C1-ESTERASE INHIBITOR REVERSES FUNCTIONAL CONSEQUENCES OF SUPERIOR MESENTERIC ARTERY ISCHEMIA/REPERFUSION BY LIMITING REPERFUSION INJURY AND RESTORING MICROCIRCULATORY PERFUSION

Michael Lauterbach,*[†] Georg Horstick,*[†] Nicola Plum,* Johannes Lotz,[‡] Enise Lauterbach,[†] Ludwig S. Weilemann,[†] and Oliver Kempski*

*Institute for Neurosurgical Pathophysiology, [†]2nd Medical Clinic, and [‡]Institute of Clinical Chemistry and Laboratory Medicine, Johannes Gutenberg—University Mainz, Germany

ABSTRACT—Activated complement contributes significantly to reperfusion injury after ischemia. This study explores functional consequences of C1-esterase inhibitor (C1-INH) treatment after superior mesenteric artery occlusion (SMAO)/reperfusion using intravital microscopy. Thirty anesthetized, spontaneously breathing, male Sprague-Dawley rats underwent SMAO for 60 min followed by reperfusion (4 h). C1-esterase inhibitor (100 and 200 IU/kg body weight) or saline (0.9%) was given as a single bolus before reperfusion. Sham-operated animals (n = 10) without SMAO served as controls. Systemic hemodynamics were monitored continuously, arterial blood gases analyzed intermittently, and leukocyte/endothelial interactions in the mesenteric microcirculation quantified at intervals using intravital microscopy. Ileal lipid-binding protein (I-LBP) levels were determined from serum samples with an enzyme-linked immunosorbent assay at the end of the experiments. C1-esterase inhibitor restored microcirculatory perfusion to baseline levels in a dose-dependent manner and reduced adherent leukocytes after SMAO/reperfusion to similar levels in both C1-INH–treated groups during reperfusion. Furthermore, C1-INH treatment efficiently prevented metabolic acidosis, reduced the need for intravenous fluids to support blood pressure, and decreased I-LBP levels in a dose-dependent manner. Survival rates were 100% in controls and after 200 IU/kg C1-INH, 90% after 100 IU/kg C1-INH, and 30% in saline-treated animals. C1-esterase inhibitor bolus infusion efficiently blunted functional consequences of mesenteric ischemia/reperfusion with I-LBP, proving to be a valuable serum marker mirroring the effect of ischemia/reperfusion and treatment at the end of the experiments.

KEYWORDS—C1-esterase inhibitor, superior mesenteric artery, ischemia, bolus treatment, ileal lipid-binding protein, fatty acid-binding protein, intravital microscopy

INTRODUCTION

Mesenteric ischemia is a life-threatening condition associated with high mortality (50%–70%) (1, 2). The high mortality of mesenteric ischemia can be explained with the high metabolic activity of the gut and its function as a large surface barrier (3). Ischemia causes tissue injury, which is significantly enhanced by reperfusion injury (4, 5). With reperfusion, oxygen radicals are produced in the previously ischemic tissue and an inflammatory response is triggered (4). Depending on the affected tissue or organs, this often leads to systemic inflammatory response and shock. In addition to the inflammatory response, shunting of the microcirculation can be observed after mesenteric ischemia and might substantially contribute to tissue damage (6). In humans, the diagnosis of mesenteric ischemia is often delayed due to a lack of appropriate diagnostic tools, which are specific, easy to use, and detect early stages of the disease. Furthermore, treatment of mesenteric ischemia is not specific, and evidence favoring one therapeutic option over another is still lacking.

Complement is known to be activated during reperfusion, and activated complement is thought to contribute significantly to reperfusion injury (7, 8). Studies using C1-esterase inhibitor (C1-INH) in the treatment of reperfusion injury after mesenteric ischemia showed reduced mucosal damage (7). C1-esterase inhibitor is a multifunctional serine protease inhibitor that is normally present in high concentrations in plasma. It is the only plasma inhibitor of C1r and C1s, the activated proteases of the first component of complement (9). It is also the major plasma inhibitor of activated Hageman factor, the first protease in the contact system (9). In addition, C1-INH is one of the major inhibitors of plasma kallikrein, the contact system protease that cleaves kininogen and releases bradykinin (9). C1-esterase inhibitor interferes with P- and E-selectin, and given as a treatment, significantly reduces leukocyte-endothelial interaction (10). Our own studies have shown that C1-INH, when given as a single intravenous bolus, is beneficial in reducing leukocyte/endothelial interaction under low-flow conditions such as hemorrhagic shock (11).

As for the diagnosis of mesenteric ischemia, fatty acid-binding protein is one of the new promising biomarker proteins, which has been shown to be specific for tissue injury (12, 13). This cytoplasmic protein is abundantly expressed in tissues with an active fatty acid metabolism such as heart, liver, and gut. However, these markers have not yet made their way into clinical practice due to a lack of rapid detection assays for point-of-care testing (13). There are a

number of different fatty acid-binding proteins, some of which colocalize in organs such as heart-type fatty acid-binding protein, liver-type fatty acid-binding protein, intestinal fatty acid-binding protein, and ileal lipid-binding protein (I-LBP) in the gut (13).

Because there are studies showing reduced tissue damage after C1-INH treatment (7), we wondered whether C1-INH treatment would also impact on microcirculatory perfusion abnormalities, which develop in the setting of mesenteric ischemia and reperfusion (6). Furthermore, we wanted to explore whether I-LBP levels would mirror functional consequences of both mesenteric ischemia/reperfusion and treatment at the end of the experiments.

To address these questions, we used our previously published animal (rat) model of mesenteric ischemia (6, 14). Briefly, leukocyte/endothelial interaction and microcirculatory perfusion were determined using intravital microscopy. Continuous hemodynamic monitoring and intermittent arterial blood gas analysis served to monitor systemic effects of mesenteric ischemia/reperfusion and treatment. Ischemia was set by tightening an occluding snare around the superior mesenteric artery (SMA) for 60 min followed by 4 h of reperfusion. Comparing 2 different doses (100 and 200 IU/kg body weight), C1-INH was given intravenously as a single bolus shortly before reperfusion. This was followed by normal saline infusion (0.9%) as needed to support arterial blood pressure. Animals treated with normal saline alone served as controls. We chose the relatively new marker I-LBP, sometimes also referred to as "ileal fatty acid-binding protein", for it is exclusively expressed in the distal third of the small intestine (15).

MATERIALS AND METHODS

Forty male Sprague-Dawley rats (body weight, 350 ± 10 g) were maintained on a standard rat chow and water *ad libitum* before the experiment. After anesthesia with urethane (1.25 g/kg body weight; i.m., single dose), the carotid artery and jugular vein were cannulated with a small PE tube for arterial blood pressure recordings, arterial blood gas analysis, and fluid replacement during the experiment via central venous line. For each sample, 250 μL of blood were drawn into heparinized syringes. Arterial blood gases [PaO$_2$, pH, cBase(ecf) (BE), hematocrit (Hct)], lactate, potassium, sodium, and chloride were analyzed by Arterial Blood Gas Laboratory Radiometer Copenhagen 615. PaO$_2$, PaCO$_2$, and arterial pH, hemoglobin concentration, lactate, potassium, and sodium were measured, whereas BE and Hct were calculated from measured values (16).

Rats received a basal infusion with albumin in physiological saline (0.3 mL/100 g body weight/h) for compensation of intraoperative albumin loss and evaporative water loss as published previously (17). The animals were placed on a heating pad, and the rectal temperature was kept constant at 37.5 ± 0.5°C by means of a feedback controlled heating unit (Homeothermic Blanket Control, Harvard, South Natick, Mass). After a median laparatomy, a Doppler-flow probe (ES-20-1.6; Triton Technology, Inc, San Diego, Calif) was placed around the abdominal aorta without impairing the normal blood flow in this vessel. A snare with a monofilament suture was placed around the SMA serving as occlusion apparatus during the ischemic period as described previously (6, 14).

Hemodynamic data [systolic arterial blood pressure, mean arterial blood pressure (MAP), and diastolic arterial blood pressure, heart rate (HR), and abdominal blood flow (ABF)] were recorded using System 6 (Triton Technology, Inc, San Diego, Calif), digitized, and registered on a beat-to-beat basis with a computer-based system (Dasylab; National Instruments Corporation, Austin, Tex). Abdominal stroke volume was calculated real time from the area under the curve of the registered pulsatile velocity curves. Abdominal blood flow (ABF) was calculated by multiplication of the abdominal stroke volume and the simultaneously registered HR (14). The Doppler-flow values showed a linear correlation with electromagnetic blood flow sensors (Skalar-Medical b.v., Delft, the Netherlands) as described previously (18). An electrocardiogram in lead II was recorded during the entire duration of the experiment to detect electrocardiogram changes of any sort.

Animals were placed on the heating pad in the left lateral recumbent position on an adjustable Plexiglas microscope stage. A segment of the ileum was exteriorized through the abdominal incision, avoiding trauma to the exposed bowel and mesentery. The ileal loop was placed onto a translucent temperature-controlled pedestal (37.5°C) and covered with oxygen- and water-impermeable plastic foil (Folio, Germany) to prevent evaporative loss of water (18). The exposed mesentery was continuously superfused with warm (37.5°C) Krebs-Henseleit buffer; the pH of the buffer was adjusted to pH 7.4 with 5% CO$_2$ in N$_2$. Animals were allowed to stabilize for 30 min after exteriorization of the ileal loop before starting the experimental protocol. Intravital microscopy was performed with epi-illumination and transillumination, observing 3 to 5 unbranched mesenteric venules (diameter, 20–35 μm; length, 125–180 μm; Zeiss Axiotech fluorescence microscope with computer-controlled scanning table; light source, AttoArc HBO 100 W) as described previously (19). The images were recorded with a high-resolution camera (Stemmer b/w VS 450) and a videocassette recorder (S-VHS Panasonic AG-7355). Off-line analysis was performed with the Cap Image software system (Version 6.01, Dr Zeintl, Heidelberg, Germany) on an IBM-compatible PC with a Matrox image processing card and real-time video tape digitization by a researcher blinded to the different groups. Erythrocyte flow velocity [centerline velocity (CLV)] was measured with a frame-to-frame method using fluorescein isothiocyanate–labeled red blood cells from donor animals (18). Wall shear rate (γ) was calculated based on the Newtonian definition: $\gamma = 8*$(mean velocity/venular diameter); mean velocity was calculated by dividing CLV by an empirical factor of 1.6 as described previously (18). Rolling leukocytes (RLs) and adherent leukocytes (ALs) were analyzed at distinct time points (see "Experimental protocol") using a frame-to-frame method for an observation period of 1 min each for epi-illumination and transillumination as published previously (18). A leukocyte was defined as adherent to venular endothelium if it remained stationary for more than 30 s under flow. Rolling leukocytes and ALs were expressed as number per squared millimeter calculated from the measured diameter and length of the observed vessels assuming cylindrical geometry. Perfusion of ileal arteries was qualitatively analyzed as absent or present but could not be quantified using the aforementioned technique.

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

Experimental protocol

Animals were randomized into 4 groups (10 animals per group). In the first group, all procedures were performed as described above without tightening the occluding snare (sham-operated group; NOCC). In the other 3 groups, the SMA was occluded by tightening an occluding snare for 60 min as described previously (6, 14). Shortly before reperfusion, either 100 IU/kg body weight C1-INH (C1-INH, Berinert P, Aventis Behring GmbH, Marburg), 200 IU/kg body weight C1-INH, or normal saline (0.9%) was given as an intravenous bolus infusion (groups; OCC+100C1-INH, OCC+200C1-INH, and OCC+S). In all groups, normal saline was infused intravenously as needed to maintain MAP above 70 mmHg (14). All

Table 1. **Sham-operated controls without ischemia (NOCC) at baseline and after 300 min**

	Baseline	300 min
AL/mm^2	208 ± 40	216 ± 33
RL/mm^2	170 ± 19	168 ± 36
CLV (mm/s)	2.3 ± 0.1	2.2 ± 0.1
MAP (mmHg)	94.5 ± 3.4	97.8 ± 4.3
HR (min^{-1})	386 ± 10	405 ± 10
ABF (mL * min^{-1})	52.3 ± 1.5	54.8 ± 2.1
pH	7.38 ± 0.01	7.37 ± 0.01
BE (mmol/L)	−1.8 ± 0.4	−3.0 ± 0.6
PaCO$_2$ (mmol/L)	40.1 ± 2.1	38.1 ± 0.8
Hct (%)	45.4 ± 1.5	41.5 ± 0.8
Lac (mmol/L)	1.4 ± 0.1	0.7 ± 0.1
K$^+$ (mmol/L)	4.1 ± 0.3	3.9 ± 0.3
Na$^+$ (mmol/L)	139.4 ± 1.2	141.4 ± 1.0
Cl$^-$ (mmol/L)	104.8 ± 1.1	109.8 ± 1.2

Lac indicates lactate.

TABLE 2. **Intravenously substituted volumes to maintain MAP over 70 mmHg were significantly larger in normal saline (0.9%) treated controls (OCC+S) as compared with animals treated with a bolus infusion of 100 or 200 IU/kg body weight C1-INH (OCC+100C1-INH and OCC+200C1-INH)**

	Time interval				
Groups	R-60 min after R	60-120 min after R	120-180 min after R	180-240 min after R	Total volume
OCC+S	14.4 ± 5.3	26.2 ± 8.0	25.6 ± 9.5	26.5 ± 13.9	66.0 ± 18.0 mL
OCC+100C1-INH	0.7 ± 0.5	5.8 ± 2.5	6.7 ± 2.8	8.4 ± 2.9	$20.6 \pm 7.3*$ mL
OCC+200C1-INH	0.5 ± 0.5	1.4 ± 1.4	1.4 ± 1.4	1.4 ± 1.4	$4.8 \pm 4.8^{\dagger,\ddagger}$ mL

R indicates 5 min after reperfusion; 60, 120, 180, and 240 min after reperfusion (R).
*Significant difference between OCC+100C1-INH and OCC+S, $P < 0.05$, unadjusted for death rate.
$\dagger$Significant differences between OCC+200C1-INH and OCC+S, $P < 0.05$, unadjusted for death rate.
$\ddagger$Significant differences between OCC+100C1-INH and OCC+200C1-INH, $P < 0.05$.

animals were followed up for a total of 5 h. At baseline—before the occlusion—of the SMA and 5 min after reperfusion (R), and 30, 60, 120, 180, and 240 min after reperfusion, 3 to 5 unbranched mesenteric venules were studied by intravital microscopy, and arterial blood gases were drawn in each group. To avoid artifacts in the microcirculation, intravital microscopy recordings and arterial blood gases were performed in sequence. Rhodamine-6-G (100 μL of a 0.005% solution, Sigma Aldrich Co) was injected via central venous line to visualize leukocytes using epi-illumination at each designated recording time point. Red blood cell CLV was recorded using fluorescein isothiocyanate–labeled red blood cells as described previously (6).

At the end of the observation, serum (200 μL) of blood drawn from the arterial line was frozen for later I-LBP enzyme-linked immunosorbent assay (HK409 ELISA Test Kit; HyCult biotechnology b.v., AA Uden, the Netherlands). After termination of the experiments, the SMA was dissected and analyzed morphologically to ensure the integrity of the vessel at the former position of the occluding snare (14).

Statistical analysis

Data from sham-operated animals (no occlusion of the SMA; NOCC) remained at baseline in all measured parameters and were not further analyzed statistically. Data from groups with SMA occlusion (OCC+S, OCC+100C1-INH, and OCC+200C1-INH) are presented as means ± SEM. Statistical analysis was performed with Sigma Stat (SPSS Science Inc, Chicago, Ill). In ischemia groups, until 120 min of reperfusion, statistical significance of changes from baseline values within each group and differences between groups were tested with 2-way analysis of variance for repeated measures (1 factor repetition; "within subjects"). A multiple-comparison procedure was performed using the Holm-Sidak method. Statistical significance was accepted at $P < 0.05$. With the high death rate 120 min after reperfusion in normal saline–treated animals subjected to 60 min of mesenteric ischemia (OCC+S), data from this group were only analyzed descriptively at time points 120 min after reperfusion. In the two other groups (OCC+100C1-INH and OCC+200C1-INH), differences between animals were tested with a Student t test at time points 120 min after reperfusion. To exclude the possibility that differences at baseline were carried forward, values at 180 and 240 min after reperfusion were subtracted from baseline values of these groups and the results compared with a Student t test. A paired Student t test was used to analyze differences between baseline and 240 min after reperfusion in OCC+100C1-INH and OCC+200C1-INH. For t tests, likewise, statistical significance was accepted at $P < 0.05$.

RESULTS

Sham-operated animals (NOCC) remained at baseline levels in all measured parameters (Table 1) and are not shown further.

Volume substitution and outcome

Animals treated with a C1-INH–bolus treatment needed substantially smaller volumes in addition to basal infusion to hold up MAP over 70 mmHg as compared with OCC+S (Table 2). Infusion rates were constant after 120 min of reperfusion until the end of the experiments only in OCC+200C1-INH, whereas animals in OCC+100C1-INH needed larger volumes over time.

C1-esterase inhibitor treatment significantly improved outcome after mesenteric ischemia. Survival rates (Fig. 1) were 30% in OCC+S, 90% in OCC+100C1-INH, and 100% in

OCC+200C1-INH (OCC+100C1-INH vs. OCC+S, $P < 0.05$; OCC+200C1-INH vs. OCC+S, $P < 0.01$), and NOCC.

Mesenteric microcirculation and global hemodynamics

Rolling and adherent leukocytes—Rolling leukocytes did not differ at baseline: OCC+S, 122 ± 24/mm²; OCC+100C1-INH, 190 ± 30/mm²; and OCC+200C1-INH, 151 ± 22/mm². During reperfusion, RL did not increase in C1-INH–treated groups whereas they tended to be higher in survivors in OCC+S at 120 and 240 min after reperfusion (data not shown).

Adherent leukocytes doubled in C1-INH–treated animals compared with baseline (Fig. 2A), with a significant difference between both C1-INH–treated animals and saline-treated animals (OCC+S) at 120 min after reperfusion. In OCC+S, survivors with persistent microcirculatory perfusion showed a 7-fold increase in AL until 240 min after reperfusion.

Centerline velocity and shear rate—Centerline velocity was unchanged from baseline during reperfusion only in OCC+200C1-INH (Fig. 2B). In OCC+100C1-INH, CLV was significantly reduced as compared with OCC+200C1-INH until the end of the experiments ($P < 0.05$). After initiation of reperfusion, CLV remained reduced down to low-flow/no-flow in OCC+S. In contrast to that, perfusion of larger (ileal) vessels returned after initiation of reperfusion in all groups and persisted
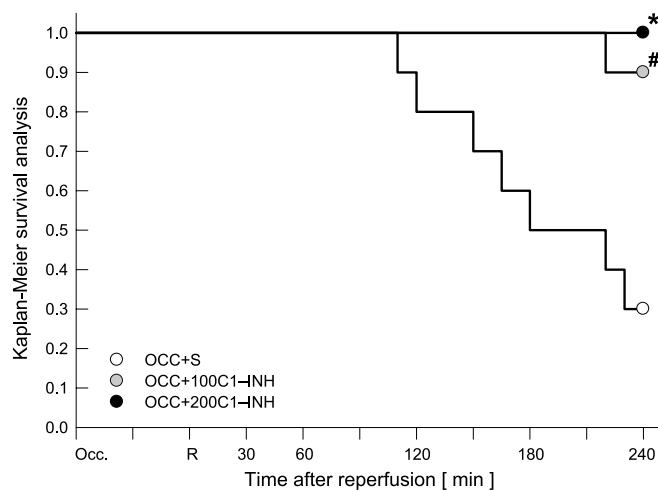

FIG. 1. **Kaplan-Meier survival analysis log rank.** Data are presented as means ± SEM. Occ. indicates SMA occlusion; R, 5 min after reperfusion. #Significant difference between OCC+100C1-INH and OCC+S, $P < 0.05$. *Significant difference between OCC+200C1-INH and OCC+S, $P < 0.01$.
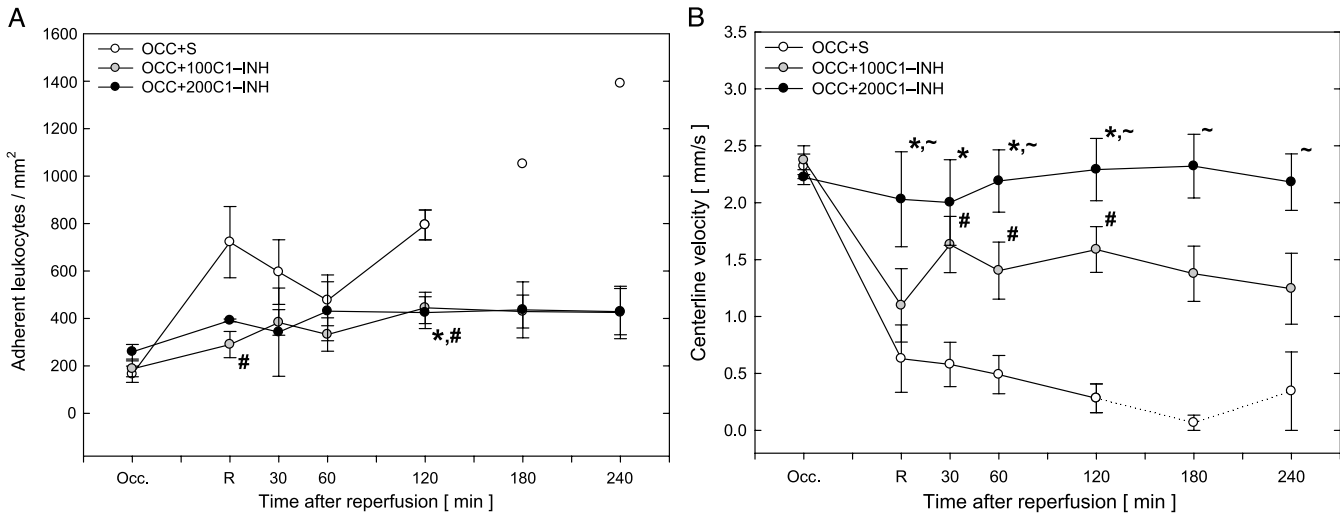
FIG. 2. **A, Adherent leukocytes/mm² were significantly lower in both C1-INH–treated animals (OCC+100C1-INH and OCC+200C1-INH) as compared with saline-treated animals (OCC+S).** B, Centerline velocity recovered after ischemia depending on the dose of C1-INH–bolus treatment. Data are presented as means ± SEM. #Significant difference between OCC+100C1-INH and OCC+S, $P < 0.05$. *Significant difference between OCC+200C1-INH and OCC+S, $P < 0.05$. ~Significant difference between OCC+100C1-INH and OCC+200C1-INH, $P < 0.05$.

until 240 min after reperfusion (data not shown). Shear rate was comparable at baseline (controls, $423 \pm 20 \text{ s}^{-1}$; C1-INH 100 IU/kg body weight, $430 \pm 27 \text{ s}^{-1}$; and C1-INH 200 IU/kg body weight, $454 \pm 18 \text{ s}^{-1}$) and paralleled CLV during the course of observation (data not shown).

Mean arterial blood pressure and heart rates—Mean arterial blood pressure did not differ between groups before and during ischemia (Fig. 3A). After initiation of reperfusion, MAP remained at baseline in OCC+200C1-INH during the experiments and decreased significantly in OCC+S as compared with OCC+200C1-INH until 60 min after reperfusion ($P < 0.05$). After 60 min after reperfusion, MAP in OCC+100C1-INH dropped to similar levels compared with OCC+S until 240 min after reperfusion. With intravenous volume substitution, a decrease of MAP below 70 mmHg was prevented in all groups without using vasopressors (see "Results": volume substitution).

Heart rates increased significantly ($P < 0.05$) in all groups from baseline (OCC+S, 398 ± 9 bpm; OCC+100C1-INH, 399 ± 7 bpm; and OCC+200C1-INH, 389 ± 4 bpm) until 240 min after reperfusion (OCC+S, 509 ± 9 bpm; OCC+100C1-INH, 470 ± 10 bpm; and OCC+200C1-INH, 454 ± 19 bpm) and tended to be lower in C1-INH–treated animals as compared with controls (OCC+S).

Abdominal blood flow—Abdominal blood flow tended to be lower in all groups during ischemia (Fig. 3B). Shortly after reperfusion, ABF decreased further in OCC+S and OCC+100C1-INH as compared with OCC+200C1-INH, with ABF being significantly lower in OCC+S compared with OCC+200C1-INH at 30 min after reperfusion ($P < 0.05$).

Arterial blood gas values

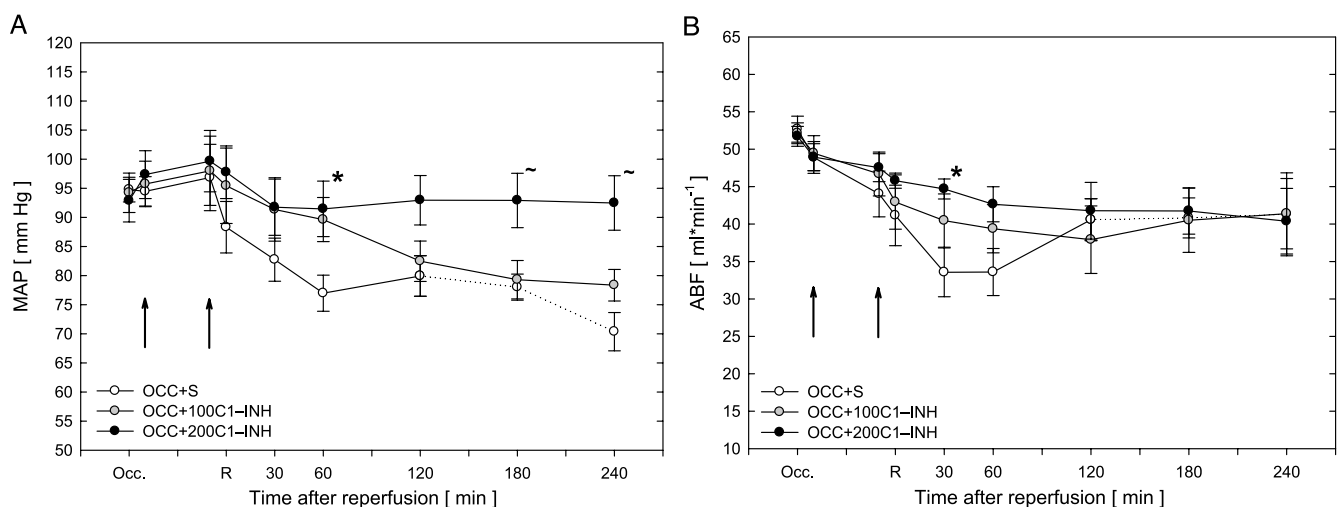Blood pH remained unchanged in OCC+200C1-INH and tended to decrease in OCC+100C1-INH. In OCC+S, blood pH

FIG. 3. **A, Mean arterial blood pressure decreased after initiation of reperfusion in saline-treated animals (OCC+S) and in OCC+100C1-INH after 60 min after reperfusion, whereas MAP remains at baseline levels in OCC+200C1-INH.** B, Abdominal blood flow decreased in saline-treated animals (OCC+S) shortly after reperfusion and recovered with time to levels similar to those in C1-INH–treated animals. Data are presented as means ± SEM. *Significant difference between OCC+200C1-INH and OCC+S, $P < 0.05$. ~Significant difference between OCC+100C1-INH and OCC+200C1-INH, $P < 0.05$. Arrows indicate OCC+10 min and R−10 min.
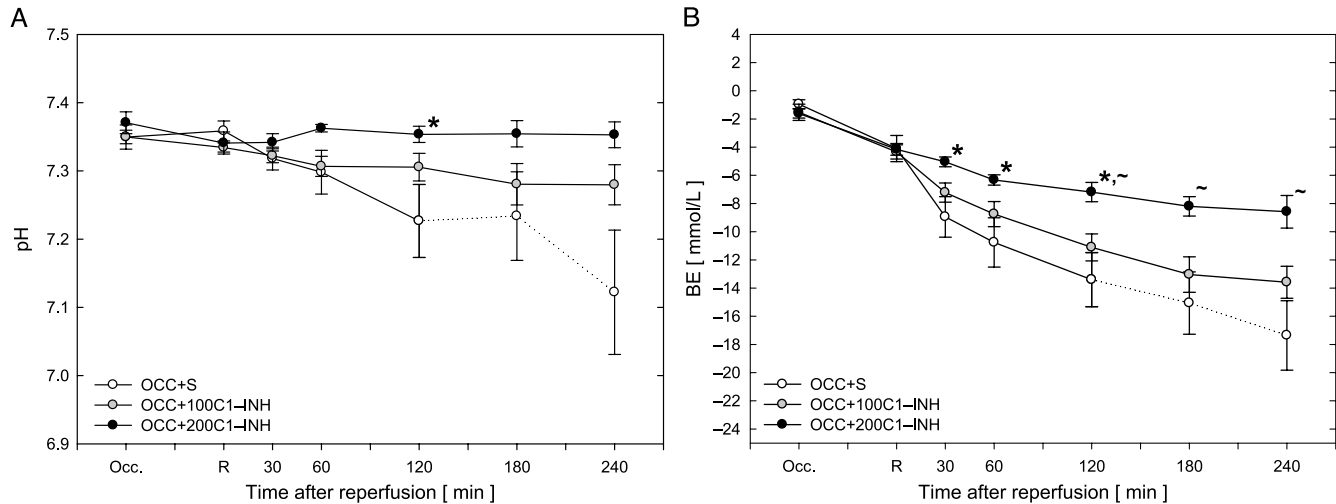
FIG. 4. **A, Arterial blood pH was sustained in OCC+200C1-INH and decreased in OCC+100C1-INH, and even more in saline-treated controls (OCC+S).** B, Base excess decreased in all groups, with the lowest values found in saline-treated animals (OCC+S). Data are presented as means ± SEM. *Significant difference between OCC+200C1-INH and OCC+S, $P < 0.05$. ~Significant difference between OCC+100C1-INH and OCC+200C1-INH, $P < 0.05$.

dropped significantly until 120 min after reperfusion ($P < 0.05$) and decreased further in this group until the end of the experiments (Fig. 4A). $PaCO_2$ values were comparable at baseline, decreased significantly, and almost linear, in all groups until 240 min after reperfusion, with no significant differences between groups (Table 3). Base excess decreased significantly during ischemia and reperfusion in all groups with significant differences between OCC+S and OCC+200C1-INH from 30 to 120 min after reperfusion, and between OCC+100C1-INH and OCC+200C1-INH from 120

to 240 min after reperfusion ($P < 0.05$; Fig. 4B). Animals from group OCC+200C1-INH showed a significantly attenuated decrease in BE values during reperfusion until the end of the experiments.

PaO_2 values increased significantly in all groups from baseline until the end of the observation period without showing significant differences between the groups at any point during the experiments (data not shown). Hematocrit was comparable at baseline and tended to increase shortly after initiation of reperfusion in OCC+S (Table 3 and data not

TABLE 3. **Selected parameters at baseline, 120, and 240 min after reperfusion (R)**

		Baseline	120 min after R	240 min after R
$PaCO_2$ (mmHg)	OCC+S	44.8 ± 1.9	31.1 ± 2.1*	25.1 ± 3.8
	OCC+100C1-INH	43.4 ± 1.5	29.2 ± 2.1*	25.9 ± 1.5*
	OCC+200C1-INH	40.8 ± 2.1	31.9 ± 1.0*	29.0 ± 1.6*
Hct (%)	OCC+S	45.8 ± 0.9	42.3 ± 2.4	32.6 ± 3.4
	OCC+100C1-INH	45.4 ± 0.6	42.6 ± 2.0	38.6 ± 1.8*
	OCC+200C1-INH	43.4 ± 0.9	40.0 ± 2.1*	38.0 ± 2.9*
Lac (mmol/L)	OCC+S	1.3 ± 0.1	1.2 ± 0.2	1.5 ± 0.2
	OCC+100C1-INH	1.4 ± 0.1	1.0 ± 0.1*	1.0 ± 0.2
	OCC+200C1-INH	1.6 ± 0.1	0.7 ± 0.1*	0.7 ± 0.1*
K^+ (mmol/L)	OCC+S	4.4 ± 0.2	5.4 ± 0.5*	6.0 ± 0.8
	OCC+100C1-INH	4.3 ± 0.1	4.5 ± 0.2[†]	5.4 ± 0.4*
	OCC+200C1-INH	4.0 ± 0.1	4.0 ± 0.1[‡]	4.0 ± 0.1[§]
Na^+ (mmol/L)	OCC+S	140.0 ± 1.1	142.0 ± 1.0	143.2 ± 0.9
	OCC+100C1-INH	138.4 ± 0.4	144.4 ± 1.4*	143.0 ± 0.8*
	OCC+200C1-INH	139.3 ± 0.8	143.3 ± 0.5	144.7 ± 0.5*
Cl^- (mmol/L)	OCC+S	105.5 ± 0.1	121.4 ± 1.2*	126.2 ± 0.5
	OCC+100C1-INH	104.3 ± 0.1	114.8 ± 1.8[†,*]	120.7 ± 1.9*
	OCC+200C1-INH	103.6 ± 0.8	114.0 ± 1.1[‡,*]	117.1 ± 3.9*

*Significant difference between baseline, 120, and 240 min after reperfusion, $P < 0.05$.
[†]Significant difference between OCC+100C1-INH and OCC+S, $P < 0.05$.
[‡]Significant difference between OCC+200C1-INH and OCC+S, $P < 0.05$.
[§]Significant difference between OCC+100C1-INH and OCC+200C1-INH, $P < 0.05$.

shown). Following this short-term increase, Hct decreased more pronounced in OCC+S until the end of the experiments as compared with C1-INH–treated groups, however, without significant differences between the groups.

Electrolytes and lactate

Lactate levels did not differ at baseline (Table 3) or until 60 min after reperfusion (data not shown). Thereafter, lactate levels decreased with the difference being significant in OCC+100C1-INH and OCC+200C1-INH between baseline and 120 min after reperfusion ($P < 0.05$). In OCC+S, lactate levels remained unchanged from baseline until the end of the experiments. Serum potassium levels constantly and significantly increased in OCC+S after initiation of reperfusion until 120 min after reperfusion ($P < 0.05$) and continued to rise in this group until the end of the experiments (Table 3). As compared with OCC+S, potassium levels increased in OCC+100C1-INH to a lesser extent, however, significantly from baseline to 240 min after reperfusion. Only in OCC+200C1-INH, serum potassium remained unchanged from baseline during the course of the observation.

Sodium levels significantly increased in animals treated with C1-INH and tended to increase in OCC+S from baseline to 240 min after reperfusion without statistically significant differences between the groups (Table 3). Serum chloride levels were comparable at baseline and increased significantly ($P < 0.05$) in all groups until 120 min after reperfusion with significant differences between OCC+S and both C1-INH–treated groups ($P < 0.05$, Table 3). All groups showed a further increase in serum chloride levels until the end of the experiments with OCC+S having the highest chloride levels among the groups.
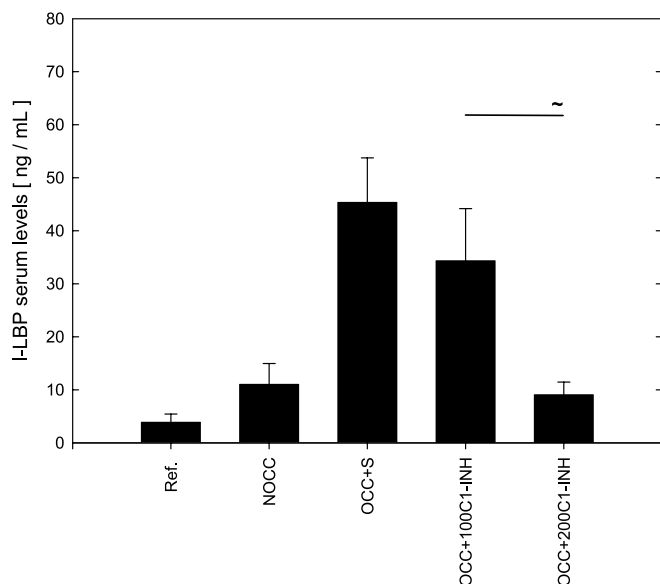

FIG. 5. **Ileal lipid-binding protein serum levels at the end of the experiments differed significantly between C1-INH–treated groups with the levels being comparable between OCC+200C1-INH and sham-operated animals without mesenteric ischemia (NOCC).** Data are presented as means ± SEM. Ref. indicates no abdominal surgery; NOCC, abdominal surgery, no mesenteric ischemia. ˜Significant difference between OCC+100C1-INH and OCC+200C1-INH, $P < 0.05$.

Ileal lipid-binding protein levels in serum

Abdominal surgery and exteriorization of the gut (NOCC) alone tended to increase I-LBP serum levels compared with animals without abdominal surgery (Ref. in Fig. 5). The highest I-LBP levels in serum were found in OCC+S. In OCC+200C1-INH, serum I-LBP levels were significantly reduced compared with OCC+100C1-INH. Ileal lipid-binding protein serum levels were comparable between OCC+200C1-INH and sham-operated animals without ischemia (NOCC; Fig. 5).

DISCUSSION

C1-esterase inhibitor–single bolus treatment significantly reduced local and systemic effects of mesenteric ischemia/reperfusion and mortality by restoring microcirculatory perfusion and reducing leukocyte/endothelial interaction. Ileal lipid-binding protein proved to be a valuable serum marker because it correlated well with functional consequences of mesenteric ischemia and C1-INH treatment, as seen in intravital microscopy at the end of the experiments.

Reperfusion injury may be an important source of organ failure and is applicable to a wide variety of problems (aortic cross clamping, myocardial reperfusion injury, and late resuscitation) (20). The beneficial effect of C1-INH has been explored in animal studies in a number of these settings, including myocardial infarction, burns, and sepsis (21–23). A study conducted by Karpel-Massler et al. (7) shows in histology a significantly reduced local inflammatory response after mesenteric ischemia/reperfusion in mice treated with C1-INH. Because neutrophils do promote inflammation, leukocyte/endothelial interaction and neutrophil recruitment are usually used as a surrogate for tissue injury. In good agreement with the findings of Karpel-Massler et al. (7), our study shows a functional reduction of leukocyte/endothelial interaction in the number of AL after C1-INH treatment. Karpel-Massler et al. (7) show no significant differences in the number of infiltrated neutrophils over a large range of C1-INH doses (200–800 IU/kg). This is in good agreement with our data showing that leukocyte adhesion is equally reduced after treatment with 100 or 200 IU/kg body weight C1-INH. However, our data show that despite the reduced leukocyte/endothelial interaction, microcirculatory perfusion is still significantly impaired after 100 IU/kg body weight as compared with 200 IU/kg body weight C1-INH. As we know from previous experiments using this model, shunting of the microcirculation reproducibly occurred after mesenteric ischemia and reperfusion with fluid resuscitation (6). Hence, our current experiments strongly suggest that C1-INH treatment successfully reduced shunting of the microcirculation after mesenteric ischemia/reperfusion; although the exact mechanism by which C1-INH reduces shunting of the microcirculation remains to be further explored.

Leukocyte/endothelial interaction and leukocyte recruitment usually augment the inflammatory response and are already efficiently reduced at lower doses of C1-INH (7). Local complement activation and deposition are reduced in a dose-dependent manner (7) and might explain the differences seen between the C1-INH–treated groups in our study.

However, local complement deposition is not completely abolished after treatment with 200 IU/kg body weight of C1-INH, as seen by Karpel-Massler et al. (7). Hence, reaching a functionally almost normal microcirculatory perfusion most likely does not require blocking complement or leukocyte recruitment completely, and complement deposition and histology might not be conclusive read-outs of what is generally considered a successful treatment.

In addition to the described local effects, our study showed marked systemic effects of C1-INH treatment with significantly reduced metabolic acidosis and mortality after SMA occlusion/reperfusion. The latter effects appeared to be dose dependent. We chose not to treat the acidosis or ventilate the animals albeit knowing that this might have significantly affected survival and hemodynamics. However, we considered pH treatment and mechanical ventilation significant confounders that might disguise the translation of local effects of ischemia and treatment to systemic parameters. Interestingly, the degree of hyperventilation, as reflected by $PaCO_2$ levels, was comparable in all groups. In animals treated with 200 IU/kg body weight C1-INH, hyperventilation was sufficient to maintain pH at baseline levels; whereas animals treated with 100 IU/kg C1-INH and even more so saline-treated animals did not achieve a similar level of compensation of metabolic acidosis. Furthermore, systemic hemodynamic parameters such as blood pressure and HR showed marked differences after C1-INH treatment as compared with controls. Mean arterial blood pressure remained at baseline levels only in animals treated with 200 IU/kg body weight C1-INH without the need for significant volumes of intravenous fluids. In animals treated with normal saline, MAP decreased shortly after reperfusion, and these animals required substantially larger volumes of intravenous fluids to support blood pressure. Although ABF appeared to be the earliest indicator of fluid loss in our study, we chose a blood pressure–guided fluid replacement strategy because this is common in clinical practice. With the volumes infused, especially in saline-treated animals, Hct tended to decrease until the end of the experiments, however, without a measurable effect on PaO_2. Different from common clinical practice, we refrained from using vasopressors to sustain blood pressure to see the effects of volume and C1-INH on microcirculatory perfusion. However, eliminating the confounding effect of vasopressors for the microcirculation most likely also increased the volumes infused to support blood pressure, which can also be harmful. In our study, we did not find any correlation between infused volumes and survival (>240 min of reperfusion) in saline-treated controls, neither positive nor negative. Some of the short-term nonsurvivors in the control group needed very little volume substitution to maintain MAP over 70 mmHg, and some of the longer-living nonsurvivors in controls required large volumes (>120 mL) for blood pressure support. Interestingly, most of the survivors in the control group did not need large volumes. Indeed, for the group of longer-living nonsurvivors with large volume infusion, vasopressors would have probably reduced the need for intravenous fluids, which could have prolonged their survival past 240 min of reperfusion, however, this would have also affected microcirculation. At least in models of hemorrhagic shock, restoring microcirculation with increased functional capillary density resulted in better prognosis (24), which questions the potential benefit of using vasopressors to save fluids.

Because we used normal saline as volume substitution, serum sodium and chloride levels significantly increased in all groups until the end of the experiments (25, 26). As hyperchloremia might contribute to metabolic acidosis, it might theoretically worsen the outcome, although this has never been clearly shown (26). However, hypovolemia and shock do promote metabolic acidosis as well. In a system with intact counter-regulatory mechanisms, the contribution of hyperchloremia to metabolic acidosis might be less important, and saline infusion might actually attenuate blood pH (27). In our experiments, BE is higher than the predicted BE calculated from pH and $PaCO_2$. This correlated well with the developing hyperchloremia during the experiments in animals that needed large volumes of intravenous fluids. However, eventual consequences of this mismatch need to be further explored.

Tissue damage usually increases serum potassium levels (27), and serum potassium levels tended to correlate with the degree of tissue injury in our study. However, acidemia also causes potassium to shift out the cells and into the blood. Depending on the acuteness of tissue injury or acidemia, renal function is able to compensate and maintain normal potassium levels. With the lack of other clinically well-established parameters, blood lactate is commonly used as a surrogate for mesenteric ischemia. However, blood lactate is not considered a reliable marker of mesenteric ischemia because increases in lactate levels are nonspecific, and lactate produced in the upper gastrointestinal tract is usually rapidly metabolized by the liver before entering systemic circulation (28, 29). Confirming previous experiments, blood lactate concentration did not increase in response to SMA ischemia (6, 14). Furthermore, blood lactate concentration does not necessarily reflect lactate concentration in ischemic tissues as no flow or shunting of the microcirculation might prevent lactate from being washed out into systemic circulation. Indeed, shunting of the microcirculation can be observed in animals undergoing 60 min of ischemia and reperfusion as shown in previous experiments (6), and might prevent lactate washout. This can serve to explain why even animals dying from mesenteric ischemia/reperfusion did not show an increase in lactate levels. These observations are in good agreement with other studies (14, 29). Compared with lactate, I-LBP, which is exclusively expressed in the distal third of the small intestine (15), highly correlated with the degree of local and systemic tissue injury after mesenteric ischemia and reperfusion in our study. As shown in our experiments, abdominal surgery alone tended to increase I-LBP levels highlighting the sensitivity of this parameter for even minimal tissue injury, although being specific at the same time. Treatment with C1-INH reduced serum I-LBP levels significantly in a dose-dependent manner. Furthermore, I-LBP levels after 200 IU/kg body weight C1-INH and in animals that did not undergo ischemia were comparable. This provides further evidence that for a successful treatment of reperfusion injury, a functional restoration of microcirculatory perfusion together with a tamed

leukocyte recruitment (compared with untreated reperfusion injury) might be sufficient to prevent extensive tissue damage and favor tissue repair.

Looking at possible adverse effects of therapy and as a paradigm, it might even be more favorable to tame the inflammatory response over trying to block it completely. In our study using 100 and 200 IU/kg body weight of C1-INH, no adverse effects were seen. Nevertheless, in addition to its function as complement inhibitor, C1-INH inhibits fibrinolysis (factors XIIa and XIa, kallikrein, and plasmin) (30, 31). The inhibition of fibrinolysis might have caused fatal outcomes in pediatric patients that were empirically treated with high doses of C1-INH in an attempt to reduce capillary leak after cardiac surgery and the use of extracorporal circulation (32). These patients died of upper venous and arterial thromboses. Although it might as well be coincidence in these cases, an excess of C1-INH not matching the actual degree of complement activation should be avoided, even if serious adverse effects were not observed in other studies with the concomitant use of heparin (33).

However, whether the effect of C1-INH in our experiments can be exclusively explained with the inhibition of complement activation or whether a possible inhibition of other serine proteases by C1-INH plays a role requires further studies. In animal studies, C1-INH is usually given before organ ischemia; and in some studies, doses are split so that 1 dose is given before, and a second dose is given after initiation of reperfusion. One study looked at brain infarct size after ischemia/reperfusion and saw a reduced effect of C1-INH when the same dose was given after initiation of reperfusion instead of a dose given before the initiation of reperfusion (34). Further studies are needed to work out the potential benefit of a late treatment.

Other limitations of our study include the use of small animals and the restriction to a 5-h total observation time. As published previously, we were able to prolong observation time over what was published before by other groups to 5 h with stable microhemodynamics and macrohemodynamics (6, 14). A further prolongation of observation time most certainly would have been more informative when looking at the outcome. However, after a 5-h total observation time, outcome is also possibly affected by the developing perfusion abnormalities in the exteriorized gut. Further studies are needed to elucidate the role of microcirculatory perfusion abnormalities and the impact of treatment.

In our study, we chose to analyze I-LBP only at the end of the experiments because we wanted to see whether I-LBP would mirror functional consequences of mesenteric ischemia and reperfusion at the end of the experiments. Drawing a repeatedly sufficient volume of blood for both blood gas analysis and I-LBP serum levels during the experiment would have affected microcirculation and outcome negatively, as seen in unpublished pilot experiments. To increase the clinical usefulness of I-LBP, a more detailed knowledge of the time course of I-LBP levels during ischemia/reperfusion and the development of rapid detection assays is mandatory because mesenteric ischemia requires immediate attention and treatment, and diagnosis of mesenteric ischemia in patients is difficult and often delayed (1, 2). A combination of the use of I-LBP or intestinal fatty acid-binding protein with a single bolus treatment of C1-INH could prove to be a very promising treatment strategy in an attempt to improve the still poor prognosis of mesenteric ischemia in patients (1, 2).

CONCLUSION

In the setting of mesenteric ischemia, C1-INH given as a bolus infusion shortly before reperfusion efficiently restored microcirculatory perfusion in a dose-dependent manner, reduced local and systemic inflammatory response, and improved outcome. Ileal lipid-binding protein proved to be a valuable marker that correlated well with the functional consequences of mesenteric ischemia/reperfusion and treatment at the end of the experiments.

ACKNOWLEDGMENTS

REFERENCES

1. Asensio JA, Berne JD, Chahwan S, Hanpeter D, Demetriades D, Marengo J, Velmahos GC, Murray J, Shoemaker WC, Berne TV: Traumatic injury to the superior mesenteric artery. *Am J Surg* 178:235–239, 1999.
2. Bassiouny HS: Nonocclusive mesenteric ischemia. *Surg Clin North Am* 77:319–326, 1997.
3. Grotz MR, Deitch EA, Ding J, Xu D, Huang Q, Regel G: Intestinal cytokine response after gut ischemia: role of gut barrier failure. *Ann Surg* 229:478–486, 1999.
4. Savoye G, Tamion F, Richard V, Varin R, Thuillez C: Hemorrhagic shock resuscitation affects early and selective mesenteric artery endothelial function through a free radical-dependent mechanism. *Shock* 23:411–416, 2005.
5. Parks DA, Granger DN: Contributions of ischemia and reperfusion to mucosal lesion formation. *Am J Physiol* 250:G749–G753, 1986.
6. Lauterbach M, Horstick G, Plum N, Weilemann LS, Münzel T, Kempski O: Shunting of the microcirculation after mesenteric ischemia and reperfusion is a function of ischemia time and increases mortality. *Microcirculation* 13:411–422, 2006.
7. Karpel-Massler G, Fleming SD, Kirschfink M, Tsokos GC: Human C1 esterase inhibitor attenuates murine mesenteric ischemia/reperfusion induced local organ injury. *J Surg Res* 115:247–256, 2003.
8. Weiser MR, Williams JP, Moore FD Jr, Kobzik L, Ma M, Hechtman HB, Carroll MC: Reperfusion injury of ischemic skeletal muscle is mediated by natural antibody and complement. *J Exp Med* 183:2343–2348, 1996.
9. Davis AE III: C1 Inhibitor and hereditary angioneurotic edema. *Annu Rev Immunol* 6:595–628, 1988.
10. Cai S, Davis AE 3rd: Complement regulatory protein C1 inhibitor binds to selectins and interferes with endothelial-leukocyte adhesion. *J Immunol* 171:4786–4791, 2003.
11. Horstick G, Kempf T, Lauterbach M, Bhakdi S, Kopacz L, Heimann A, Malzahn M, Horstick M, Meyer J, Kempski O: C1-esterase-inhibitor treatment at early reperfusion of hemorrhagic shock reduces mesentery leukocyte adhesion and rolling. *Microcirculation* 8:427–433, 2001.
12. Niewold TA, Meinen M, van der Meulen J: Plasma intestinal fatty acid binding protein (I-FABP) concentrations increase following intestinal ischemia in pigs. *Res Vet Sci* 77:89–91, 2004.
13. Pelsers MM, Hermens WT, Glatz JF: Fatty acid-binding proteins as plasma markers of tissue injury. *Clin Chim Acta* 352:15–35, 2005.
14. Lauterbach M, Horstick G, Plum N, Heimann A, Becker D, Weilemann LS, Münzel T, Kempski O: Prolonged recirculation is required to detect secondary metabolic and hemodynamic deterioration after superior mesenteric artery occlusion. *Clin Hemorheol Microcirc* 32:1–12, 2005.
15. Agellon LB, Toth MJ, Thomson AB: Intracellular lipid binding proteins of the small intestine. *Mol Cell Biochem* 239:79–82, 2002.
16. Kofstad J: Base excess: a historical review—has the calculation of base excess been more standardised the last 20 years? *Clin Chim Acta* 307:193–195, 2001.
17. Horstick G, Lauterbach M, Kempf T, Ossendorf M, Kopacz L, Heimann A, Lehr HA, Bhakdi S, Horstick M, Meyer J, et al: Plasma protein loss during surgery: beneficial effects of albumin substitution. *Shock* 16:9–14, 2001.

18. Horstick G, Kempf T, Lauterbach M, Ossendorf M, Kopacz L, Heimann A, Lehr HA, Bhakdi S, Meyer J, Kempski O: Plastic foil technique attenuates inflammation in mesenteric intravital microscopy. *J Surg Res* 94:28–34, 2000.

19. Lauterbach M, Horstick G, Kempf T, Weilemann LS, Münzel T, Kempski O: Anti-inflammatory treatment with standardized human serum protein solution reduces local and systemic inflammatory response after hemorrhagic shock. *Eur Surg Res* 38:399–406, 2006.

20. Khalil AA, Aziz FA, Hall JC: Reperfusion injury. *Plast Reconstr Surg* 117: 1024–1033, 2006.

21. Jansen PM, Eisele B, de Jong IW, Chang A, Delvos U, Taylor FB Jr, Hack CE: Effect of C1 inhibitor on inflammatory and physiologic response patterns in primates suffering from lethal septic shock. *J Immunol* 160:475–484, 1998.

22. Radke A, Mottaghy K, Goldmann C, Kovacs B, Janssen A, Klosterhalfen B, Hafemann B, Pallua N, Kirschfink M: C1 inhibitor prevents capillary leakage after thermal trauma. *Crit Care Med* 28:3224–3232, 2000.

23. Horstick G, Berg O, Heimann A, Gotze O, Loos M, Hafner G, Bierbach B, Petersen S, Bhakdi S, Darius H, et al: Application of C1-esterase inhibitor during reperfusion of ischemic myocardium: dose-related beneficial versus detrimental effects. *Circulation* 104:3125–3131, 2001.

24. Kerger H, Waschke KF, Ackern KV, Tsai AG, Intaglietta M: Systemic and microcirculatory effects of autologous whole blood resuscitation in severe hemorrhagic shock. *Am J Physiol* 276:H2035–H2043, 1999.

25. Brill SA, Stewart TR, Brundage SI, Schreiber MA: Base deficit does not predict mortality when secondary to hyperchloremic acidosis. *Shock* 17: 459–462, 2002.

26. Moviat M, van Haren F, van der Hoeven H: Conventional or physicochemical approach in intensive care unit patients with metabolic acidosis. *Crit Care* 7:R41–R45, 2003.

27. O'Neill PJ, Cobb LM, Ayala A, Morrison MH, Chaudry IH: Aggressive fluid resuscitation following intestinal ischemia-reperfusion in immature rats prevents metabolic derangements and down regulates interleukin-6 release. *Shock* 1:381–387, 1994.

28. Sommer T, Larsen JF: Detection of intestinal ischemia using a microdialysis technique in an animal model. *World J Surg* 27:416–420, 2003.

29. Jakob SM, Merasto-Minkkinen M, Tenhunen JJ, Heino A, Alhava E, Takala J: Prevention of systemic hyperlactatemia during splanchnic ischemia. *Shock* 14:123–127, 2000.

30. Pixley RA, Schapira M, Colman RW: The regulation of human factor XIIa by plasma proteinase inhibitors. *J Biol Chem* 260:1723–1729, 1985.

31. Schapira M, de Agostini A, Colman RW: C1 inhibitor: the predominant inhibitor of plasma kallikrein. *Methods Enzymol* 163:179–185, 1988.

32. Aerzteschaft AdD: Schwerwiegende Thrombenbildung nach Berinert HS. *Dtsch Aerztebl* 97:B864, 2000.

33. Tassani P, Kunkel R, Richter JA, Oechsler H, Lorenz HP, Braun SL, Eising GP, Haas F, Paek SU, Bauernschmitt R, et al: Effect of C1-esterase-inhibitor on capillary leak and inflammatory response syndrome during arterial switch operations in neonates. *J Cardiothorac Vasc Anesth* 15:469–473, 2001.

34. De Simoni MG, Rossi E, Storini C, Pizzimenti S, Echart C, Bergamaschini L: The powerful neuroprotective action of C1-inhibitor on brain ischemia-reperfusion injury does not require C1q. *Am J Pathol* 164:1857–1863, 2004.