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C1-Esterase-Inhibitor Treatment at Early Reperfusion of Hemorrhagic Shock Reduces Mesentery Leukocyte Adhesion and Rolling

GEORG HORSTICK,*† TIBOR KEMPF,† MICHAEL LAUTERBACH,† SUCHARIT BHAKDI,‡ LAZLO KOPACZ,† AXEL HEIMANN,† MICHAEL MALZAHN,† MARINA HORSTICK,*† JÜRGEN MEYER,* AND OLIVER KEMPSKI

*2nd Medical Clinic, †Institute for Neurosurgical Pathophysiology, and ‡Institute of Microbiology and Hygiene, Johannes Gutenberg-University Mainz, Germany

ABSTRACT

Objective: Complement activation probably plays a pathogenic role in multiple organ failure in shock. This study evaluates the effects of C1-esterase-inhibitor treatment on leukocyte-endothelial interaction in the mesenteric microcirculation in hemorrhagic shock.

Methods: Rats underwent median laparotomy and exteriorization of an ileal loop for intravital microscopy of the mesenteric microcirculation. Volume controlled hemorrhagic shock was provoked by arterial blood withdrawal (2.5 mL/100 g body wt. for 60 minutes) followed by a 4-hour reperfusion period. C1-INH (100 IU/kg body wt. i.v.) or 0.9% NaCl i.v. were administered as a bolus at the beginning of reperfusion. Reperfusion time mimicked a "pre-hospital" phase of 30 minutes followed by a quasi "in-hospital" phase of 3.5 hours. The "in-hospital" phase was initiated by substitution of blood followed by fluid resuscitation with normal saline.

Results: Application of C1-INH markedly reduced rolling and adherent leukocytes to numbers approaching baseline values. Vmax and shear rate of the mesenteric microcirculation improved in both groups after reperfusion with a trend to higher values in the C1-INH group (n.s. $p = 0.08$).

Conclusion: C1-INH applied in a bolus dose of 100 IU/kg body wt. i.v. abrogated enhanced leukocyte adhesion and rolling in the mesenteric microcirculation after hemorrhagic shock. Single bolus treatment with a complement inhibitor may provide clinical benefit when applied at an early stage of reperfusion during hemorrhagic shock. **Microcirculation (2001) 8, 427–433.**

KEY WORDS: complement inhibition, C1-esterase-inhibitor, leukocyte-endothelial interaction, mesentery microcirculation

INTRODUCTION

Following the concept that hemorrhagic shock initiates activation of the complement system and that this event probably plays an important role in the pathogenesis of multiple organ failure (37,39), several inhibition studies employing soluble CR1 have indicated that suppression of complement activation may indeed be beneficial for treatment of hemorrhagic shock (10,33).

This approach is very costly, however. A possible alternative is the application of C1-INH. The use of this inhibitor appears possible because a major path to complement activation in shock likely involves C-reactive-protein and, concomitantly, C1 (5). Application of C1-INH indeed appears to provide benefit in myocardial ischemia and reperfusion (4,17). C1-INH therapy has been reported in sepsis (13,21), liver ischemia (22), hemorrhagic pancreatitis (36), bone marrow transplantation (25), thermal trauma (27), cerebral infarction (15), and cardiopulmonary

For reprints of this article, contact Dr. Georg Horstick, 2nd Medical Clinic, Johannes Gutenberg-University Mainz, Langenbeckstrasse 1, 55131 Mainz, Germany; e-mail: horstick@mail.uni-mainz.de
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bypass operation (2,3,34). Here, we demonstrate a remarkable reduction of leukocyte-endothelial cell interactions in the rat mesentery under hemorrhagic shock by single-dose application of C1-INH.

MATERIALS AND METHODS

Experimental Conditions

16 male Sprague Dawley rats were maintained on standard rat chow and water *ad libitum* until the night before the experiment. Rat chow was removed 10 to 12 hours before commencement of the experiment to reduce bowel movements during intravital microscopy. After anaesthesia with urethane (1.25 g/kg i.m., single dose) the femoral artery and jugular vein were cannulated with a small PE-tube for arterial blood gas analysis, measurements of plasma protein and arterial and central venous blood pressure. For each sample 250 μL of blood was drawn into heparinized syringes. Arterial blood gases (PaO_2, sO_2, pCO_2, pH, BE), hemoglobin (Hb), hematocrit (Hct), lactate, potassium, and sodium were analyzed with Arterial Blood Gas Laboratory Radiometer Copenhagen 615. After median laparotomy, a Doppler flow-transducer was placed around the abdominal aorta. Hemodynamic data [abdominal blood flow (ABF), descending aorta], heart rate (HR), systolic, mean and diastolic arterial blood pressure (SAP, MAP, DAP), and central venous pressure (CVP) were recorded on a beat-to-beat basis using System-6 (Triton Technology, Inc., San Diego, CA, USA). Analog data were digitized and recorded online with a computer based system (DasyLab®, National Instruments Corporation, Austin, TX, USA). An ECG in lead II was recorded during the experiment to detect morphological ECG-changes (16). Rectal temperature was kept constant at 37.5 ± 0.5 °C by means of a feedback controlled homeothermic blanket control unit (Harvard, South Natick, MA, USA).

Animals were placed on a heating pad in a left lateral recumbent position on an adjustable plexiglas microscope stage. A segment of the ileum was exteriorized through the abdominal incision and prepared for *in vivo* microscopic observation as previously published (18). Rats received a basal infusion with albumin in physiological saline for compensation of intraoperative albumin loss and evaporative water loss (0.3 mL/h/100 g body wt.) after the CVP line was introduced as previously described (19). Animals were randomized before reperfusion to either treatment with C1-INH at a dose of 100 IU/kg body wt. (group 1, $n = 6$, body wt., 296 ± 5 gr) or normal saline i.v. (group 2, $n = 6$, body wt., 298 ± 15 gr) administered at the beginning of reperfusion after hemorrhagic shock of 60 minutes. Four animals died during the phase of systemic hypotension before reperfusion could be achieved.

Intravital microscopy was performed with epi- and transillumination observing 3 to 5 unbranched mesenteric venules (diameter 25–40 μm; length 100–150 μm) (Zeiss Axiotech fluorescence microscope with computer controlled scanning table, light source AttoArc HBO 100 W). For study of macromolecular leakage we injected fluorescein isothiocyanate (FITC)-labeled bovine albumin (50 mg/kg; Sigma Chemical Co., St. Louis, MO, USA) intravenously at the end of the experiment to avoid phototoxic side effects and scanned for further 30 minutes. The fluorescent intensity was measured within a defined area of the venule under study and in the adjacent perivascular interstitium. The vascular albumin leakage index was calculated from the ratio of: (perivascular intensity—background intensity)/(venular intensity—background intensity) ×100. Off-line analysis of coded videorecordings was performed in a blinded manner (18).

All investigative procedures and the animal facilities conformed with the *Guide of the Care and Use of Laboratory Animals* published by the US National Institutes of Health. The protocol was approved by the regional animal care and use committee.

Experimental Protocol

Following exteriorization and fixation of the mesentery, rats were allowed to stabilize for 30 minutes and mesentery microcirculation was observed for 5 hours of experimental time. Volume-controlled hemorrhagic shock was provoked during the first 10 minutes. 2.5 mL/kg body wt. of arterial blood was drawn from the femoral artery and transferred to citrate plasma tubes (citrate:blood = 1:10). After 60 minutes of systemic hypotension, reperfusion was initiated. The reperfusion phase was divided into a "pre-hospital" phase of 30 minutes and the "in-hospital" phase starting with administration of red blood cells (RBC) to compensate the initial blood loss (Fig. 1A). During the first 5 minutes of reperfusion, either C1-INH or normal saline was administered followed by normal saline infusion for 25 minutes until a mean arterial blood pressure of 70 mm Hg was reached. In all animals, fluid resuscitation was performed with normal saline i.v. First, 2 times the shed blood volume were infused for 30 minutes. Thereafter, the equivalent of 1 shed blood

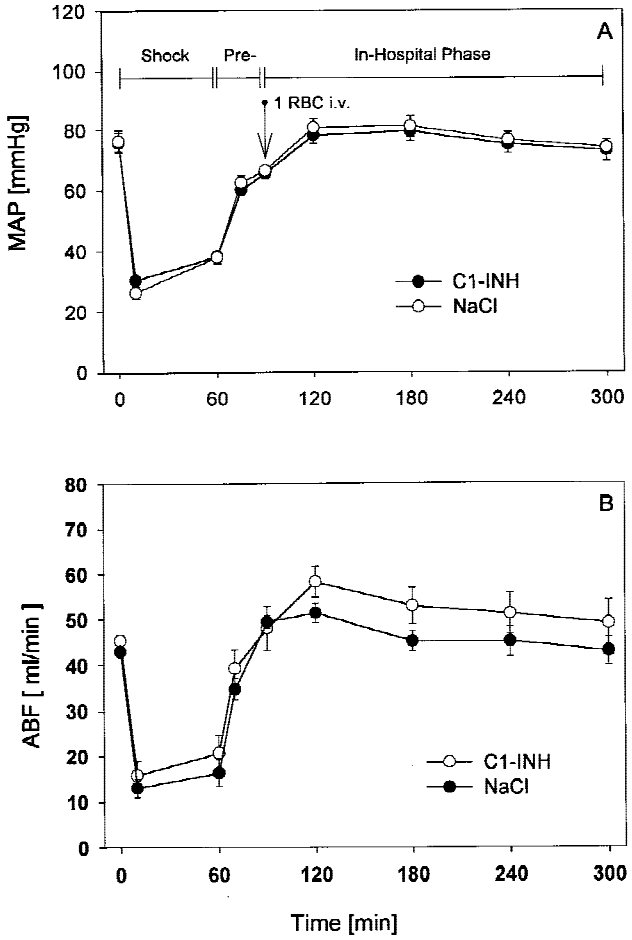

Figure 1. Mean arterial blood pressure (A, upper panel) and abdominal blood flow (B, lower panel).

volume was infused over 2 hours. Maintenance infusion with normal saline was continued throughout the experiment so that MAP did not drop below 70 mm Hg (Fig. 1A). After 5 hours we injected 50 mg/kg body wt. of FITC-labeled bovine albumin for detection of molecular leakage and concluded the experiment.

Statistical Analysis

Data are presented as mean ± SEM. Statistical analysis was performed with Sigma Stat® (Jandel Corporation, Erurath, Germany). Statistical significance of changes from baseline values within each group was tested with analysis of variance (ANOVA) for repeated measures. Differences between groups were statistically analyzed by one way ANOVA comparing several groups. If values did not show a normal distribution, ANOVA for nonparametric values (Kruskall-Wallis-Test) with the multiple comparison method (Student-Newman-Keuls-Test) was used. Statistical significance was accepted at an error probability of $p < 0.05$.

RESULTS

Hemodynamics

Mean Arterial Blood Pressure, Heart Rate, and Central Venous Pressure. Immediately following induction of hemorrhagic shock, MAP dropped to 40 mm Hg until 60 minutes of experimental time. Following reperfusion, MAP increased during the first 30 minutes and no differences were observed between the two experimental groups (Fig. 1A). HR at baseline was similar between groups at the beginning (C1-INH: 361 ± 8 bpm, NaCl: 348 ± 16 bpm) and increased significantly during systemic hypotention after 60 minutes (C1-INH: 394 ± 17 bpm, NaCl: 393 ± 12 bpm, $p < 0.05$) and stayed on a plateau until the end (C1-INH: 421 ± 15 bpm, NaCl: 409 ± 14 bpm). The CVP did not differ between both groups and was within normal ranges.

Abdominal Blood Flow. The ABF measured in the descending aorta is depicted in Fig. 1B. There were no differences between the two groups at any time during the experiment. Baseline values were 43.0 ± 1.2 mL/min in the NaCl group and 45.3 ± 1.3 mL/min in the C1-INH group. After 60 minutes of hemorrhagic shock, ABF decreased in both groups (C1-INH: 20.6 ± 4.1 mL/min, NaCl: 16 ± 2.9 mL/min). ABF increased after fluid resuscitation at 90 minutes (C1-INH: 48.1 ± 4.9 mL/min, NaCl: 49.4 ± 1.4 mL/min) and remained on a plateau until the end of the experiments (C1-INH: 49.2 ± 5.0 mL/min, NaCl: 43.1 ± 3.1 mL/min).

Arterial Blood Gas Values

PaO_2 values of both groups did not differ at the beginning of the experiment (C1-INH: 76 ± 2 mm Hg, NaCl: 74 ± 2 mm Hg) and did not significantly change throughout the experiments (C1-INH: 87 ± 2 mm Hg, NaCl: 88 ± 2 mm Hg). Analogous values were obtained for SaO_2. Arterial paCO2 of all groups were similar at baseline (C1-INH: 42.8 ± 0.7 mm Hg, NaCl: 41.2 ± 1.2 mm Hg) and did not differ between groups throughout the experiments (C1-INH: 34.2 ± 1.3 mm Hg, NaCl: 33.9 ± 1.0 mm Hg). The decrease in paCO2 during the experiments was significant in both groups ($p < 0.05$).

Base-excess values mirrored the paCO2 data and decreased in both groups significantly over time, without differences becoming apparent between the two groups (Fig. 2A). pH-values were similar between the two groups (Beginning: C1-INH: 7.36 ± 0.01,

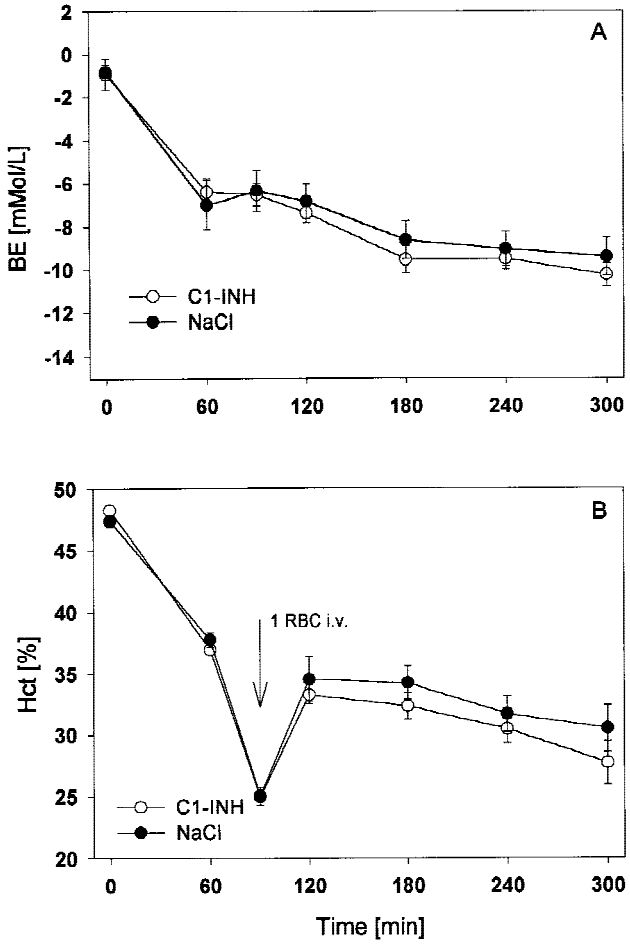

Figure 2. Arterial base excess (A, upper panel) and hematocrit (B, lower panel).

NaCl: 7.37 ± 0.01; end:C1-INH:7.26 ± 0.01, NaCl: 7.29 ± 0.01, n.s. between groups).

Hematocrit values were equal at baseline (C1-INH: 48 ± 0.3 vol%, NaCl: 47 ± 0.5 vol%) and decreased significantly 60 minutes after induction of hemorrhagic shock with a further decrease after the first 30 minutes of fluid resuscitation (Fig. 2B; C1-INH: 25 ± 0.2 vol%, NaCl: 25 ± 0.7 vol%). After the "prehospital" phase with blood transfusion, the hematocrit returned to ≥30 vol% in both groups.

Microcirculation

Rolling and Adherent Leukocytes. Rhodamine-labeled leukocytes were counted using epiillumination. Cell counts were normalized to the endothelial surface assuming cylindrical geometry. Numbers of rolling and adherent leukocytes showed no significant differences between groups at the beginning of the experiment. A marked increase of rolling leukocytes was registered with NaCl, which remained detectable over the next 4 hours of observation. The numbers of rolling leukocytes approximately doubled baseline values in this group. In contrast, the numbers of rolling leukocytes did initially increase in the C1-INH group during the first 30 minutes of reperfusion, but then dropped significantly, returning to baseline values after 2 hours (Fig. 3A, $p < 0.05$). Parallel findings were made with regard to adherent leukocytes. While the number of these cells markedly increased in the NaCl group, they remained essentially at baseline levels in the C1-INH group (Fig. 3B, $p < 0.05$).

Velocity, Shear Rate and Vascular Albumin Leakage. Measured venular diameters did not differ significantly during the experiment. Maximum velocity and shear rate were similar in both groups with a trend towards higher values in the C1-INH group at the end of the experiment (Fig. 4A and B, n.s. $p = 0.08$). During systemic hypotension, blood flow in

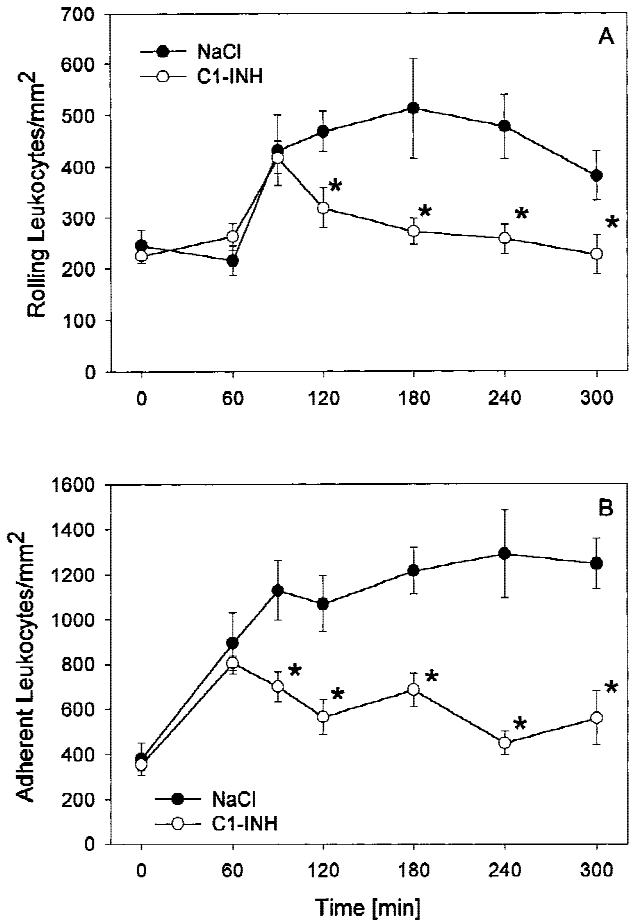

Figure 3. Rolling (A, upper panel) adherent leukocytes (B, lower panel): Data for rolling and adherent leukocytes presented significantly lower values in the C1-INH group versus the vehicle treated group (*$p < 0.05$).

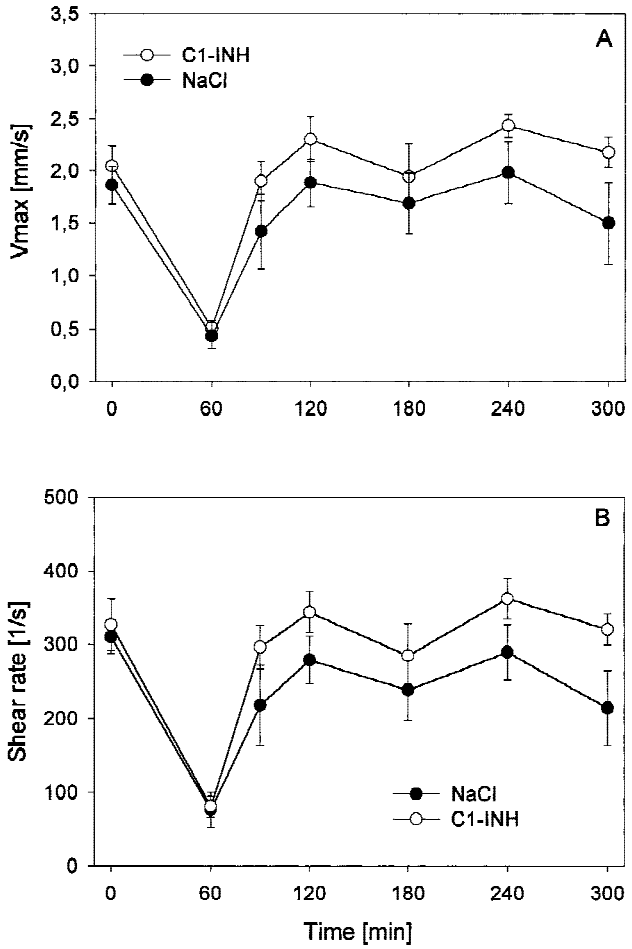

Figure 4. Maximum velocity (A, upper panel) and shear rate (B, lower panel).

mesenteric venules remained at a severe low-flow situation without complete interruption of microvascular perfusion (Fig. 4A: C1-INH: 0.5 ± 0.07 mm/s, NaCl: 0.44 ± 0.1 mm/s). No relevant capillary leakage could be detected after injection of FITC-labeled albumin. Volume substitution was not different between groups.

DISCUSSION

Two main questions were addressed in this study. First, does application of C1-INH reduce the local inflammatory response in the mesentery microcirculation, as reflected by leukocyte-endothelium interaction? Second, does C1-INH application have any side effects when applied at a bolus dose of 100 IU/kg body wt. i.v. in hemorrhagic shock? The results obtained raise the possibility that early single-shot reperfusion therapy with C1-INH at the beginning of fluid resuscitation for hemorrhagic shock could represent a simple and safe measure to attenuate the local mesenteric inflammatory response syndrome.

Fluid resuscitation after hemorrhagic shock is accompanied by complement activation, with possible deleterious consequences in the mesenteric microcirculation (33,37). Previous studies with experimental myocardial ischemia have revealed that C1-INH administration at reperfusion effectively abrogates local complement activation, and this is accompanied by marked beneficial effects in the ischemic tissue (17). In the present study, leukocyte-endothelial cell interactions were strikingly reduced in the reperfused mesentery (Fig. 3A and B). Enhanced leukocyte-endothelium cell-cell interaction is a reflection of a local inflammatory response syndrome and parallels contact activation and cytokine release (6,9,11,30). The number of rolling and adherent leukocytes was almost completely decreased to baseline values, indicating a very pronounced anti-inflammatory effect. C1-INH suppresses C1, which may be activated via C-reactive-protein (CRP) during shock (8,32,38). Additionally, C1-INH inhibits factor XII, factor XI, and kallikrein (26,28,29,35). C1-INH treatment in endotoxin-induced shock and hemorrhagic pancreatitis has been shown to be effective particularly in combination with antithrombin III administration (30,36). C1-INH reduces release of C3a, C4a, and C5a anaphylatoxins that mediate several inflammatory reactions including neutrophil chemotaxis (12,14,20,24) and production of cytokines (7,23,31). These properties together may account for the marked beneficial effects of C1-INH in blunting the systemic inflammatory response.

Correct dosage is pivotal to success, however. Previous clinical studies with C1-INH treatment (300 to 500 IU/kg body wt. i.v.) aimed at preventing capillary leakage syndrome resulted in great vein thrombosis (1,34). In contrast, C1-INH applied at a dose of 100 IU/kg i.v. in our experimental model presented no adverse effects on global or local hemodynamics.

The mortality rate during systemic hypotension under hemorrhagic shock was about 25% to 30% in our experiments. All other animals survived until the end of the experiment. The initial C1-INH bolus was followed by an intensified fluid resuscitation strategy with severe hemodilution mirrored by the hematocrit values (Fig. 2B). Therefore, activated coagulation factors might have been diluted by fluid administration.

Similar results were obtained in a study of liver ischemia and reperfusion using 100 IU/kg C1-INH

(22). Liver microcirculation improved significantly in contrast to our study at reperfusion. The basic difference between both studies is the complete "no-flow" ischemia in contrast to the "low-flow" situation under hemorrhagic shock (Figs. 1B and 4A).

In conclusion, C1-esterase inhibitor at a single dose of 100 IU/kg body wt. i.v. effectively inhibited increased leukocyte rolling and adhesion in the mesenteric microcirculation in experimental hemorrhagic shock. Bolus therapy with a complement inhibitor at early reperfusion may emerge as a beneficial measure for treatment of hemorrhagic shock in the clinical setting.

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