07</td>
<td>2.07 ± 0.18*</td>
<td>2.40 ± 0.29*</td>
<td>n.s.</td>
</tr>
<tr>
<td>Neutrophil infiltration</td>
<td>1.00 ± 0.00</td>
<td>1.07 ± 0.07</td>
<td>1.10 ± 0.10</td>
<td>n.s.</td>
</tr>
<tr>
<td>Brush border loss</td>
<td>1.20 ± 0.2</td>
<td>4.00 ± 0.33*</td>
<td>3.40 ± 1.35*</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

*P < 0.05 vs. Ctrl (ANOVA, post hoc SNK test).
rose beyond day 1 and were to some extent less pronounced when the classical pathway was inhibited by a C1q inhibitor (23). Similar findings were published for creatinine levels in nonhuman primates with *E. coli*-induced sepsis (16). In experimental HS and synchronic LPS challenge, a deposition of C3 cleavage products was reported in the kidney, known as a frequent early failing organ (24). Concomitantly, after murine renal ischemia/reperfusion injury, C3b accumulated in the brush borders of the proximal tubules (25). Even though histological changes such as acute tubular necrosis or glomerular changes are hard to detect as early as 4 h after shock, some morphological alterations were evident. A tendency of improved early signs of morphological kidney alterations was noted in the Cp40 group, although an assessment of the development of full acute kidney injury after HS would require a longer observation period. The exact mechanism how Cp40...
improves kidney function is not yet known and needs to be addressed in future analyses.

Importantly, the gastrointestinal tract has been considered a critical engine of HS-related multiple organ failure. Not only protease release (26) but also epithelial damage and gut barrier failure with subsequent bacterial translocation have been proposed as pathophysiological drivers of HS toward multiple organ dysfunction syndrome (MODS). In the past, complement inhibition on the level of C5 activation revealed some protective effects on HS-induced intestinal damage (27). Recently, TLR2-dependent intestinal expression and deposition of C3 have been proposed as central pathomechanism for intestinal damage and systemic inflammatory response after HS (28). In the present study, blinded assessment by an independent pathologist found significant signs of mucosal edema in the control group, predominantly in the small intestine, which were completely abolished in the case of Cp40 treatment. The macroscopic findings were reflected by the histological (intestinal damage score) and biochemical data (iFABP in serum and inflammatory mediators in peritoneal fluids), indicating protection of HS-induced intestinal tissue damage by Cp40 (Fig. 4). Accordingly, in a porcine pressure-controlled model of HS, indirect C3 targeting by C1 inhibition (29) or by DAF (13) revealed a dose-dependent protection against HS-associated intestinal and pulmonary damage. These protective effects on the intestine and various other organs by C1 inhibition were still present when the pigs were objected to further multiple injuries in addition to HS (30). In rodent HS models, the soluble complement receptor 1 was capable to reduce small bowel injury and intestinal neutrophil influx (31), to enhance compromised mucosal blood flow, to prevent postresuscitation vasoconstriction and thereby gut ischemia, and to improve intestinal endothelial function (32). In this context, it had been proposed that HS may engage a vicious cycle that leads to activated complement-dependent bacterial translocation from the gut and results in subsequent endotoxemia (33). Notably, C3 targeting has been suggested to reduce endotoxin levels after HS by improving the gut-barrier pathway through a hitherto undescribed mechanism (34). As recently shown, intestinal ischemia/reperfusion injury in mice led to mucosal injury as early as 30 min after ischemia, which was associated with an increased appearance of C3, or C3 fragments, within intestinal epithelial cells (35). The reperfusion injury was significantly reduced in C3-deficient mice or by C3 depletion (35).

The study presented here has some intrinsic limitations that need to be considered. First, the number of animals per group was kept rather small. As a consequence, we have assumed normal distribution, and some of the observed effects might have been underestimated. Second, to allow for a rational use of local resources, the observation period was limited to 5 h after induction of HS and thus might have been too short for manifestation of organ failure, thereby not enabling an assessment of potential long-term effects of Cp40 therapy. Third, no dose-escalation protocol could be applied without significantly expanding the number of experimental animals (see above). However, we have used an established dose, based on previous dose findings studies, which could completely inhibit circulating C3 as confirmed by serum complement analyses (see above).

Overall, present data suggest that C3 inhibition by Cp40 may improve early organ dysfunction in a NHP model of HS and holds a promising translational potential for an improved clinical outcome in HS patients.

ACKNOWLEDGMENTS

The authors thank Sonja Braunmüller, Bettina Klohs, and Anne Rittlinger for excellent technical support.

REFERENCES


