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Hydroxyethyl Starch (130 kD), but Not Crystalloid Volume Support, Improves Microcirculation during Normotensive Endotoxemia

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Background: **Increased leukocyte–endothelial cell interaction (LE) and deterioration of capillary perfusion represent key mechanisms of septic organ dysfunction. The type of volume support, however, which may be used during septic disorders, remains controversial. Using intravital microscopy, the authors studied the effect of different regimens of clinically relevant volume support on endotoxin-induced microcirculatory disorders, including the synthetic colloid hydroxyethyl starch (HES, 130 kD) and a crystalloid regimen with isotonic saline solution (NaCl).**

Methods: **In Syrian Golden hamsters, normotensive endotoxemia was induced by intravenous application of *Escherichia coli* lipopolysaccharide (LPS, 2 mg/kg). The microcirculation was analyzed in striated muscle of skinfold preparations. HES 130 kD (Voluven®, 16 ml/kg, n = 7) or isotonic saline (NaCl, 66 ml/kg, n = 6) were infused 3 h after LPS exposure over a 1-h period (posttreatment mode). Animals receiving LPS without volume therapy served as control subjects (n = 8, control). LE, functional capillary density (FCD), and macromolecular leakage were repeatedly analyzed in the awake animals during a 24-h period using intravital fluorescence microscopy.**

Results: **HES 130 kD significantly reduced LPS-induced arteriolar and venular leukocyte adherence ($P < 0.05$), whereas NaCl resuscitation had no effect when compared with nontreated control animals. The LPS-induced decrease in FCD and increase in macromolecular leakage were also significantly attenuated by HES 130 kD but not by NaCl. Improvement of LPS-induced microcirculatory disorders by HES was unlikely the result of macro- and microhemodynamic changes because arterial blood pressure, heart rate, and venular wall shear rate did not differ between HES- and NaCl-treated animals.**

Conclusions: **Thus, our study provides microhemodynamic and cellular mechanisms of HES 130 kD-mediated protection on microcirculation during endotoxemia, even when used in a clinically relevant posttreatment mode during normotensive conditions.**

IMPROVEMENTS in critical care medicine, including modern organ support devices, have not decreased overall the mortality of septic multiple organ dysfunction.[1] Besides removal of the septic focus, effective resuscitation combined with specific vasoconstrictor therapy is considered the standard of adjuvant therapy, aiming at optimized microvascular perfusion.[2,3] Thus, improvement of nutritional blood flow is thought to be essential to prevent tissue injury and subsequent development of septic multiple organ system failure.[4]

Despite extensive research in this area, there are no conclusive studies referring to the ideal type of volume support during septic disorders.[5] Currently, it is unclear whether a pure crystalloid resuscitation protocol is superior to an approach that includes synthetic colloids (*e.g.*, hydroxyethyl starch [HES] solutions) for volume resuscitation. Because human albumin solutions have been shown to increase mortality in critically ill patients, many intensive care unit (ICU) specialists abandoned human albumin solutions from their use during resuscitation in this cohort of patients.[6] In contrast to albumin, application of synthetic colloids, such as HES, induces a downregulation of inflammatory mediators *in vitro* and *in vivo*.[7–12] It is unclear, however, whether these antiinflammatory effects of HES are because of a more rapid and effective improvement of nutritional blood flow or result from direct blockade of sepsis-associated inflammatory processes.[10–12]

Since their first extensive use during World War II, HES solutions have been optimized to (1) guarantee a stable volume effect, (2) lower plasma accumulation and tissue deposition, and (3) increase renal excretion. These modifications influence plasma half-life, antiinflammatory properties, and incidence of side effects. Because of its low molecular weight (130 kD), the preparation of HES used in the present study is unlikely to accumulate in plasma and tissue. Moreover, clinical application revealed a low incidence of anaphylactic reactions and coagulatory disorders and also promising antiinflammatory properties when compared with other HES and dextran solutions.[13,14]

Given that leukocyte–endothelial cell interaction (LE) and capillary perfusion failure represent key mechanisms of septic organ dysfunction, we analyzed microhemodynamic and cellular *in vivo* HES 130 kD effects in comparison with a crystalloid protocol. Because reversal of systemic hypotension would also influence microcirculatory disorders and thus would not allow discrimination between the effects of HES 130 kD on macrohemodynamics and microcirculation *per se*, we tested the efficacy of HES 130 kD under normotensive conditions

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by using an endotoxin dose, which exerts LE and capillary dysfunction but not systemic hypotension and shock.

Materials and Methods

All experiments adhered to the international standards for good animal care and laboratory practice and were performed in line with the National Institutes of Health guidelines on the use of experimental animals. The study was approved by the Landratsamt Homburg/Saar, Saarland, Germany (K110/180–07).

Animals

Six- to eight-week-old Syrian golden hamsters with a body weight of 60–80 g were used for the study. The animals were housed one per cage and had free access to tap water and standard pellet food (Altromin, Lage, Germany) throughout the experiment.

Experimental Protocol

To investigate the effects of HES and crystalloids (isotonic saline, NaCl) during endotoxemia, one group of animals (n = 7, HES group) was assigned to receive intravenous endotoxin (lipopolysaccharide [LPS]; 2 mg/kg body weight each, *Escherichia coli* serotype 0128:B12, Sigma, Deisenhofen, Germany) directly after monitoring of baseline parameters (0 h). In these animals, HES (Voluven®, 6% HES 130 kD, 16 ml/kg, Fresenius Kabi Deutschland GmbH, Bad Homburg, Germany) was given 3 h after LPS injection during a 1-h period. In a second group of animals (n = 6, crystalloid application with 0.9% isotonic saline solution [NaCl]), intravenous LPS was given (2 mg/kg body weight each, *E. coli* serotype 0128:B12), but isotonic NaCl solution (66 ml/kg) instead of HES 130 kD was administered over a 1-h period starting 3 h after LPS exposure. In a third group of animals (n = 8, control group [control]), LPS (2 mg/kg body weight each) was given without additional volume support. In all three groups, analysis of microcirculation by intravital microscopy was performed at baseline (before LPS, 0 h) and 30 min, 3 h, 4 h, 8 h, and 24 h after LPS exposure.

Microcirculation Model

For intravital fluorescence microscopy, we used the dorsal skinfold chamber preparation, which contains one layer of striated muscle and skin and allows for quantitative analysis of the microcirculation in the awake animal over a prolonged period.[15] Microcirculatory analysis included LE (rolling and adherence of leukocytes) in arterioles and venules, capillary perfusion (functional capillary density), and microhemodynamics in postcapillary venules.[16]

The chamber and its implantation procedure have been described previously by Endrich *et al.* in detail.[17] The preparation used in this study was similar, except for minor modifications, and has been described also in earlier reports.[18] Briefly, during sodium pentobarbital anesthesia (50 mg/kg body weight intraperitoneally; Nembutal, Abbott, Wiesbaden, Germany), the animals were fitted with two symmetrical titanium frames positioned on the dorsal skinfold, sandwiching the extended double layer of skin. One layer of skin was completely removed in a circular area of 15 mm diameter, and the remaining layers (consisting of striated skin muscle and subcutaneous tissue) were covered with a removable cover slip incorporated into one of the titanium frames. Fine polyethylene catheters (PE10, 0.28 mm internal diameter) were inserted into the jugular vein, passed subcutaneously to the dorsal site of the neck, and fixed to the titanium frames. The chambers and catheters were implanted at least 72 h before the experiment. The animals tolerated the chambers and catheters well and showed no signs of discomfort. In particular, no effects on sleeping and feeding habits were observed.

Intravital Fluorescence Microscopy

For the *in vivo* microscopic analysis, the awake hamsters were immobilized in plexiglas tubes, and the skinfold preparation was attached to the microscope stage. Then the stage was placed on a computer-controlled microscope desk, which allowed repeated scanning of identical segments of microvessels during the experiment. After intravenous injection of 0.2 ml of 5% fluorescein isothiocyanate (FITC)-labeled dextran (MW 150 kD; Pharmacia, Uppsala, Sweden) and *in vivo* staining of leukocytes by 0.2 ml of 0.1% rhodamin 6G (Sigma Chemical Co., Deisenhofen, Germany), intravital microscopy was performed using a modified Leitz Orthoplan microscope with a 100-W HBO mercury lamp attached to a Ploemo-Pak illuminator with an I2 blue and a N2 green filter block (Leitz, Wetzlar, Germany) for epi-illumination. The observations were recorded by means of a charge-coupled device video camera (FK 6990, Cohu, Prospective Measurements, San Diego, CA) and transferred to a video system for off-line evaluation. The microscopic images were recorded on videotape and analyzed during playback by using special computer software (CAPIMAGE version 6.0, Dr. Heinz Zeintl, Dipl. eng., Zeintl Company, Heidelberg, Germany). A 25-fold water immersion objective (Leitz Inc., Wetzlar, Germany), resulting in a total magnification of ×690 at a 14-inch monitor screen, was used to select at least four regions of interest per chamber, whereby each site contained at least six postcapillary or collecting venules, three precapillary arterioles, and a network of nutritive capillaries. These regions were repeatedly analyzed over a 24-h period.

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Microcirculatory Analysis

Vessel diameters (μm) were analyzed by length measurements, orientated perpendicularly to the vessel path (CAPIMAGE). As an indicator of endothelial integrity (microvascular permeability), macromolecular leakage of FITC dextran was densitometrically analyzed in identical vessel segments by means of CAPIMAGE 10 min after intravenous injection of the high molecular weight FITC dextran. Leakage was quantified as the ratio of fluorescence intensity outside the vessel segment *versus* that inside the vessel segment.[16]

Functional capillary density (FCD), which served as the measure of quality of microvascular perfusion, was defined as the length of all erythrocyte-perfused nutritive capillaries per observation area (CAPIMAGE) and is given in cm^{-1}.[19]

To further characterize microhemodynamic changes, erythrocyte velocity in postcapillary venules was measured in the center line of the microvessels using CAPIMAGE. Individual venular blood flow was then calculated from vessel diameter and erythrocyte velocity, including a Baker–Wayland factor of 1.6. Venular wall shear rate (γ) was calculated based on the Newtonian definition: $\gamma = 8 \times V/D$, where V represents the erythrocyte velocity of the individual microvessel and D is the respective diameter. Arteriolar erythrocyte velocities always exceeded 2 mm/s and, therefore, could not be analyzed by the off-line technique used.

Leukocytes were classified as rolling or adherent cells, according to their interaction with the microvascular endothelium. Rolling leukocytes were defined as cells moving along the endothelial lining at a velocity slower than two fifths of the surrounding red cell column and are given as percentage of leukocytes passing the observed vessel segment within 20 s.[18] Adherent leukocytes were defined as cells that did not detach from the endothelial lining within a 20-s observation period and are given as number of cells/mm^2 of endothelial surface, calculated from the respective vessel diameter and length (100 μm) of the vessel segment studied, assuming cylindrical geometry.

Macrohemodynamic Analysis

Because macrohemodynamic effects of HES and NaCl could not be evaluated during the intravital microscopic procedure, three additional groups of animals were studied. One group of animals (n = 4, macrohemodynamic HES group [mHES]) was assigned to receive intravenous endotoxin (LPS, 2 mg/kg body weight each) directly after monitoring of baseline parameters (0 h). HES (Voluven®, 6% HES 130 kD; 16 ml/kg) was given 3 h after LPS injection over a 1-h period. In a second group of animals (n = 4, macrohemodynamic crystalloid group [mNaCl]), intravenous LPS was given (LPS, 2 mg/kg body weight each), but isotonic NaCl solution (66 ml/kg) instead of HES 130 kD was administered over a 1-h period starting at 3 h after LPS exposure. In a third group of animals (n = 4, macrohemodynamic control group [mControl]), LPS was given without additional volume support. Mean arterial blood pressure (MAP) and heart rate (HR) were measured at baseline (0 h) and 1 h, 3 h, 4 h, 8 h, and 24 h after LPS injection *via* carotid artery catheters, which had been inserted during pentobarbital anesthesia (50 mg/kg).

Laboratory Analysis

In HES, NaCl, and control animals, laboratory analysis was performed at the end of the observation period (24 h) in plasma samples obtained by laparotomy and blood withdrawal from the abdominal aorta during sodium pentobarbital anesthesia. Repeated blood sampling was not possible because of the limited circulating blood volume of the hamster. Therefore, 8-h laboratory analysis in the three treatment groups, corresponding to the most pronounced effect of LPS on leukocyte adherence, had to be obtained from additional animals (HES 8 h [n = 4], NaCl 8 h [n = 4], Control 8 h [n = 4]), which were treated similar as the HES, NaCl, and control animals. Blood gas analysis (BGA 348, Chiron Diagnostics, San Diego, CA) was performed in heparinized samples. Total erythrocyte, leukocyte, and platelet counts and hemoglobin were determined by a Coulter Counter (AcTdiff, Coulter, Bad Homburg, Germany). Analysis of laboratory parameters in blood from eight healthy animals without treatment served as normal values.

Statistical Analysis

All parameters are given as mean ± SD. If not indicated otherwise, mean values of the observed microscopic parameters were calculated for each animal (> 6 venules, > 3 arterioles per site of observation), and statistical analysis was performed between HES, NaCl, and control animals by using the mean values of the respective animals. To detect significant differences between experimental groups at the different time points, a one-way analysis of variance (ANOVA) was performed if the Levene test (test of equality of variance) did not show significance. If there were unequal variances, the Kruskal–Wallis one-way ANOVA was performed. *Post hoc* comparisons were performed with the Student–Newman–Keuls test (equal variance) or the Mann–Whitney U test (unequal variance). Accordingly, significant differences within the respective experimental groups between the different time points to baseline were tested by paired Student *t* test or paired Wilcoxon test, including Bonferroni probabilities to correct for repeated measurements. A significance level of $P < 0.05$ was used.

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Table 1. Effect of Resuscitation with Hydroxyethyl Starch (HES) and Isotonic Saline (NaCl) on Arteriolar and Venular Diameters during Endotoxemia

	Arteriolar Diameters (μm)			Venular Diameters (μm)		
	HES	NaCl	Control	HES	NaCl	Control
Baseline	33.7 ± 9.9	38.8 ± 15.3	41.6 ± 16.1	32.9 ± 5.2	37.8 ± 7.3	37.4 ± 5.4
30 min	33.2 ± 7.1	41.2 ± 17.1	40.0 ± 18.0	35.3 ± 5.0	40.5 ± 8.9	37.9 ± 4.6
3h	32.7 ± 8.5	40.5 ± 17.2	39.4 ± 14.4	37.6 ± 5.3†	50.5 ± 16.2†	42.6 ± 8.5†
4h	31.9 ± 9.6	38.2 ± 15.6	34.8 ± 17.6	36.8 ± 4.9†*	47.7 ± 12.1	50.8 ± 8.0†
8h	31.1 ± 8.9	39.4 ± 10.0	34.7 ± 12.6†	34.3 ± 5.5	43.2 ± 9.5	40.5 ± 7.3
24h	32.8 ± 13.9	46.2 ± 15.1	40.6 ± 18.7	29.7 ± 5.5*	44.2 ± 9.4	36.6 ± 8.2

Data are mean ± SD. HES represents animals (n = 7) that received LPS (2 mg/kg body wt) and HES (16 ml/kg body wt/1h) 3h after LPS exposure, whereas NaCl represents animals (n = 6) that received LPS (2 mg/kg body wt) and isotonic saline (66 ml/kg body wt/1h) 3h after the induction of endotoxemia. Control represents animals (n = 8) which received LPS (2 mg/kg body wt) only.

* = significant differences ($P < 0.05$) of HES *versus* control and NaCl animals (ANOVA, Student Newman–Keuls test). † = significant intragroup differences ($P < 0.05$) from baseline (paired Student *t* test).

Results

Effect of Hydroxyethyl Starch and Crystalloid Volume Support on Arteriolar and Venular Diameters

At baseline, arteriolar and venular diameters did not differ between HES, NaCl, and control animals (control) (table 1). There was no significant difference in arteriolar diameters between HES, NaCl, and control animals at the different time points analyzed (table 1).

In venules, LPS induced an approximate 20% increase in diameter, which was significant after 4 h in control animals. HES, but not NaCl, significantly reduced this increase in venular diameters at 4 h and 24 h after LPS exposure.

Effect of Hydroxyethyl Starch and Crystalloid Volume Support on Rolling Leukocyte Fraction in Arterioles and Venules

At baseline, in all experimental groups, 0.3–4% of leukocytes showed loose interaction with the vascular endothelium of arterioles, representing the fraction of spontaneously rolling leukocytes (table 2). In all groups, LPS administration decreased arteriolar and venular leukocyte rolling fraction at 30 min after LPS exposure, which correlated well with peripheral leukopenia. Thereafter, there was an impressive increase in leukocyte rolling, which was most pronounced in control animals. HES and NaCl treatment showed significantly lower values of arteriolar leukocyte rolling at 4 h and 8 h after LPS administration (table 2). After 24 h, HES treatment produced a decrease in rolling fraction to baseline conditions, whereas NaCl and control animals still showed 10–30% rolling leukocytes (table 2).

In venules, baseline analysis revealed that 20–30% of leukocytes have loose interaction with the vascular endothelium (table 2). After a decrease in rolling fraction at 30 min, a secondary increase in venular leukocyte rolling fraction with maximum values after 8 h and 24 h of endotoxemia was observed. HES and NaCl administration did not significantly reduce venular leukocyte rolling when compared with nontreated control animals.

Effect of Hydroxyethyl Starch and Crystalloid Volume Support on Adherent Leukocyte Counts in Arterioles and Venules

The number of adherent leukocytes in arterioles and venules at baseline did not significantly differ between the three experimental groups (figs. 1 and 2). In control

Table 2. Rolling Leukocytes in Arterioles and Venules during Resuscitation with Hydroxyethyl Starch (HES) and Isotonic Saline (NaCl) during Endotoxemia

	Rolling Leukocytes in Arterioles (%)			Rolling Leukocytes in Venules (%)		
	HES	NaCl	Control	HES	NaCl	Control
Baseline	0.3 ± 0.4	3.0 ± 1.5	4.6 ± 6.2	23.6 ± 14.3	17.5 ± 7.1	30.8 ± 28.0
30 min	0.0 ± 0.0	0.3 ± 0.8	0.0 ± 0.0	0.0 ± 0.0†	3.0 ± 6.4‡	5.1 ± 7.4†
3h	2.0 ± 3.4	3.0 ± 6.9	5.6 ± 13.1	39.7 ± 33.6	24.3 ± 26.7	29.6 ± 26.2
4h	13.0 ± 26.6*	4.3 ± 10.1*	54.3 ± 41.7	53.7 ± 39.3	20.0 ± 21.0*	63.0 ± 10.2†
8h	39.4 ± 43.1*	39.4 ± 29.3*†	71.6 ± 35.4†	84.5 ± 20.7†	58.4 ± 29.8†	73.8 ± 19.5‡
24h	1.0 ± 2.0*	33.8 ± 34.6†	11.4 ± 27.0	64.8 ± 12.4‡	72.8 ± 26.1†	79.2 ± 10.0†

Data are mean ± SD. HES represents animals (n = 7) that received LPS (2 mg/kg body wt) and HES (16 ml/kg body wt/h) 3h after LPS exposure, whereas NaCl represents animals (n = 6) that received LPS (2 mg/kg body wt) and isotonic saline (66 ml/kg body wt/h) 3h after induction of endotoxemia. Control represents animals (n = 8) that received LPS (2 mg/kg body wt) only.

* = significant differences ($P < 0.05$) of HES and NaCl *versus* control (Kruskal–Wallis ANOVA, Mann–Whitney U test). † (paired Wilcoxon test) and ‡ (paired Student *t* test) indicate significant intragroup differences ($P < 0.05$) from baseline.

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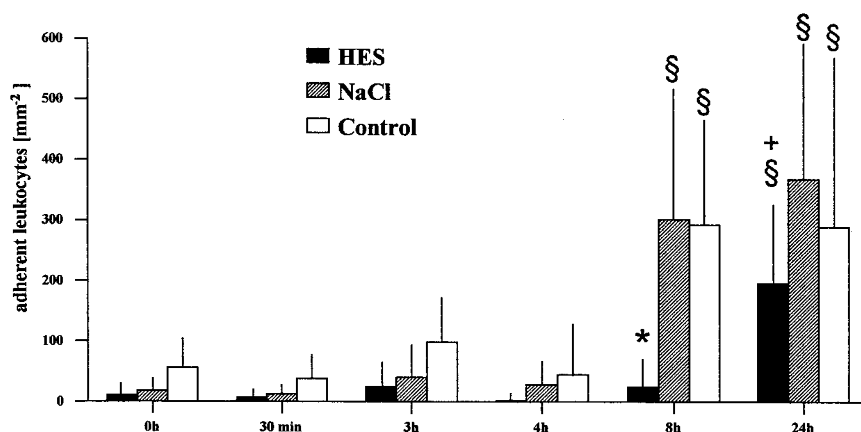

Fig. 1. Effect of hydroxyethyl starch (HES) and isotonic saline (NaCl) resuscitation on lipopolysaccharide (LPS)-induced arteriolar leukocyte adherence is shown. LPS (n = 8, control [white bars]) significantly increased arteriolar leukocyte adherence (adherent leukocytes, leukocytes that did not detach from the endothelial cell lining within a 20-s observation period [given as number of cells/mm^2]) with a peak at 8 h and 24 h after LPS administration. HES (n = 7 [black bars]) significantly attenuated LPS-induced leukocyte adherence, whereas isotonic saline (n = 6, NaCl [dashed bars]) did not influence LPS-induced leukocyte adherence compared with control group. Data are mean ± SD. $^+$Indicates significant differences (P < 0.01) of HES *versus* NaCl (ANOVA, Student–Newman–Keuls test), and *indicates significant differences (P < 0.05) of HES *versus* NaCl and control (Kruskal–Wallis ANOVA, Mann–Whitney U test). §Indicates significant intragroup differences (P < 0.05) from baseline (paired Wilcoxon test).

and NaCl animals, LPS administration led to a profound increase of arteriolar leukocyte adherence over time. At 8 h after LPS exposure, this increase of leukocyte adherence was significantly attenuated by HES 130 kD treatment (fig. 1). Also at 24 h, the number of adherent leukocytes in the arterioles was lower after HES treatment when compared with NaCl-treated animals (fig. 1).

In venules, LPS exposure produced an approximate 10-fold increase in leukocyte adherence (control animals). Crystalloid volume administration (NaCl) tended to even further increase venular leukocyte adherence when compared with control animals, whereas HES 130 kD treatment produced a significant decrease in the number of adherent leukocytes at 8 h and 24 h (fig. 2).

Effect of Hydroxyethyl Starch and Crystalloid Volume Support on Functional Capillary Density

Functional capillary density did not differ significantly between HES 130 kD, NaCl, and control animals at baseline. In control animals, LPS administration significantly reduced FCD by approximately 50% at 24 h (fig. 3). Crystalloid volume support (NaCl) did not improve FCD when compared with control animals. In contrast, HES 130 kD prevented this severe decrease in capillary perfusion with a FCD of approximately 80–90% of baseline values.

Effect of Hydroxyethyl Starch and Crystalloid Volume Support on Erythrocyte Velocity, Wall Shear Rate, and Blood Flow in Postcapillary Venules

Erythrocyte velocity did not differ significantly between HES, NaCl, and control animals at baseline (table 3). In all groups, erythrocyte velocity was found significantly reduced at 30 min and 3 h after LPS exposure, although without significant differences between the groups. Erythrocyte velocities progressively recovered until 8 h in all groups. Strikingly, at 24 h, HES 130 kD- and NaCl-treated animals showed a significant secondary increase in erythrocyte velocity compared with baseline values, although this difference did not prove statistical significance compared with the control group, which is probably because of the high SD (table 3).

At baseline, venular wall shear rate did not differ significantly between the experimental groups (table 4). In

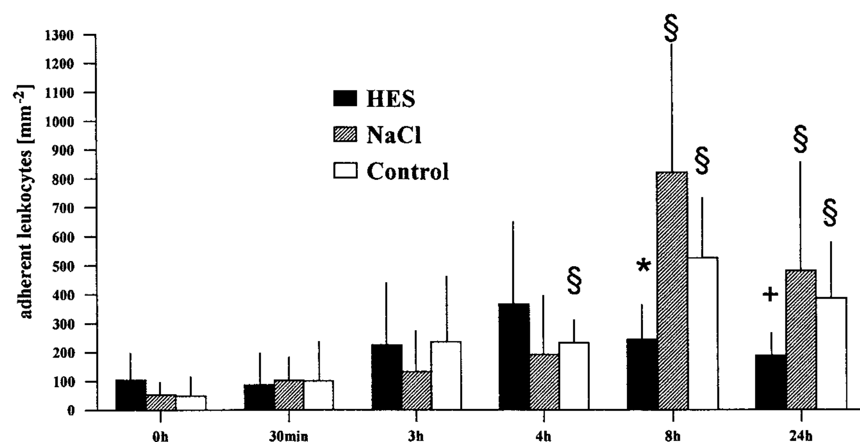

Fig. 2. Effect of hydroxyethyl starch (HES) and isotonic saline (NaCl) resuscitation on lipopolysaccharide (LPS)-induced venular leukocyte adherence is shown. LPS (n = 8, control [white bars]) significantly increased venular leukocyte adherence (adherent leukocytes, leukocytes that did not detach from the endothelial cell lining within a 20-s observation period [given as number of cells/mm^2]) with a peak at 8 h after LPS administration. HES (n = 7 [black bars]) significantly attenuated LPS-induced leukocyte adherence. Isotonic saline (n = 6, NaCl [dashed bars]) tended to increase leukocyte adherence when compared with control group. Data are mean ± SD. *Indicates significant differences (P < 0.05) of HES *versus* control and NaCl (ANOVA, Student–Newman–Keuls test), $^+$indicates significant differences (P < 0.05) of HES *versus* NaCl (Kruskal–Wallis ANOVA, Mann–Whitney U test), and §indicates significant intragroup differences (P < 0.05) from baseline (paired Wilcoxon test).

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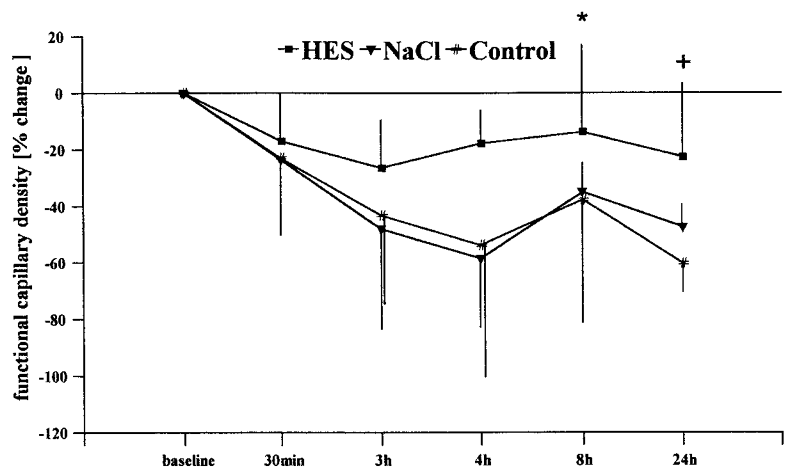

Fig. 3. Effect of hydroxyethyl starch (HES) and isotonic saline (NaCl) resuscitation on lipopolysaccharide (LPS)-induced microvascular perfusion injury is shown. LPS (n = 8, control) significantly decreased functional capillary density (measured as the length of all erythrocyte-perfused nutritive capillaries per observation area and given in cm^{-1}) by about 50%. HES (n = 7) significantly attenuated LPS-induced microvascular perfusion injury over time, whereas crystalloid application (n = 6, NaCl) failed to attenuate microvascular perfusion injury as indicated by functional capillary densities (FCDs) in the range of control animals. Data are mean ± SD. *Indicates significant differences ($P < 0.05$) of HES *versus* NaCl and control animals (Kruskal–Wallis ANOVA, Mann–Whitney U test), and $^{+}$indicates significant differences ($P < 0.05$) of HES *versus* control animals (Kruskal–Wallis ANOVA, Mann–Whitney U test).

all groups, venular wall shear rates were significantly reduced at 30 min and 3 h after LPS exposure, although without significant differences between the groups. There was no acute effect of HES 130 kD and NaCl on shear rates at 4 h. Thereafter, venular wall shear rates comparably increased until 8 h in all experimental groups. At 24 h after LPS exposure, HES 130 kD- and NaCl-treated animals, but not control animals, revealed a significant increase in venular wall shear rate, which was almost twofold higher compared with baseline values (table 4).

At baseline, venular blood flow did not differ between HES, NaCl, and control animals (table 5). In all groups, blood flow in venules was found reduced at 30 min after LPS exposure but recovered progressively until 3 h. At 24 h, HES 130 kD- and NaCl-treated animals showed a significant increase of venular blood flow when compared with baseline values, which led to significantly higher flows in NaCl animals compared with control animals (table 5).

Effect of Hydroxyethyl Starch and Crystalloid Volume Support on Macromolecular Leakage in Venules

Lipopolysaccharide administration produced a significant increase in macromolecular leakage in venules when compared with baseline values (mean value of increase, 25–30% after 8 h). Treatment with HES 130 kD was associated with a significantly reduced venular leakage (mean value of increase, 10%) at 8 h and 24 h compared with control animals, whereas crystalloid fluid replacement (NaCl-treated animals) showed increased venular leakage compared with control animals as early as 1 h after treatment (t = 4 h, fig. 4). This more pronounced increase in venular leakage persisted until 8 h, whereas at 24 h leakage values of NaCl-treated animals were comparable with those of control animals.

Effect of Hydroxyethyl Starch and Crystalloid Volume Support on Macrohemodynamics

At baseline, HR and MAP were comparable between mHES, mNaCl, and mControl animal groups (table 6).

Table 3. Venular RBC-Velocity during Resuscitation with Hydroxyethyl Starch (HES) and Isotonic Saline (NaCl) during Endotoxemia

	RBC-Velocity (μm/s)		
	HES	NaCl	Control
Baseline	243 ± 129	216 ± 68	293 ± 87
30 min	138 ± 105*	111 ± 98*	145 ± 134*
3h	155 ± 163*	151 ± 224*	165 ± 55*
4h	234 ± 103	161 ± 237	220 ± 99
8h	291 ± 171	305 ± 219	238 ± 132
24h	523 ± 144†	553 ± 272†	248 ± 105

Data are mean ± SD. HES represents animals (n = 7) that received LPS (2 mg/kg body wt) and HES (16 ml/kg body wt/1h) 3h after LPS exposure, whereas NaCl represents animals (n = 6) that received LPS (2 mg/kg body wt) and isotonic saline (66 ml/kg body wt/1h) 3h after induction of endotoxemia. Control represents animals (n = 8) that received LPS (2 mg/kg body wt) only.

* (paired Wilcoxon test) and † (paired Student *t* test) indicate significant intragroup differences ($P < 0.05$) from baseline.

Table 4. Venular Wall Shear Rate during Resuscitation with Hydroxyethyl Starch (HES) and Isotonic Saline (NaCl) during Endotoxemia

	Wall Shear Rate (s^{-1})		
	HES	NaCl	Control
Baseline	55 ± 25	40 ± 24	67 ± 29
30 min	30 ± 24*	15 ± 15*	39 ± 24*
3h	29 ± 28*	21 ± 32*	33 ± 8†
4h	46 ± 21	35 ± 37	36 ± 28
8h	64 ± 40	46 ± 29	54 ± 31
24h	132 ± 56†	93 ± 55†	60 ± 34

Data are mean ± SD. HES represents animals (n = 7) that received LPS (2 mg/kg body wt) and HES (16 ml/kg body wt/h) 3h after LPS exposure, whereas NaCl represents animals (n = 6) that received LPS (2 mg/kg body wt) and isotonic saline (66 ml/kg body wt/1h) 3h after induction of endotoxemia. Control represents animals (n = 8) that received LPS (2 mg/kg body wt) only.

* (paired Wilcoxon test) and † (paired Student *t* test) indicate significant intragroup differences ($P < 0.05$) from baseline.

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Table 5. Venular Blood Flow during Resuscitation with Hydroxyethyl Starch (HES) and Isotonic Saline (NaCl) during Endotoxemia

	Venular Blood Flow (nL/s)		
	HES	NaCl	Control
Baseline	0.16 ± 0.12	0.23 ± 0.06	0.20 ± 0.09
30 min	0.10 ± 0.07‡	0.13 ± 0.13†	0.11 ± 0.10‡
3h	0.14 ± 0.17	0.25 ± 0.34	0.15 ± 0.09
4h	0.19 ± 0.09	0.21 ± 0.29	0.17 ± 0.11
8h	0.20 ± 0.10	0.30 ± 0.39	0.15 ± 0.08
24h	0.30 ± 0.14‡	0.59 ± 0.31*†	0.15 ± 0.03

Data are mean ± SD. HES represents animals (n = 7) that received LPS (2 mg/kg body wt) and HES (16 ml/kg body wt/h) 3h after LPS exposure, whereas NaCl represents animals (n = 6) that received LPS (2 mg/kg body wt) and isotonic saline (66 ml/kg body wt/h) 3h after induction of endotoxemia. Control represents animals (n = 8) that received LPS (2 mg/kg body wt) only.

* = significant differences ($P < 0.05$) of NaCl *versus* control (Kruskal–Wallis ANOVA, Mann–Whitney U test). † (paired Wilcoxon test) and ‡ (paired Student *t* test) indicate significant intragroup differences ($P < 0.05$) from baseline.

LPS exposure did not induce significant changes in HR and MAP over time. Accordingly, there were no significant differences between the experimental groups at the different time points studied (table 6).

Effect of Hydroxyethyl Starch and Crystalloid Volume Support on Laboratory Parameters

The HES, NaCl, and control animals did not show major disturbances in blood gas analysis, Ca^{2+} and K^{+} concentrations, and platelet count after 8 h of endotoxemia, the time point at which the most pronounced leukocytic response was observed (table 7). HES 8 h, NaCl 8 h, and control 8 h animal groups showed a decrease in total leukocyte count and Na^{+} concentrations. In contrast, only saline resuscitation significantly decreased K^{+} concentration, erythrocyte count, and hemoglobin concentration.

At 24 h, there were no differences in erythrocyte count, hemoglobin concentration, and total leukocyte count (table 8). HES 130 kD treatment increased P_{O_2} and produced a tendency toward lower P_{CO_2} levels when compared with NaCl-treated animals and nontreated control animals. The LPS-induced decrease in platelet count was prevented neither by HES 130 kD nor by NaCl, as reflected by significantly lower values in all experimental groups when compared with normal values.

Discussion

Leukocyte-endothelial cell interaction is a prerequisite for transendothelial migration and tissue infiltration.[4,20] During septic disorders, endothelial activation results in increased neutrophil rolling along the endothelium and subsequent adherence (firm interaction) with concurrent release of various cytotoxic agents.[21] Thereby, the endothelium is injured and induces a further increase of leukocyte adherence, resulting in a marked deterioration of the microcirculation.

The herein-used normotensive endotoxemia model simulates sepsis without relevant macrohemodynamic disturbances.[22] This implies that the observed increase in LE and the impairment of capillary perfusion are not primarily mediated by endotoxin-induced hypotension or septic shock but are more likely caused by a direct effect of endotoxin on the microcirculation. In the present study, relevant macrohemodynamic changes during resuscitation could be excluded by repeated measurements of hemodynamics in all experimental groups. Astonishingly, we could also not detect a relevant systemic blood pressure increase during the HES 130 kD and NaCl infusion. The lack of response to volume support may be the result of a nonapparent change in endothelial properties after endotoxin exposure. This explanation, however, is not likely because nonvolume-treated control animals did not show a decrease in systemic blood pressure on endotoxin exposure. The fact that endotoxin-induced macrohemodynamic changes were clearly absent without intergroup differences in the model used indicates that microcirculatory effects

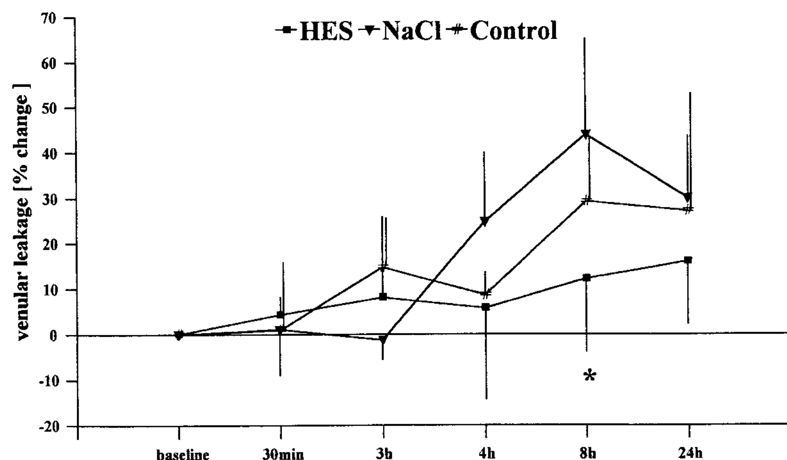

Fig. 4. Effect of hydroxyethyl starch (HES) and isotonic saline (NaCl) resuscitation on lipopolysaccharide (LPS)-induced venular leakage is shown. LPS (n = 8, control) significantly increased venular leakage (measured as the extravasation of the macromolecular fluorescein isothiocyanate [FITC] dextran) by about 30% during the 24-h observation period. HES (n = 7) significantly attenuated LPS-induced FITC dextran extravasation, whereas crystalloid application (n = 6, NaCl) further increased leakage after 1 h (time point, 4 h) and 5 h (time point, 8 h) of treatment and showed FITC extravasation in the range of control animals after 24 h. Data are mean ± SD. *Indicates significant differences ($P < 0.05$) of HES *versus* NaCl and control animals (Kruskal–Wallis ANOVA, Mann–Whitney U test).

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Table 6. Macrohemodynamic Effects of Resuscitation with Hydroxyethyl Starch (HES) and Crystalloid Volume Support (NaCl) during Endotoxemia

	MAP			HR		
	HES	NaCl	Control	HES	NaCl	Control
Baseline	97 ± 7	101 ± 7	110 ± 11	350 ± 61	359 ± 85	360 ± 56
1h	100 ± 8	103 ± 7	102 ± 14	339 ± 79	427 ± 92	340 ± 53
3h	106 ± 18	93 ± 4	108 ± 13	413 ± 29	373 ± 53	349 ± 45
4h	97 ± 19	92 ± 16	111 ± 11	402 ± 75	379 ± 109	405 ± 59
8h	94 ± 11	100 ± 13	102 ± 12	369 ± 88	372 ± 136	390 ± 49
24h	87 ± 10	89 ± 19	109 ± 14	372 ± 93	344 ± 113	375 ± 62

Data are mean ± SD. HES represents animals (n = 5) that received LPS (2 mg/kg body wt) and HES (16 ml/kg body wt/h) 3h after the induction of endotoxemia, whereas mNaCl represents animals (n = 5) that received LPS (2 mg/kg body wt) and isotonic saline (66 ml/kg body wt/h) 3h after induction of endotoxemia. Control animals (n = 4) received LPS (2 mg/kg body wt) without volume. Mean arterial pressure (MAP) and heart rate (HR) were measured via carotid artery catheters. There were no significant differences between the groups at the respective time points. Also, intragroup comparisons to baseline did not show significant changes.

exerted by volume support are not caused by reversal of macrohemodynamic disturbances.

To increase the clinical relevance of the experimental protocol, a posttreatment mode of therapy, beginning 3 h after LPS exposure, was chosen. To study the mechanisms and efficacy of antiinflammatory drugs in endotoxemia, the use of the skinfold chamber and intravital microscopy has to be considered an ideal tool because this model allows repeated analysis of microvascular LE and capillary perfusion impairment in the awake animal over a prolonged period.[16,18,22–24] It may be argued that the study of the microcirculation of skin muscle and subcutaneous tissue may not necessarily be representative for endotoxin-induced microcirculatory disorders in vital organs such as the heart, the lung, and the liver. However, it seems reasonable to suppose that general mechanisms of endotoxin-induced leukocyte and endothelial cell activation exist in different target organs despite potential differences in quantity and time kinetics of endotoxin response in the microvasculature.[25] Besides, only intravital microscopy of peripheral tissue in the awake animal allows this long-term experimental setting (24 h), excluding side effects of anesthesia and surgical trauma on the inflammatory response.[22]

Endotoxin exposure initially induced a rapid leukopenia, which was observed as early as 30 min after LPS administration. Later, however, there was an impressive upregulation of LE with increased leukocyte rolling and adherence, which was associated with a deterioration of capillary perfusion. Although the postcapillary venules are considered the primary target for LE in inflammation, the present experiments demonstrate leukocyte adherence not only in venules but also in arterioles. This is in line with data of previous experiments[22] and indicates that in contrast to an unspecific inflammatory challenge, such as ischemia–reperfusion,[18] LPS may induce a more specific endothelial activation, targeting not only venules but also arterioles.

The HES 130 kD significantly down-regulated the increase in arteriolar and venular leukocyte adherence. Amazingly, a corresponding amount of crystalloid solution (isotonic saline) did not produce such protection. Because the animals were hemodynamically stable without intergroup differences, it is unlikely that the reduc-

Table 7. Laboratory Parameters during Resuscitation with Hydroxyethyl Starch (HES) and Isotonic Saline (NaCl) in Endotoxemic Hamsters after 8h Endotoxemia

	HES 8h	NaCl 8h	Control 8h	Normal Values
*p*H	7.41 ± 0.03	7.39 ± 0.07	7.39 ± 0.07	7.39 ± 0.05
Po_2 (mmHg)	73.0 ± 5.7	74.5 ± 14.8	69.5 ± 7.7	83.7 ± 20.8
Pco_2 (mmHg)	39.4 ± 6.4	40.2 ± 6.0	45.6 ± 8.6	56.9 ± 11.1
Na^+ (mM)	132 ± 0‡	134 ± 0‡	130 ± 0‡	139 ± 4
K^+ (mM)	4.7 ± 0.2	3.8 ± 0.3*	5.4 ± 0.5	4.8 ± 0.6
Ca^{2+} (mM)	1.4 ± 0.1	1.3 ± 0.1	1.3 ± 0.9	1.5 ± 0.4
Red blood cell count ($\times 10^6/\mu l$)	7.0 ± 0.2	6.4 ± 0.1†‡	7.3 ± 0.2	7.4 ± 1.0
Hemoglobin (g/dl)	15.3 ± 0.2	13.9 ± 0.6†	15.9 ± 0.4	15.3 ± 1.5
Platelet count ($\times 10^3/\mu l$)	564 ± 24	469 ± 205	532 ± 56	653 ± 196
Total leukocyte count ($\times 10^3/\mu l$)	2.7 ± 0.3‡	2.1 ± 1.0‡	3.1 ± 0.6‡	5.7 ± 1.8

Data are mean ± SD. HES 8h represents animals (n = 4) that received LPS (2 mg/kg body wt) and HES (16 ml/kg body wt/h) 3h after the induction of endotoxemia, whereas NaCl 8h represents animals (n = 4) that received LPS (2 mg/kg body wt) and isotonic saline (66 ml/kg body wt/h) 3h after induction of endotoxemia. Control 8h represents animals (n = 4) which received LPS (2 mg/kg body wt) only. Normal standard values were derived from eight healthy animals without any treatment.

* = significant differences ($P < 0.05$) *versus* control animals (ANOVA, Student Newman–Keuls test), † indicates significant differences ($P < 0.05$) *versus* HES and control animals (ANOVA, Student Newman–Keuls test), ‡ = significant differences ($P < 0.05$) *versus* normal values (Kruskal–Wallis ANOVA, Mann–Whitney U test).

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Table 8. Effect of Resuscitation with Hydroxyethyl Starch (HES) and Isotonic Saline (NaCl) on Laboratory Parameters 24h after Induction of Endotoxemia

	HES	NaCl	Control	Normal Values
*p*H	7.34 ± 0.07	7.27 ± 0.07	7.31 ± 0.12	7.39 ± 0.05
Po_2 (mmHg)	82.5 ± 12.1*	57.8 ± 18.2	65.2 ± 4.3	83.7 ± 20.8
Pco_2 (mmHg)	48.3 ± 10.0	73.4 ± 17.0	77.7 ± 37.5	56.9 ± 11.1
Na^+ (mM)	139 ± 3	144 ± 6	141 ± 12	139 ± 4
K^+ (mM)	4.0 ± 1.0	3.6 ± 0.9	4.7 ± 0.9	4.8 ± 0.6
Ca^{2+} (mM)	1.6 ± 0.9	1.6 ± 1.3	1.4 ± 0.4	1.5 ± 0.4
Red blood cell count ($\times 10^6/\mu l$)	6.3 ± 0.7	5.9 ± 0.8	7.1 ± 0.6	7.4 ± 1.0
Hemoglobin (g/dl)	13.3 ± 1.5	12.6 ± 1.5	14.6 ± 1.6	15.3 ± 1.5
Platelet count ($\times 10^3/\mu l$)	338 ± 65	357 ± 121	336 ± 93	653 ± 196†
Total leukocyte count ($\times 10^3/\mu l$)	3.1 ± 2.4	5.9 ± 5.5	8.5 ± 1.0	5.7 ± 1.8

Data are mean ± SD. HES represents animals (n = 7) that received LPS (2 mg/kg body wt) and HES (16 ml/kg body wt/h) 3h after the induction of endotoxemia, whereas NaCl represents animals (n = 6) that received LPS (2 mg/kg body wt) and isotonic saline (66 ml/kg body wt/h) 3h after induction of endotoxemia. Control represents animals (n = 8) which received LPS (2 mg/kg body wt) only. Normal standard values were derived from eight healthy animals without any treatment.

* = significant differences ($P < 0.05$) of HES *versus* NaCl (ANOVA, Student Newman–Keuls test), † = significant differences ($P < 0.05$) of normal values *versus* NaCl, HES and controls (Kruskal–Wallis ANOVA, Mann–Whitney U test).

tion of the leukocytic response by HES 130 kD is caused by changes in macrohemodynamics. To account for the higher acute volume effect of the colloid solution, the ratio of 1:4 between colloid and crystalloid has been used. From a clinical point of view, the dose of HES 130 kD used in the present study corresponds to 1 l/70 kg, whereas isotonic saline was given at a dose of 4 l/70 kg. By this, we most closely simulated a typical clinical resuscitation protocol.

The observed reduction of endotoxin-induced leukocyte adherence by HES 130 kD corresponds well with the results of experimental studies on hemorrhagic shock, which demonstrated that HES attenuates the hemorrhage-induced increase in plasma interleukin-6 (IL-6) concentrations.[26] Because IL-6 concentrations are known to reflect the inflammatory leukocytic response, IL-6 downregulation may indicate a direct antiinflammatory effect of HES on leukocyte activation. Schmand *et al.*,[26] however, could not exclude that the decreased IL-6 concentrations may only reflect a more rapid shock reversal and, thus, an improved macrohemodynamic profile. However, Traber *et al.*[8] also reported an antiinflammatory effect of HES as measured by the attenuation of sepsis-induced lung lymph flow. In this study, the lung lymph flow has been used to estimate the increased microvascular permeability as an indicator of the inflammatory mediator response (prostanoid and eicosanoid release).

A direct effect of HES on granulocyte activation seems highly likely. Hofbauer *et al.*[10] showed *in vitro* a reduction of granulocyte chemotaxis through endothelial cell monolayers by HES used in clinically relevant concentrations. In line with this, endothelial cell culture studies by Dieterich *et al.*[9] demonstrated a HES-associated downregulation of adhesion molecules, which mediate the inflammatory interaction between leukocytes and endothelial cells, including ICAM-1. Accordingly, during resuscitation of trauma patients, HES was also reported to significantly reduce soluble ICAM-1 and soluble vascular cell adhesion molecule-1 (VCAM-1) concentrations over time.[11,27]

Circulating adhesion molecules not only represent excellent markers of inflammatory activation of endothelial cells and leukocytes but also directly modulate the inflammatory process. Whereas the multistep process of leukocyte recruitment during inflammation is initiated by adhesion molecules from the selectin family (E-selectin, L-selectin, and P-selectin), which mediate leukocyte rolling, the subsequent firm leukocyte adherence at the endothelial surface involves endothelial adhesion molecules from the immunoglobulin G (IgG) superfamily (ICAM-1, VCAM-1) and the leukocytic β2-integrin family (CD11, CD18). Because in the present study HES 130 kD reduced leukocyte adherence in arterioles and venules without significant effects on leukocyte rolling, its antiinflammatory effect may be mainly attributed to the inhibition of β2-integrins or IgG superfamily adhesion molecules. However, earlier steps of LE may also be influenced as shown by Collis *et al.*,[12] demonstrating *in vitro* that HES inhibits the endothelial cell activation by downregulation of P-selectin expression.

To exclude the possibility that the effect of HES 130 kD on leukocyte adherence is mainly mediated by changes in microvascular flow conditions, we analyzed local microhemodynamics that might influence adhesion: LPS decreased erythrocyte velocity at 30 min and 3 h in all experimental groups to a comparable degree. Further, HES 130 kD and NaCl did not influence erythrocyte velocity at 4 h and 8 h, as reflected by the lack of intergroup differences when compared with nontreated control animals. Because the most pronounced reduction of arteriolar and venular leukocyte adherence by HES 130 kD was observed at 8 h after LPS administration, this effect is highly unlikely to be primarily caused by changes in local microhemodynamics. In particular, a major role of lower wall shear forces to explain the antiadhesive properties of HES 130 kD can be excluded. Although at 24 h HES 130 kD–treated animals

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showed increased erythrocyte velocities and wall shear rates when compared with control animals and baseline levels, the attenuation of leukocyte adherence at this time point may also not be the result of improved microhemodynamic conditions because crystalloid-treated animals showed a comparable improvement of microhemodynamics, although without attenuation of leukocyte adherence. Because direct measurements of wall shear rates in the microvasculature did not reveal differences between experimental groups, it is unlikely that these groups differed in either cardiac outputs or systemic resistances.

The reduction of endotoxin-induced LE by HES 130 kD was associated with a less-pronounced decrease in FCD, indicating improved capillary perfusion conditions. This HES 130 kD–mediated improvement of endotoxin-induced perfusion failure is also unlikely to be caused by microhemodynamic (wall shear rate) or macrohemodynamic (MAP) changes because these parameters did not differ between the groups, but rather it may be the result of the HES-mediated reduction of endothelial and leukocytic activation.

Quantitative analysis of microvascular perfusion indicated that crystalloid support, which could not prevent the LPS-induced increase in LE, was also unable to protect capillary perfusion. This result correlates with the findings of Marik *et al.*,[28] who demonstrated that in patients with abdominal aortic aneurysm repair, HES volume resuscitation is superior to improve microvascular blood flow and tissue oxygenation when compared with crystalloid therapy.

Systemic capillary leakage is an early sign of inflammation after injury and is proportional to the severity of the insult.[29] The skinfold chamber model allowed the quantitative assessment of macromolecular leakage by repeated intravital microscopic determination of FITC dextran extravasation. Whereas crystalloid-treated animals and nontreated control animals showed a 30% increase of macromolecular leakage in venules, this increase was significantly attenuated in venules of HES 130 kD–treated animals. The observation of reduced macromolecular leakage after HES 130 kD therapy is in line with experimental and clinical studies, demonstrating prevention of capillary leak syndrome by HES in posttraumatic, postischemic, and septic conditions.[29,30] Oz *et al.*[30] reported a reduction of postischemic capillary leakage, analyzed by FITC dextran 150 extravasation in the rat cremaster muscle preparation. This finding is in line with the results of a histologic study in the rat limb, which demonstrated that various MW fractions of HES are capable of reducing abnormal microvascular permeability after ischemia and reperfusion.[31] Also, in a model of myocardial reperfusion injury, HES was shown effective at protecting cellular morphology and at reducing myocardial water content.[32]

Therefore, it seems reasonable to speculate that the attenuation of LE by HES 130 kD results in a reduction of cytotoxic mediator release and, subsequently, amelioration of increased capillary permeability. Because after 24 h the erythrocyte velocity was not only increased in HES 130 kD–treated but also in NaCl-treated animals, the selective protection from LPS-induced increase of microvascular permeability by HES 130 kD seems not to be the result of a decreased wall shear stress, which theoretically could be related to less delivery of solute. Changes in capillary wall permeability are more likely to be mediated by a direct interaction of HES 130 kD with the endothelium, which has been characterized as "plugging the leaks."[33] In support of the present data, Webb *et al.*[34] demonstrated in a pig model of hyperdynamic sepsis that pentastarch (pentafraction of HES) is superior compared with native HES to reduce structural organ damage, as indicated by a decrease in white cell capillary plugging and a reduction of tissue edema.

It has to be emphasized that our findings with the new HES 130 kD preparation may not be extended to other HES preparations that clearly differ in concentration, degree of substitution, and molecular weight distribution.[14] Thus, the mean molecular weight of HES 130 kD amounts 130 ± 20 kD with a relatively small range of distribution. The molecular weight and the range of the respective HES fraction determines renal excretion and volume effect, as well as tissue deposition and hemostatic changes, and may also be critical for specific microcirculatory effects on leukocytes and endothelial cells.

In summary, our experiments show a relevant protective *in vivo* effect of HES on endotoxin-induced microcirculatory disorders. HES 130 kD is effective (1) to prevent LPS-induced leukocyte adherence, (2) to attenuate LPS-induced capillary perfusion failure, and (3) to reduce LPS-induced macromolecular leakage. Because there were no signs of *in vivo* HES 130 kD toxicity or bleeding diathesis, HES 130 kD may offer an antiinflammatory potential when used during experimental sepsis. These protective HES 130 kD effects should deserve clinical testing with human sepsis in well-designed trials.

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