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EXTRACORPOREAL PLASMA TREATMENT FOR THE REMOVAL OF ENDOTOXIN IN PATIENTS WITH SEPSIS: CLINICAL RESULTS OF A PILOT STUDY

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ABSTRACT—Despite the advances in therapeutic approaches in the management of inflammatory conditions, the incidence of sepsis is on increase in the intensive care units (ICU). In a pilot study, we investigated whether the use of an apheresis system based on DEAE-cellulose is capable of reducing the plasma concentration of endotoxin in patients with severe sepsis. We enrolled 15 intensive care patients with severe sepsis and plasma endotoxin concentrations >0.3 EU/mL. In addition to standard ICU therapy, a total of 83 apheresis treatments were performed. About 1.7 volumes of plasma (6000 mL) were treated at each apheresis session. A significant reduction in plasma endotoxin levels from a median of 0.61 to 0.39 EU/mL (−35%) could be achieved ($P < 0.001$). Long-term comparison of the initial and post-treatment levels after a series of five to six individual apheresis treatments also showed a statistically significant decline in circulating endotoxin, interleukin (IL)-6, C-reactive protein (CRP), fibrinogen, and an increase in cholesterol levels. Except for a transient and reversible increase of prothrombin time, no adverse events were observed in patients undergoing this new adsorption apheresis treatment. Our data show that reduction of endotoxin by extracorporeal DEAE-cellulose-based plasma treatment may prove a promising therapeutic tool for patients suffering from bacterial sepsis and proven endotoxemia.

KEYWORDS—DEAE-cellulose, adsorption apheresis, endotoxin removal, sepsis

INTRODUCTION

Severe sepsis associated with multiple organ dysfunction leads to high morbidity and mortality in intensive care units despite the advances in therapeutic approaches (1). Gram-negative bacteria cause about one-half of all serious human infections, and complications of severe sepsis account for as many as 200,000 deaths per year in the United States (2). Moreover, in the past two decades, many bacteria, including gram-negative species, have acquired resistance to diverse antibiotics (3). Even when antibiotics are effective in a severe gram-negative infection, the lipid A moiety, which is the active substructure of the lipopolysaccharide (LPS), may be shed from dying bacteria and cause overt activation of macrophages and endothelial cells (4, 5), thereby triggering the systemic immunological response (SIRS) (6, 7). LPS concentrations at the beginning of sepsis correlate with the acute physiology and chronic health evaluation (APACHE) II score, inflammatory markers, and inversely with circulating cholesterol levels (8–10). Modulations of these markers are closely correlated with the prognosis and outcome of the disease (11). Newer therapeutic strategies attempting to reduce activation of coagulation and thus avoid disseminated intravascular coagulation were more successful (12, 13). Clinical studies using antibodies to endotoxin proved disappointing (14, 15), possibly due to the time of administration or to their inability to neutralize LPS activity sufficiently. A different approach using extracorporeal adsorption apheresis for elimination of endotoxin from plasma is currently under investigation in clinical studies (16–18).

A cartridge containing polymyxin B-immobilized fibers (PMX-F) for the selective binding and removal of LPS directly from whole blood was investigated in Japan for the treatment of septic shock (8, 16, 19–24). Data from *in vitro* experiments demonstrate a high adsorption rate of LPS from whole blood by this system (16). In a sepsis model, treated rats survived, whereas controls died (21). Also, in patients with sepsis, a 45% reduction of circulating LPS concentrations and a decrease in 28-day mortality has been reported (8). This elegant approach seems to be a promising strategy for the treatment of patients with sepsis.

In the Matisse® system, the binding ability of LPS to human albumin is used for its elimination from whole blood (18, 25). A 25% to 36% reduction of LPS was achieved after perfusion of 1.5 volumes of patients' blood. However, in a controlled study, including 145 patients with gram-negative sepsis, the APACHE II score did not decrease significantly during serial apheresis treatment in comparison with the control group and the survival rate showed no difference (17). On the other hand, in a subgroup of 104 patients suffering from peritonitis, the SOFA score declined clearly in patients undergoing hemoperfusion.

In vitro experiments carried out in our laboratory demonstrated that recirculation of plasma through DEAE-cellulose mats, an adsorber material that not only possesses anion-exchange groups, but also hydrophobic properties (26, 27), effectively removed LPS from plasma at pH 5.12 (28). The combination of electrostatic and hydrophobic interaction potentiates the selectivity and affinity of this adsorber material for the binding of LPS. Such an adsorber is part of the H.E.L.P.

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system (B. Braun, Melsungen, Germany) that is in clinical use for the treatment of severe hypercholesterolemia for more than a decade. In a preliminary pilot study, we showed that the H.E.L.P. treatment reduces LPS levels in patients suffering from sepsis (28). Recently, Wang et al. (29) showed that this therapy efficiently also reduces proinflammatory and prothrombotic markers from the circulation of patients with chronic heart disease. In this study, we addressed the question of whether the DEAE-cellulose adsorber material effectively reduces LPS concentration also under physiological pH conditions in *in vitro* studies and in patients with sepsis without undesired side effects.

MATERIALS AND METHODS

In vitro *experiments*

The binding capacity of the DEAE-cellulose adsorber (H.E.L.P. Heparin Adsorber; B. Braun) for endotoxin from plasma was evaluated *in vitro* using tritium-labeled endotoxin from *Escherichia coli* K12 strain LCD 25 (^{3}H-LPS; List Biological Laboratories, Quadratech, Surrey, UK) with a specific activity of 4×10^5 dpm/μg. The labeled endotoxin was added to 200 mL of heparinized plasma obtained from healthy volunteers to a final concentration of 25 ng/mL. After recirculating three plasma volumes at a flow rate of 20 mL/min through the adsorber cartridge, reduction of radioactivity from plasma and concentration of LPS (LAL assay) were measured.

Clinical study

The study protocol was reviewed and approved by the local institutional review board. Informed consent of patients or their next relative was obtained before the therapy.

We prospectively included 15 patients at the intensive care unit of the Department of Surgery (University Hospital Grosshadern, Munich, Germany) with clinically established severe sepsis (ACCP/SCCM) (30) and a plasma concentration of LPS > 0.30 EU/mL. The presence of at least two of the four criteria for SIRS and at least one sepsis-induced organ dysfunction were criteria for inclusion. The following indices were used for organ failure: lungs, artificial ventilation >1 day after admission; circulation, catecholamine therapy despite adequate fluid support; coagulation, prothrombin time <60% of normal value or partial thromboplastin time >45 s or platelet count <100 G/L; kidneys, acute increase of creatinine >2mg/dL or continuous veno-venous hemofiltration (CVVH); liver, bilirubin >2 mg/dL plus prothrombin time <60% of control or cholinesterase <1500 U/L; and central nervous system, acute disorientation, stupor, or coma. The clinical characteristics are summarized in Table 1. Exclusion criteria were an age less than 18 years, pregnancy, presence of an acute cerebrovascular event, acute coronary syndrome, severe cardiac arrhythmia, bradycardia, prothrombin time <60%, gastrointestinal hemorrhage, or an indication for imminent surgery.

The standard therapy included administration of antibiotics, vasoactive drugs, mechanical ventilatory support, hemofiltration, corrective measures for metabolic abnormalities, and other supportive therapy that appeared to be necessary. Conventional therapy was maintained during the DEAE-cellulose apheresis. None of the patients received steroids or other immunosuppressive drugs. The APACHE II score was evaluated daily for each patient (31).

Adsorption apheresis procedure

The apheresis therapy was initiated when patients with severe sepsis did not respond to conventional therapy and fulfilled the inclusion criteria on at least 2 consecutive days. A double-lumen catheter was inserted into the femoral vein or vena cava superior by the Seldinger technique. DEAE-cellulose adsorption apheresis was performed with a continuous plasma separation unit (Diapact CRRT; B. Braun) for 2 to 4 h at a blood flow rate of 60 to 120 mL/min through the venovenous catheter. Plasma was separated from blood by a passage through a 0.55-μm hollow fiber (Haemoselect 0.2 m^2; B. Braun) and was then passed through a cartridge (11 × 6 cm I.D.), at a flow rate of 25 mL/min, packed with the anion exchange material, i.e., DEAE-modified cellulose (H.E.L.P Heparin Adsorber; B. Braun). Endotoxin-depleted plasma was continuously mixed with the cellular blood components and reinfused into the patient. In general, patients received five serial aphereses within 1 week. The time interval between two apheresis treatments was at least 24 h. Anticoagulation was achieved by intravenous infusion of the prostacyclin analog epoprostenol (Flolan) at a rate of 20 μg/h during apheresis treatment. Two patients were given heparin during the apheresis treatment (500 I.U./h) to maintain flow through the filter.

Clinical chemical analysis and assay procedures

Peripheral blood samples were collected before, immediately after, and on the morning of each day after the apheresis procedure. All laboratory tests were performed at the Institute of Clinical Chemistry (University Hospital Grosshadern) according to standard procedures. Serum IL-6 concentrations were measured by an enzyme-linked immunoabsorbant assay method (Biosource Europe, Nivelles, Belgium). For bacterial endotoxin measurement, blood samples were aseptically taken from an arterial vascular access at 8:00 a.m. and immediately before and after apheresis treatment. LPS was measured using a kinetic limulus amebocyte lysate assay (LAL-QCL; Cambrex, Walkersville, MD). Briefly, whole blood was collected in 4-mL LPS-free vacutainers containing heparin (Chromogenix, Mölndal, Sweden) and centrifuged at 1800*g* for 10 min to separate the plasma from cellular components. Plasma was collected with an LPS-free micropipette and aliquoted into a 1.8-mL LPS-free Cryotube (Nunc, Wiesbaden, Germany). Lyophilized *polyphemus* amebocyte lysate (LAL solution, Kinetic-QCL; Cambrex), was dissolved in 2.6 mL of buffered solution. The lyophilized LPS standard (200 EU, *E. coli* 055:B5) was aseptically dissolved in 4 mL of LPS-free purified water. The standard LPS solution was diluted to a concentration of 50, 5, 0.5, 0.05, and 0.025 EU/mL. Plasma was diluted 10-fold by adding 10 mM LPS-free $MgCl_2$ (Cambrex). The diluted plasma was heated in water for 10 min at 75°C and was centrifuged at 1800*g* for 1 min, and the resulting supernatant was further diluted (1:5). Finally, 100 μL of 50-fold diluted plasma and 100 μL of prepared LAL solution were pipetted into a microplate (Nunc). The activation of the *Limulus* clotting enzyme by LPS catalyzes the cleavage of *p*-nitroaniline from the substrate (Ac-Ile-Glu-Ala-Arg-pNa), and adsorbance was measured photometrically at 405 nm. Positive product control as a monitor for

TABLE 1. **Characteristics of the patients**

Patient	Age (years)/gender	Diagnosis	Septic focus	Bacteria (gram)	LPS* (EU/mL)
1	76 men	Rupture of aortic aneurysm	*Pneumonia*	(−)	0.80
2	60 women	Ileus	*Peritonitis* Pneumonia	(+)	0.59
3	58 women	Diverticulitis	*Colon*	(+)	1.75
4	58 men	None	*Abscesses* Pneumonia	(+)	0.71
5	70 women	Thyroid carcinoma	Mediastinitis	(+/−)	0.49
6	66 men	Thoracic aneurysm of the aorta	*Pneumonia*	(+/−)	0.42
7	53 men	Carcinoma of papilla vateri	*Peritonitis* Pneumonia	(+/−)	0.63
8	62 men	Abdominal hernia	*Peritonitis* Pneumonia	(+/−)	0.33
9	62 men	Ileus	*Pneumonia* Pleura empyema	(+)	0.53
10	62 women	Liver carcinoma	*Peritonitis*	(+)	0.80
11	55 men	Pleura mesothelioma	*Pneumonia*	(+)	0.52
12	78 men	Colon perforation	*Peritonitis*	(+/−)	1.84
13	64 men	Bronchus carcinoma	*Pleura empyema*	(+/−)	0.57
14	50 women	Rectum carcinoma	*Peritonitis* Pneumonia	(−)	1.84
15	57 men	Pleura mesothelioma	*Pleura empyema* Pneumonia	(+)	0.38

*LPS concentration on day 1 (8 a.m.) before the first apheresis treatment. The primary septic focus is printed in italics.
−, gram-negative strain.
+, gram-positive strain.

plasma LPS inhibition or enhancement was determined by LPS measurement in a vial containing 100 μL of prepared LAL solution, 100 μL of diluted plasma sample (1:50), and 10 μL of LPS standard solution (diluted to a concentration of 5 EU/mL). The LPS recovery was 100% + 25%. The limit of detection for LPS determination using this method was 0.13 EU/mL.

Statistical analysis

Results are expressed as the median and 75th percentile. The difference in median values was examined by Wilcoxon's signed rank test for paired subjects. Values of $P < 0.05$ were considered statistically significant.

RESULTS

Elimination of LPS from human plasma after passage through the DEAE-cellulose adsorber

In vitro experiments established a high efficiency for LPS adsorption by the DEAE-cellulose adsorber (Fig. 1). The initial plasma concentration of ^{3}H-LPS was 25 ng/mL (250 EU/mL), corresponding to a specific activity of 96 dpm/ng LPS. After recirculating three plasma volumes, a reduction in LPS concentration and ^{3}H activity of 99% and 79% ($P < 0.05$), respectively, was noted.

To confirm the quantitative adsorption of ^{3}H-LPS, the fractions of treated plasma were incubated with Polymyxin-B gel (Polymyxin-B gel; Pierce, Rockford, IL). Neither ^{3}H radioactivity nor LPS concentration were further reduced, indicating that the DEAE-cellulose material had eliminated LPS from plasma in a quantitative manner.

Elimination of endotoxin in patients

The application of our study inclusion criteria (severe sepsis and LPS >0.3 EU/mL) resulted in the selection of patients with multiple organ dysfunction syndrome. Organ functions were affected as follows: lungs, 15/15 (all patients were mechanically ventilated); circulation, 15/15; coagulation, 14/15; central nervous system, 14/15; kidneys, 13/15; liver, 11/15. Ten patients received continuous veno-venous hemofiltration because of acute renal failure, 5/15 during the 5 study days. However, during apheresis therapy, CVVH was interrupted.

All patients required catecholamine therapy due to septic shock during the study, days 1 to 6, and 59 of 83 apheresis procedures were done under norepinephrine therapy. The median norepinephrine doses before and postapheresis were not different (0.7 vs. 0.8 mg/h, $P > 0.05$). We performed a total of 83 apheresis treatments, i.e., 5.5 treatments per patient. On average, the 1.7-fold plasma volume (6000 mL) was treated during each apheresis. Median values of hematocrit before and after apheresis did not differ (median before apheresis: 0.26, 25th percentile: 0.24, 75th percentile: 0.28 and after apheresis: median 0.26, 25th percentile: 0.24, 75th percentile: 0.28).

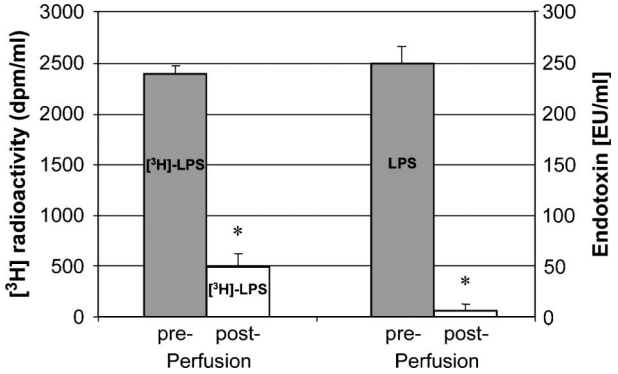

FIG. 1. ***In vitro* removal of LPS by perfusion of three plasma volumes through the DEAE-cellulose cartridge**. Median pre- and postperfusion levels of LPS (dpm/mL, left and LAL test in EU/mL, right), n = 6, *$P < 0.05$ (Student's *t* test).

All patients in this study had high plasma concentrations of LPS 1 day before the first apheresis treatment (median: 0.61 EU/mL, 25th percentile: 0.45 EU/mL 75th percentile: 0.75 EU/mL). There was a significant reduction of LPS concentrations after each apheresis treatment (median concentration before and after the first apheresis: 0.62 and 0.38 EU/mL [$P < 0.001$], second apheresis: 0.58 and 0.37 EU/mL [$P < 0.001$], third apheresis 0.57 and 0.43 EU/mL [$P < 0.001$], fourth aphe-resis: 0.71 and 0.39 EU/mL [$P < 0.001$], and fifth apheresis: 0.54 and 0.39 EU/mL [$P < 0.001$], respectively). The median LPS concentration before apheresis treatment was 0.61 EU/mL and post-treatment was 0.39 EU/mL (35% reduction, $P < 0.001$). Figure 2 shows pre- and post-treatment levels of LPS (EU/mL) in plasma during a series of five apheresis treatments in all patients. The long-term LPS profile was monitored by measuring LPS plasma levels every morning on the first day of the apheresis until day 13. Plasma LPS concentrations decreased slowly during the treatment period from 0.59 EU/mL (range 0.33-1.84 EU/mL) on day 1 to 0.49 EU/mL on day 6 (n.s.) and further to 0.33 EU/mL on day 13 ($P < 0.05$).

APACHE II score and mortality

Before the apheresis treatment, the patients had a median APACHE II score of 22 points (25th percentile, 20 points; 75th percentile, 28 points). The score decreased to 18 points after the treatment series (day six, 25th percentile: 11 points; 75th percentile: 23 points) and to 17 points (25th percentile, 12 points; 75th percentile, 21 points) on day 13. However, this decrease was not statistically significant. Heart rate, mean arterial pressure, and body temperature showed no significant changes during the therapy. Table 2 shows the median norepinephrine doses and mean arterial pressure before and after apheresis treatments. No instability of the circulation and no deterioration of organ functions were observed during apheresis. One patient died, due to a refractory septic shock on day 2 before the second apheresis treatment was started. The 28-day mortality after apheresis therapy was 26.7% (4/15 patients).

Inflammatory and coagulatory markers

Clinical chemical and hematological parameters are presented in Table 3. All patients showed a pronounced leukocytosis of 14.8 G/L on the day before the first apheresis treatment, which in the long term was not affected by the treatments (Table 3). However, a transitory increase in leukocyte count (median +5.9 G/L) was generally observed at the end of each apheresis procedure, which returned to base level values by the following morning. None of the patients showed leukopenia at any time of the study period. IL-6 (146.5 pg/mL) and C-reactive protein (CRP) (15.6 mg/dL) plasma concentrations were significantly decreased after DEAE adsorber apheresis treatment, whereas the changes in LBP were statistically insignificant. Figure 3 shows long-term results of IL-6 and CRP in 15 patients over 13 days.

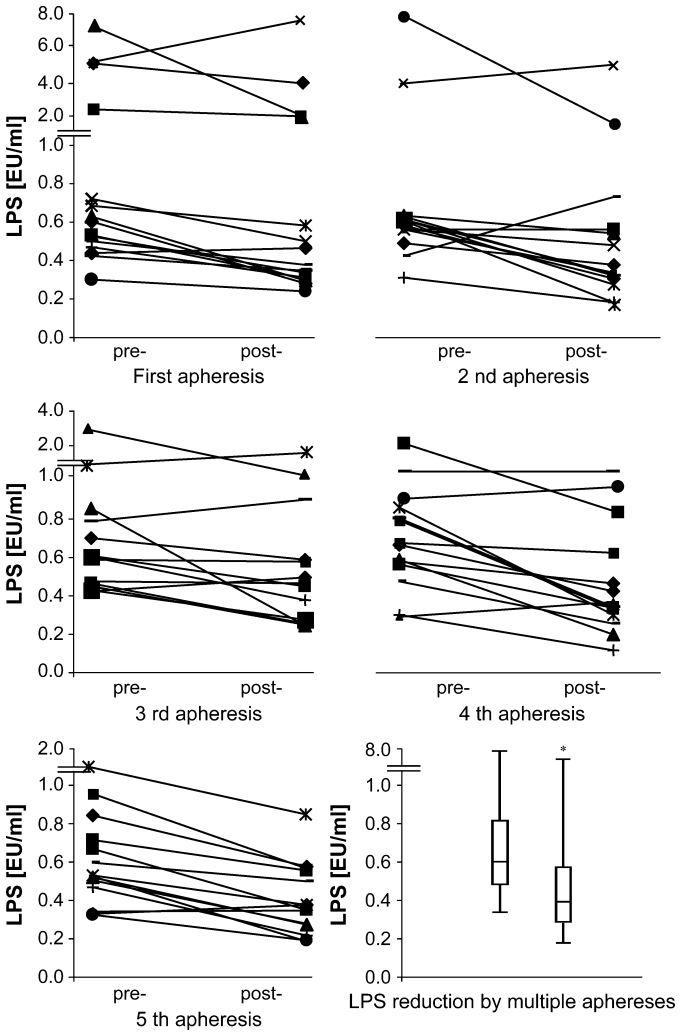

FIG. 2. **Pre- and posttreatment levels of LPS (EU/mL) in plasma of 15 patients during a series of five apheresis treatments**. LPS reduction by multiple aphereses (bottom right), median and interquartile range, $^*P < 0.001$.

Five patients were treated with CVVH during the apheresis period (days 1-5). The plasma concentration of CRP, IL-6, and LPS of these five patients and those 10 patients without CVVH are shown in Table 4.

In addition, circulating cholesterol levels, which are a sensitive indicator of severe inflammation (11) and were significantly below normal levels before apheresis, also increased after the apheresis treatments (Table 3).

We noted a significant reduction of prothrombin time after each therapy. Prothrombin time (percentage of control) decreased from a pretreatment median of 70% of normal value to a posttreatment median value of 41% ($P < 0.05$). However, it returned to normal values by the next day. Transitory reduction of prothrombin time was possibly due to adsorption of clotting factors (32). However, there were no significant changes in aPTT and D-Dimer levels over time (Table 3). Lipoproteins and immunoglobulin concentrations did not change.

DISCUSSION

Although the pathogenesis of sepsis is complex, it is now generally accepted that LPS-induced overproduction of proinflammatory factors, including TNF-α, IL-1, IL-6, and IL-8, released from the septic focus, amplify the cascade of inflammatory response (33, 34). This concept is supported by clinical observations and studies in experimental animals. Administration of endotoxin leads to increased production of cytokines, and injection of purified cytokines such as TNF-α and IL-1 provokes a sepsis-like response (35).

During recent years, many strategies have been developed to minimize or to interrupt the influence of LPS in the pathogenesis of sepsis. In the present investigation, we treated 15 patients with circulating LPS concentrations above 0.30 EU/mL using a DEAE-cellulose adsorber apheresis module. This cut-off concentration for LPS cannot be directly compared with data from other studies because of methodological differences in the analysis of LPS in plasma.

Our *in vitro* experiments with ^{3}H-labeled LPS conclusively showed that LPS is quantitatively adsorbed from human plasma during perfusion of the DEAE-cellulose adsorber investigated. This finding was further confirmed by the LAL test and the use of the LPS-specific adsorber Polymyxin B.

From the literature, it is known that only in 70% to 80% of patients suffering from gram-negative sepsis LPS could be detected (10, 24). Gram-negative bacteria were isolated from the septic focus in eight patients enrolled in this study, and in seven patients, only gram-positive bacteria were isolated from the septic focus. LPS, however, was detected in all patients. An explanation for this finding could be the translocation of LPS from the gastrointestinal tract into the circulation.

All patients in this study were suffering from septic shock for several days and were treated according to intensive care unit standards for a relatively long period of 12.7 + 14.1 days (mean + SD) before the apheresis treatments started. All of our patients tolerated the apheresis therapy well and no severe adverse event occurred. There was no deterioration of hemodynamic parameters and no significant changes of norepinephrine dosage was necessary during apheresis treatment.

The pre- and post-treatment plasma levels of LPS clearly demonstrate the potential of extracorporeal apheresis using the DEAE-cellulose adsorber investigated in reducing circulating LPS effectively (~35%, $P < 0.001$). The high significance of this reduction makes it extremely unlikely that it is caused by normal fluctuation. Thus, the median LPS concentration increased significantly from the post-treatment level of 0.39 to 0.57 EU/mL in the morning of the next day and further to 0.61 EU/mL before the next apheresis. This spontaneous increase in endotoxin concentration during this time period can only be explained by continuous endotoxin release into the circulation and the discontinued extracorporeal treatment.

Because isovolemic infusion therapy has been performed during each apheresis therapy and no change in hematocrit has been occurred, a dilutional effect can be excluded.

It is almost impossible to measure serum concentrations of all components that can be theoretically removed by this treatment modality. We measured specific clinically relevant inflammatory, coagulation parameters, and other parameters of liver function. LPS reduction was correlated with a significant decline of IL-6, CRP, and fibrinogen levels. Serial

TABLE 2. **Median norepinephrine doses (n = 59) and mean arterial pressure (n = 83) before and after apheresis treatments**

	Norepinephrine (mg/h)		Mean arterial pressure (mmHg)	
Patient	Preapheresis	Postapheresis	Preapheresis	Postapheresis
1	0.40	1.20	73	74
2	2.80	2.80	86	81
3	0.40	0.36	82	89
4	1.23	1.25	87	92
5	1.40	1.05	98	80
6	0.24	0.30	99	89
7	0.15	0.10	91	80
8	0.30	0.35	73	79
9	0.46	0.60	75	90
10	2.00	2.00	81	82
11	0.28	0.35	78	79
12	1.40	1.40	67	82
13	0.40	0.40	72	82
14	0.40	0.40	71	66
15	2.80	2.40	84	84

TABLE 3. **Clinical chemical and hematological parameters**

	Day 1 (preapheresis)	Day 6	Day 13
Erythrocytes (T/L)	3.18 (3.35)	3.03 (3.36)	3.17 (3.40)
Hemoglobin (g/dL)	9.60 (10.50)	9.65 (10.70)	10.20 (10.60)
Thrombocytes (G/L)	163 (351)	214 (275)	150 (261)
Leukocytes (G/L)	14.8 (18.3)	16.3 (26.6)	13.7 (18.9)
CRP (mg/dL)	15.6 (18.4)	7.0 (9.4)#	8.4 (12.2)
IL-6 (pg/mL)	146.5 (245)	96.8 (207)#	57.0 (189)*
LBP (μg/mL)	47.4 (57.3)	31.0 (50.7)	30.0 (39.5)
Amylase (U/L)	90.0 (193)	87.5 (243)	79.5 (270)
Lipase (U/L)	77.0 (169)	57.0 (153)	97.0 (267)
Bilirubin (mg/dL)	1.98 (3.2)	2.0 (3.4)	1.5 (5.8)
Creatinine (mg/dL)	1.9 (2.1)	1.3 (2.0)	1.2 (1.7)
Total protein (g/dL)	4.80 (5.00)	4.40 (4.80)	n.d.
Cholesterol (mg/dL)	96.0 (116)	104.0 (123)	128.0 (162)*
Prothrombin time (% of control)	75.0 (80)	60.0 (70)#	80.0 (85)
aPTT	38.5 (45.0)	39.0 (42.0)	38.0 (42.0)
Fibrinogen (mg/dL)	566.0 (680)	397.0 (529)#	n.d.
D-Dimer (μg/mL)	3.3 (6.8)	3.2 (6.1)	n.d.

Laboratory data (8 a.m.) on days 1, 6, and 13 in 15 patients treated with DEAE cellulose apheresis.
Median and 75th percentile are in parentheses; #, day 6 vs. day 1, $P < 0.05$; *, day 13 vs. day 1, $P < 0.05$; n.d., not determined.

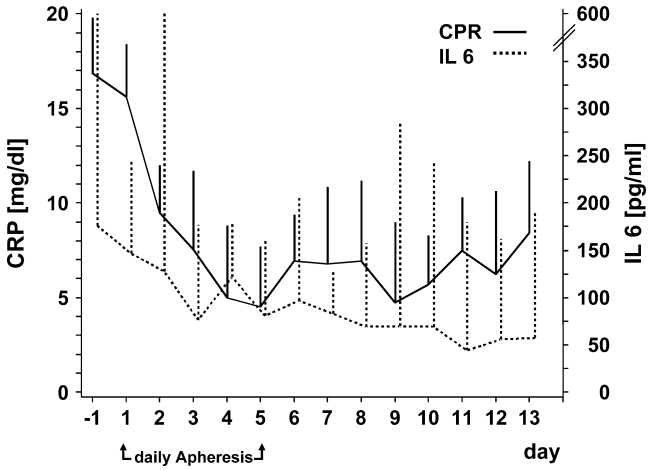

FIG. 3. **Long-term profile of daily CRP and IL-6 plasma levels in 15 patients (median and 75th percentile)**. Posttreatment values were measured each morning (8 a.m.) after the day of treatment.

TABLE 4. **Comparison of CRP, IL-6, and LPS levels (8 a.m.) on days 1 and 6 in 15 patients treated with and without CVVH**

	+CVVH (n = 5)		−CVVH (n = 10)	
Parameters	Day 1 (preapheresis)	Day 6	Day 1 (preapheresis)	Day 6
CRP (mg/dL)	17.9 (18.4)	5.9 (12.5)	15.0 (17.6)	7.0 (9.4)#
IL-6 (pg/mL)	217 (245)	171 (2507)	114 (245)*	47 (142)#
LPS (EU/mL)	1.75 (1.84)	0.51 (1.65)	0.53 (0.71)	0.44 (0.56)#

Median and 75th percentile are in parentheses.
*, Day 1 (+CVVH) vs. day 1 (−CVVH), $P < 0.05$.
#, Day 6 vs. day 1, $P < 0.05$.

measurements of serum bilirubin levels did not indicate a relevant change in liver function that could explain the observed decrease in IL-6 and CRP. Cholesterol levels, which are closely related with liver function, even increased over time, a finding that renders deterioration of liver function highly unlikely. Also, fibrinogen levels decreased over time. Serum fibrinogen reduction over time may also reflect a change in the proinflammatory state secondary to LPS elimination because fibrinogen represents an acute phase reactant. This hypothesis is also strengthened by missing changes in PTT and d-Dimers. Both parameters would be expected to decrease during consumption coagulopathy.

To investigate whether continuous hemofiltration can modify the efficacy of our LPS elimination system, we analyzed patient groups with and without concomitant hemofiltration therapy in terms of elimination efficacy. There was no obvious difference between either patient groups. This finding is in line with several reports in the literature showing that, e.g., IL-6 and other inflammatory cytokines may be eliminated into the ultrafiltrate, but without effectively influencing plasma concentrations of these mediators (37, 38).

From a theoretical point of view, anticoagulation of the extracorporeal elimination system with prostacyclin could per se modulate the proinflammatory response. *In vitro* and in animal models prostacyclin is known to modulate leukocyte-endothelial cell interaction and modulate the monocytic proinflammatory response to endotoxin (39, 40). An anti-inflammatory effect of prostacyclin infusion per se cannot be excluded by our study design because prostacyclin application was part of the elimination procedure. However the profound reduction in LPS concentration cannot be explained by prostacyclin infusion per se. There are no data from the literature showing that prostacyclin infusion can reduce LPS or IL-6 concentrations in humans. Moreover, two study patients without prostacyclin infusion who received heparin as an anticoagulant showed a similar response to treatment. The reduction in CRP concentrations may be in part mediated by prostacyclin effects. We can also not exclude that the reduction of CRP in part is due to the binding to the DEAE-cellulose adsorber. It has been shown that under acidic conditions (pH 5.12), H.E.L.P. apheresis eliminates CRP very effectively (29).

Because of the pilot nature of the present study and small number of patients, no attempts were made to provide information about the improvement of organ dysfunction and long-term prognosis. However, it is worth it to note that 28-day mortality in our patients was 26.7% in comparison with 55% to 72% in a recent multicenter study in patients with septic shock (36).

We noted a significant increase of prothrombin time after each apheresis. This could be due to the adsorption of clotting factors as shown for H.E.L.P. apheresis (32). However, prothrombin time returned to normal values at the next day and we observed no bleeding complications during DEAE-cellulose apheresis treatment. Yet, it is important to monitor coagulation parameters during each treatment to avoid the risk of bleeding.

Our *in vitro* investigations with the DEAE adsorber using tritium-labeled endotoxin revealed that endotoxin can be reduced to almost zero level when processing three plasma volumes. The performed treatment of 1.7 plasma volumes only resulted in 35% reduction of LPS. We processed only a limited plasma volume to avoid potentially detrimental changes in coagulation parameters in this first pilot trial. By simultaneous supplementation of coagulation factors (fresh frozen plasma and prothrombin complex), it should be possible to treat larger plasma volumes and thereby increase the efficacy with regard to LPS elimination.

In conclusion, therapeutic apheresis can be safely applied in patients with severe sepsis to reduce endotoxin plasma concentrations and thus the endotoxin load over time. Studies in a bigger cohort of patients are necessary to evaluate the impact of apheresis treatment on clinical outcome in endotoxemic patients.

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