Early albumin infusion improves global and local hemodynamics and reduces inflammatory response in hemorrhagic shock

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Objective: To evaluate the effects of an early, short-term albumin infusion on mesenteric microcirculation and global hemodynamics in hemorrhagic shock.

Design: A prospective, randomized study.

Setting: Animal laboratory at a university medical clinic.

Subjects: Seventeen Sprague-Dawley rats weighing 250–400 g.

Interventions: The 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 weight for 60 mins), followed by a 4-hr reperfusion period. Albumin (20%) or 0.9% NaCl was administered intravenously as a continuous infusion for 30 mins at the beginning of reperfusion. Reperfusion time mimicked a "prehospital" phase of 30 mins followed by a quasi "in-hospital" phase of 3.5 hrs. The "in-hospital" phase in both groups was initiated by substitution of blood followed by reperfusion with normal saline.

Measurements and Main Results: Central hemodynamics, mesenteric microcirculation, and arterial blood gas parameters were monitored before, during, and 60 mins after hemorrhagic shock, and for a 240-min follow-up period after initiation of reperfusion. Application of albumin markedly reduced rolling and adherent leukocytes, maximum velocity, and shear rate in the mesenteric microcirculation. Later, after improvement of mesenteric microcirculation, an intermittent increase of central venous pressure and abdominal blood flow and decrease of hematocrit was observed.

Conclusions: Albumin treatment of hemorrhagic shock improves microcirculation and global hemodynamics and attenuates the inflammatory response to reperfusion. It may provide clinical benefit when applied at an early stage of reperfusion during hemorrhagic shock. (Crit Care Med 2002; 30:851–855)

KEY WORDS: reperfusion; hemorrhagic shock; leukocyte-endothelial cell interaction; microcirculation

The treatment of shock with albumin is controversial. Albumin has been shown to worsen pulmonary function in pathologic conditions such as acute pancreatitis and abdominal sepsis (1, 2). However, other studies have shown the attenuation of pulmonary transmicrovascular flux by albumin infusion (3) and that albumin application during hemorrhagic shock did not cause an increase in extravascular lung water or intrapulmonary shunts (4). Further studies have highlighted the anti-inflammatory effect of albumin when substituted for plasma protein during abdominal surgery (5) and in critically ill patients (6, 7).

We tested the hypothesis that reperfusion of hemorrhagic shock with albumin will improve global hemodynamic and microcirculatory disturbances. The study showed that albumin infusion as an early volume replacement strategy was accompanied by marked beneficial effects on the local mesenteric microcirculation together with a reduction of inflammatory-type reactions.

MATERIALS AND METHODS

Experimental Conditions. Seventeen male Sprague-Dawley rats were maintained on standard rat chow and water *ad libitum* until the night before the experiment. Rat chow was removed 10–12 hrs before the beginning of the experiment to reduce intestinal peristalsis during intravital microscopy. After anesthesia with urethane (1.25 g/kg intramuscularly, single dose), the femoral artery and jugular vein were cannulated with a small polyethylene tube for arterial blood gas analysis and measurement of plasma protein and arterial and central venous blood pressure. From each rat, 250 μL of blood was drawn into heparinized syringes. Arterial blood gases (Pao_2, So_2, Pco_2, pH, and base excess), hemoglobin, hematocrit, lactate, potassium, and sodium were analyzed with an arterial blood gas analyzer (ABL615, Radiometer, Copenhagen, Denmark).

After median laparotomy, a Doppler flow transducer was placed around the abdominal aorta. Hemodynamic data (abdominal blood flow, descending aorta), heart rate, systolic, mean and diastolic arterial blood pressure, and central venous pressure were recorded on a beat-to-beat basis using System 6 (Triton Technology, San Diego, CA). Analog data were digitized and recorded online with a computer-based system (DASYLab, National Instruments Corporation, Austin, TX). An electrocardiogram in lead II was recorded during the experiment to detect morphologic electrocardiographic changes (8). Rectal temperature was kept constant at $37.5 \pm 0.5°C$ by means of a feedback-controlled homeothermic blanket control unit (Harvard Apparatus, South Natick, MA).

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 (9).

Rats received a basal infusion with albumin in physiologic saline for compensation of intraoperative albumin loss and evaporative water loss (0.3 mL/hr/100 g body weight) after the central venous pressure catheter was introduced as previously described (5).

Animals were randomized before reperfusion to intravenous infusion with either albumin (group 1, n = 6) or normal saline (group 2, n = 6) administered at the beginning of reperfusion after 60 mins of hemorrhagic shock. Five animals died during the phase of systemic hypotension before reperfusion could be achieved and were, hence, excluded from the study. Mean body weights of group 1 (albumin) and group 2 (NaCl) were 281 ± 6 g and 286 ± 11 g, respectively.

Intravital microscopy was performed with epi- and transillumination observing three to five unbranched mesenteric venules (diameter: 25–40 μm; length: 100–150 μm) (Axiotech fluorescence microscope with computer-controlled scanning table [Carl Zeiss, Jena, Germany]; AttoArc HBO 100 W light source [Atto Bioscience, Rockville, MD]). Rhodamine-labeled leukocytes were counted using epi-illumination. Cell counts were normalized to the endothelial surface assuming cylindrical geometry. Off-line analysis of coded video recordings was performed in a blinded manner (5, 9).

All investigative procedures and the animal facilities conformed with the *Guide for the Care and Use of Laboratory Animals* (10). The protocol was approved by the regional animal care and use committee.

Experimental Protocol. After exteriorization and fixation of the mesentery, rats were allowed to stabilize for 30 mins and mesentery microcirculation was observed for 5 hrs of experimental time. Volume-controlled hemorrhagic shock was provoked during the first 10 mins. Then, 2.5 mL/kg body weight of arterial blood was drawn from the femoral artery and transferred to citrate plasma tubes (citrate to blood ration 1:10). After 60 mins of systemic hypotension, reperfusion was initiated. The reperfusion phase was divided into a "prehospital" phase of 30 mins and an "in-hospital" phase, starting with administration of red blood cells to compensate for the initial blood loss (Fig. 1A). During the first 30 mins of reperfusion ("prehospital" phase), either 20% albumin or normal saline was administered intravenously until a mean arterial blood pressure of 70 mm Hg was reached. Afterward, all animals received fluid substitution at the beginning of the "in-hospital" phase with normal saline intravenously. The minimum infusion was normal saline at two times the shed blood volume infused for the first 30 mins after initial treatment with albumin or normal saline. Thereafter, normal saline at the equivalent of at least the shed blood volume was infused over 2 hrs, followed by maintenance infusion with normal saline throughout the experiment so that mean arterial pressure did not drop below 70 mm Hg (Fig. 1A).

Statistical Analysis

Data are presented as means ± SEM. Statistical analysis was performed with SigmaStat (SPSS Science, Chicago, IL). Statistical signif- icance 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 (Kruskal-Wallis test) with the multiple comparison method (Student-Newman-Keuls test) was used. Statistical significance was accepted at an error probability of $p < .05$.

RESULTS

Hemodynamics

Mean Arterial Blood Pressure, Heart Rate, Central Venous Pressure, and Volume Substitution. Immediately after induction of hemorrhagic shock, mean arterial pressure dropped below 40 mm Hg until 60 mins of experimental time. After reperfusion, mean arterial pressure increased during the first 30 mins, and no differences were observed between the two experimental groups (Fig. 1A). Heart rate at baseline was similar between groups at the beginning (albumin: 369 ± 6 beats per minute [bpm], NaCl: 358 ± 16 bpm), increased significantly during systemic hypotension after 60 mins (albumin: 403 ± 13 bpm, NaCl: 396 ± 13 bpm, $p < .05$), and then plateaued until the end (albumin: 421 ± 8 bpm, NaCl: 414 ± 15 bpm).

Central venous pressure increased in both groups at the beginning of reperfusion and was significantly higher at 60 mins of reperfusion in the albumin group. After and additional 60 mins, central venous pressure equalized in both groups until the end (Fig. 1B).

Total volume substitution of group 1 (albumin, 36 ± 2 mL) was significantly lower vs. group 2 (NaCl, 60 ± 6 mL). The amount of albumin for blood pressure increase during the first 30 mins after reperfusion was significantly lower than the amount of NaCl substitution (Table 1; time interval 1–1.5 hrs: albumin 10 ± 0.2 mL, NaCl 18 ± 1 mL). Volume substitution of the time intervals at 3–4 and 4–5 hrs of reperfusion were not different between groups.

Abdominal Blood Flow. Abdominal blood flow measured in the descending aorta is depicted in Figure 2. There were no differences between the two groups at the beginning and after 1 hr of systemic hypotension (baseline values: albumin 50.2 ± 2.7 mL/min, NaCl 45.2 ± 3.0 mL/min; 60 min of shock: albumin: 20.7 ± 2.1 mL/min, NaCl: 19.9 ± 3.0 mL/min). Abdominal blood flow increased after reperfusion at 90 mins in both groups (albumin 44.8 ± 2.9 mL/min, NaCl 50.0 ± 1.1 mL/min) and was significantly higher in the albumin group at 60 and 120 mins after the beginning of reperfusion. (albumin 67.0 ± 3.9 and 64.4 ± 4.6 mL/min, NaCl 53.8 ± 3.4 and 49.1 ± 4.3 mL/min, at 60 and 120 mins, respectively).

Microcirculation

Velocity and Shear Rate. Measured venular diameters did not differ significantly during the experiment. Maximum velocity and shear rate at baseline were similar in both groups and decreased in parallel during systemic hypotension (maximum velocity: albumin 0.7 ± 0.1 mm/sec, NaCl 0.6 ± 0.1 mm/sec). Thirty minutes after reperfusion, maximum velocity exceeded baseline values in the albumin group (2.8 ± 0.2 mm/sec), whereas values in the NaCl group remained below baseline values (1.4 ± 0.4 mm/sec). Elevation of maximum velocity in the albumin group continued until the end of the experiment, and was significantly higher than the NaCl group (Fig. 3A). Shear rate recovered immediately in the albumin group and remained signifi-
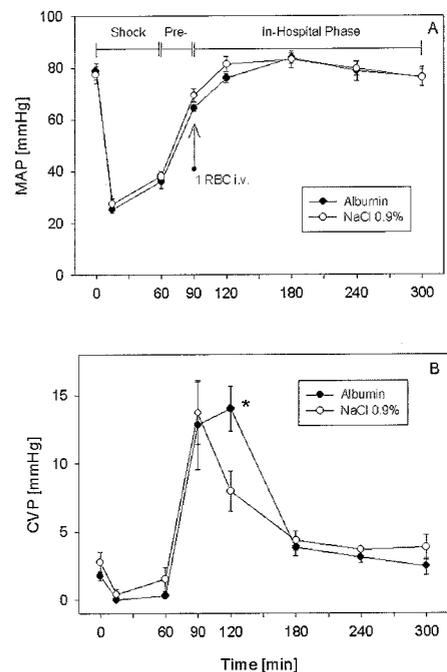

Figure 1. *A*, mean arterial blood pressure (*MAP*), and *B*, central venous pressure (*CVP*). Data for central venous pressure presented significantly higher values in the albumin group (*$p < .05$).

Table 1. Volume substitution during reperfusion in group 1 (albumin) and group 2 (NaCl) at different time intervals

	Time Interval					
Group	1–1.5 hrs	1.5–2 hrs	2–3 hrs	3–4 hrs	4–5 hrs	Total Volume
Group 1, albumin	10 ± 0.2 mL	10 ± 0.3 mL	5 ± 0.3 mL	5 ± 0.3 mL	5 ± 2 mL	36 ± 2 mL
Group 2, NaCl	18 ± 1 mL[a]	12 ± 1 mL	10 ± 2 mL[a]	11 ± 2 mL	9 ± 2 mL	60 ± 6 mL[a]

[a]$p < .05$, group 1 vs. group 2.

Figure 4. *A*, rolling leukocytes, and *B*, adherent leukocytes. Data for rolling and adherent leukocytes presented significantly lower values in the albumin group vs. the normal saline group (*$p < .05$).

cantly reduced in NaCl-treated animals (Fig. 3*B*).

Rolling and Adherent Leukocytes. 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 during hypotension, with further increase in the NaCl group over the next 4 hrs of reperfusion. The numbers of rolling leukocytes approximately doubled from baseline values in this group (Fig. 4*A*, $p < .05$) and the increase of adherent leukocytes was even more pronounced in the NaCl group (five to six times baseline values) in contrast to the albumin group (Fig. 4*B*, $p < .05$).

Arterial Blood Gas Values

Pao_2 values for both groups did not differ at the beginning (albumin 82 ± 3 mm Hg, NaCl 86 ± 2 mm Hg) and the end of the experiment (albumin 95 ± 3 mm Hg, NaCl 87 ± 2 mm Hg). Analogous values were obtained for Sao_2.

Arterial $Paco_2$ of all groups were similar at baseline (albumin 40.9 ± 0.8 mm Hg, NaCl 40.7 ± 0.6 mm Hg) and did not differ between groups throughout the experiments (albumin 30.5 ± 1.6 mm Hg, NaCl 34.2 ± 0.9 mm Hg). The decrease in $Paco_2$ during the experiments was significant in both groups ($p < .05$).

Base excess values mirrored the $Paco_2$ data and decreased in both groups significantly over time, without differences becoming apparent between the two groups (Fig. 5*A*). pH values were similar between the two groups (experiment beginning: albumin 7.36 ± 0.01, NaCl 7.37 ± 0.01; end: albumin 7.33 ± 0.01, NaCl 7.30 ± 0.01; NS).

Hematocrit values were comparable at baseline (albumin 49 ± 1.2%, NaCl 51 ± 0.5%) and decreased 60 mins after induction of hemorrhagic shock (Fig. 5*B*; albumin 33 ± 0.6%, NaCl 38 ± 0.6%, $p < .05$). Hematocrit values were significantly different between the albumin and NaCl groups
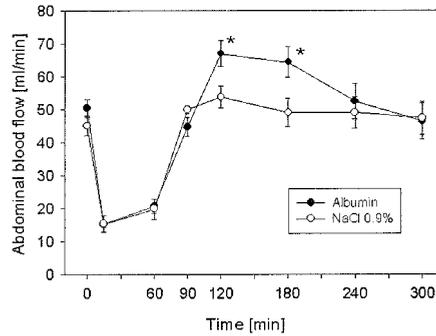

Figure 2. Abdominal blood flow. Abdominal blood flow was higher in the albumin group vs. normal saline between 120 and 180 mins (*$p < .05$).
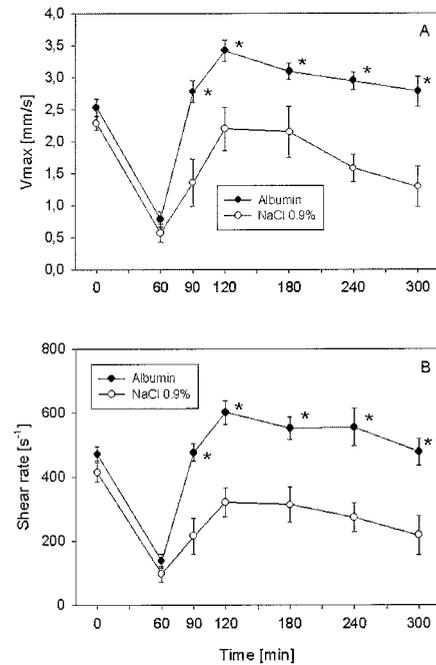

Figure 3. *A*, maximum velocity (*Vmax*), and *B*, shear rate. Values for maximum velocity and shear rate were significantly higher in the albumin group (*$p < .05$).

after the first 60 mins of reperfusion (albumin 23 ± 1.1%, NaCl 35 ± 4.4%, $p < .05$), which continued throughout 2 hrs of reperfusion. At the end of the "in-hospital"
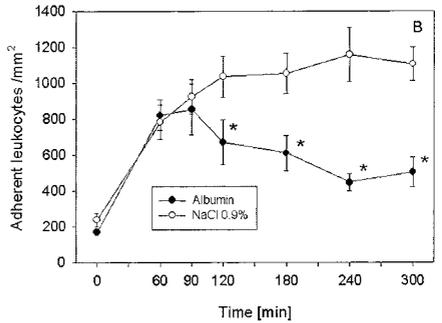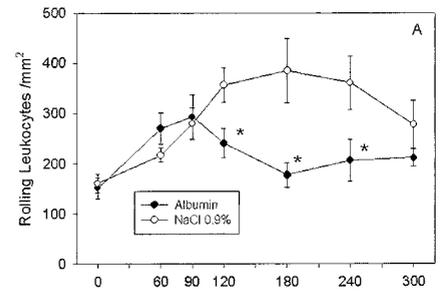
phase, hematocrit returned to approximately 30% in both groups.

DISCUSSION

The study shows that early albumin infusion in reperfusion of hemorrhagic shock is superior to normal saline. Albumin application, performed as a short-term infusion for 30 mins during initial reperfusion after systemic hypotension, improved mesenteric microcirculation and global hemodynamics throughout the complete follow-up period. The beneficial effect of albumin on mesenteric microcirculation was measured before the increase of abdominal blood flow and central venous pressure was observed. Mesenteric microcirculation was continuously enhanced, in contrast to the transient effect of central venous pressure, abdominal blood flow, and decrease of hematocrit during reperfusion. Initial reduction of volume substitution in the albumin group was balanced during the last 2 hrs of reperfusion. Enhanced reduction of adherent leukocytes from the beginning to the end of the reperfusion time showed the anti-inflammatory effect of albumin (5, 11–13). The increase of velocity to baseline values under albumin treatment during the complete reperfu-
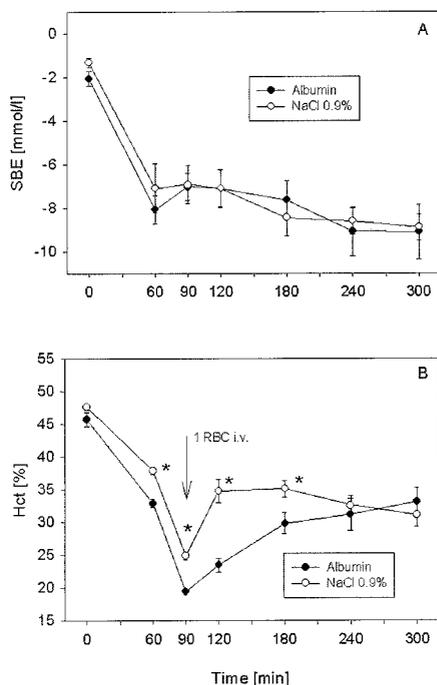
Figure 5. *A*, standard base excess (*SBE*), and *B*, hematocrit (*Hct*). Data for hematocrit were significantly different between groups between 60 and 180 mins (*$p < .05$).

sion mirrors the microcirculatory protection in contrast to crystalloid treatment.

The results of our study denied any deleterious effects on arterial oxygen content during the first hours of hemorrhagic shock therapy (3, 4). There was no alteration of oxygen diffusion throughout the whole follow-up period. The early reperfusion strategy with albumin infusion for treatment of hemorrhagic shock is likely to be quite different from those encountered in abdominal sepsis (14), when systemic inflammatory response syndrome is already completely developed and albumin infusion can worsen pulmonary function (1, 2).

Leukocyte-endothelium cell-cell interaction is known to be one important reaction at the beginning of an inflammatory response syndrome, parallel to contact activation and cytokine release (15–18). Reductions of adherent and rolling leukocytes in the mesenteric microcirculation after albumin administration suggest an anti-inflammatory effect in the treatment of hemorrhagic shock. This could be the result of multiple effects, such as free radical scavenger activity and suppression of the release of reactive oxygen species (11). Furthermore, it has been shown that albumin reduces adhesion of leukocytes to endothelial cells *in vitro* (12, 19), and inhibition of ischemia reperfusion-induced leukocyte-endothelial cell adhesion with polynitroxyl albumin was reported *in vivo* (13).

The reactions in volume-controlled shock (20) may be different from those in uncontrolled shock situations (21, 22). Reperfusion treatment in our study was pressure controlled (approximately 70 mm Hg after 30 mins of reperfusion). The observed intermittent increase of central venous pressure, hemodilution, and abdominal blood flow during reperfusion under albumin infusion could be a hyperoncotic effect with increase of intravascular volume. Further experiments and clinical studies are required to test whether early albumin infusion is truly beneficial in a clinical setting.

CONCLUSIONS

Albumin infusion at early reperfusion improved microcirculatory perfusion and global hemodynamics and reduced leukocyte-endothelium cell-cell interaction. Our results point to the potential clinical relevance of early albumin administration in patients with hemorrhagic shock.

ACKNOWLEDGMENTS

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