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Treatment of Septic Patients with an Arginine-Based Endotoxin Adsorber Column Improves Hemodynamics and Reduces Oxidative Stress: Results of a Feasibility Study

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Key Words
Sepsis · Shock, septic · Endotoxemia · Plasmapheresis · Adsorption · Advanced oxidation protein products

Abstract
Background: Mortality of severe sepsis and septic shock is unacceptably high. Adsorptive removal of endotoxin may interrupt the inflammatory cascade triggered by lipopolysaccharide. ***Methods:*** Prospective feasibility study with plasma separation and adsorption (PSA) treatment using a novel arginine-coated adsorber column was performed in a tertiary care gastroenterological intensive care unit. ***Results:*** 10 patients with severe sepsis/septic shock (median APACHE II score: 27, hospital mortality 40%) were treated with PSA on 5 consecutive days. There were no serious adverse events. No patient died during the treatment period. During treatment sessions, mean arterial pressure and cardiac power index increased while vasopressors could be reduced. Advanced oxidation protein products and in vitro pro-apoptotic activity of plasma decreased. We could not demonstrate any changes in endotoxin levels. ***Conclusions:*** PSA resulted in a reduction of indicators of oxidative stress and pro-apoptotic activity of the plasma and an improvement in hemodynamic parameters, suggesting increased myocardial contractility and reduced septic vasodilation.

Introduction

Due to the aging of the populations in the industrialized nations and the prevalence of risk factors such as immunosuppression, sepsis syndrome has an increasing incidence with a larger proportion of severe cases [1]. Progress in therapy has reduced mortality over the past decades, but mortality remains unacceptably high. In an analysis of the placebo arms of recent trials, mortality of patients with septic shock has been found to be 46% [2].

The sepsis syndrome is probably caused by an inflammatory cascade, triggered by blood-borne exogenous antigens, such as lipopolysaccharide (endotoxin), in Gram-negative infection. Pattern recognition receptors on antigen-presenting cells and endothelial cells get stimulated and lead to the activation of lymphocytes, the release of pro-inflammatory cytokines such as interleukin (IL)-1 and tumor necrosis factor-α (TNF-α), activation of complement and coagulation factors [3]. These events culminate in an uncontrolled inflammatory response causing circulatory compromise, organ dysfunction, and death. Established treatment strategies rely on eradication of infection, hemodynamic resuscitation and support of the function of failing organs. There also have been various approaches focused on the attenuation of the inflammatory response targeting the involved cytokine cascades or

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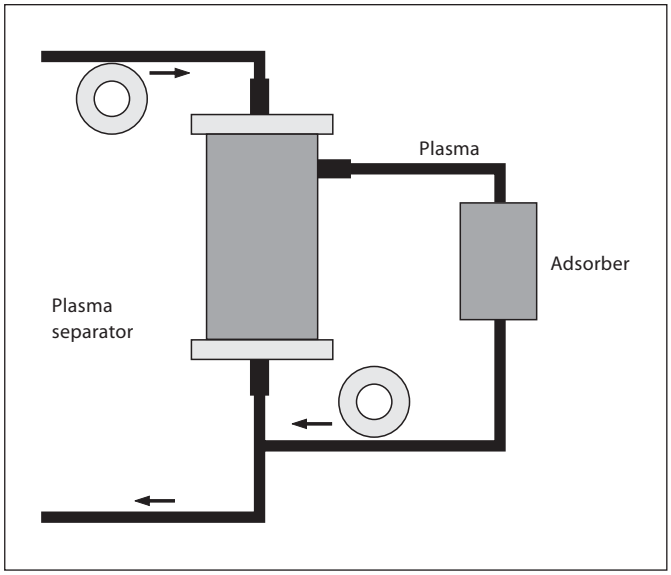

Fig. 1. Schematic representation of the extracorporeal circuit. Patient plasma is filtered though a plasma separator, passed over the adsorber column and reinfused back to the patient.

eliciting antigens, such as endotoxin or cytokines. A number of studies have evaluated methods of endotoxin adsorption using hemofiltration, plasma separation or perfusion of whole blood or separated plasma through adsorption columns containing polymyxin B, albumin or various resins as active agents [4–7]. Results were conflicting and there is a lack of controlled clinical trials proving the efficacy of these approaches. We conducted a security and feasibility study to evaluate the use of a novel adsorption column containing macroporous polymeric beads with a covalently immobilized arginine ligand. This adsorber has a high binding capacity for endotoxin due to formation of ionic and hydrogen bond interactions between the lipid A moiety of endotoxin and the guanidinium groups of the arginine ligand.

Patients and Methods

All patients admitted to our intensive care unit with a presumptive diagnosis of sepsis were evaluated for participation in this study. Inclusion criteria were two or more systemic inflammatory response syndrome (SIRS) criteria [8], oligo-anuric kidney failure and a dialysis catheter in place, a plasma concentration of endotoxin of 0.1 U/ml or more, informed written consent of the patient or a legal representative as approved by the institutional ethics committee. Patients were excluded if they were less than 18 years old, if pregnancy could not be ruled out or if they were actively bleeding. For safety reasons, patients receiving activated recombinant protein C were also excluded from this phase I study.

Adsorption therapy was initiated with a blood flow rate of 150 ml/min through a venous double-lumen catheter. Plasma was separated from blood by a plasma filter and passed through an Enso® adsorber cartridge (Gambro, Hechingen, Germany) with a flow of 1.5 l/h and continuously reinfused to the patients (fig. 1). Treatment sessions were 2 h each and were repeated on 5 consecutive days. Between adsorption treatments, continuous veno-venous hemodiafiltration was performed as needed. Routine monitoring included body temperature, urinary output, respiratory frequency, heart rate (HR) and blood pressure. Hemodynamic management included fluid resuscitation to keep central venous pressure (CVP) >8 mm Hg, use of norepinephrine to obtain a mean arterial pressure above 65 mm Hg and use of dobutamine if $cvSO_2$ was <70% or if there were signs of peripheral hypoperfusion, according to the guidelines of the surviving sepsis campaign.

Simplified acute physiology score 3 (SAPS3) scores were calculated upon admission, acute physiology and chronic health evaluation II (APACHE II) scores were calculated for the first 24 h after admission, and organ dysfunction was assessed on a daily base using the sequential organ failure assessment (SOFA) score. Transpulmonary thermodilution was used for hemodynamic monitoring [9], employing a commercially available system (PiCCO, Pulsion Medical Systems, Munich, Germany) and cardiac output (CO), HR, mean arterial pressure (MAP), systemic vascular resistance (SVR), global end-diastolic volume (GEDV) and CVP were recorded before and after each treatment. Cardiac power index (CPI) as a value integrating MAP and CI was calculated using the following formula: CPI = CI × MAP/451 [10]. Before and after each treatment, blood samples were collected for routine biochemistry and blood counts. Endotoxin was measured using a modified commercial kit (Charles River Endosafe, Inc., Charleston, S.C., USA). IL-6 and IL-1 receptor antagonist (IL-1ra) were measured using commercial ELISA kits.

Determination of Plasma-Dependent Pro-Apoptotic Activity and Oxidant and Carbonyl Stress-Related Plasma Components

A post-hoc analysis was performed on collected plasma samples examining pro-apoptotic activity in a U937 human monocytic cell line. U937 cells were cultured as described before [11]. Cultured monocytes were incubated with plasma samples obtained before, and after, each adsorption treatment and cultured for 96 h. Apoptosis was evaluated after 72 and 96 h by staining with Hoechst 33342 and fluorescence microscopy. It was expressed as percent of the population, counting at least 300 cells in at least 6 random selected fields. Advanced oxidation protein products (AOPPs) and plasma carbonyl residues (RCOs) were determined in the paired samples as described elsewhere [12, 13].

Preclinical Tests of Adsorber Columns

Preceding the clinical use, biocompatibility and adsorption capacity of the adsorber materials had been evaluated in several in vitro experiments. Static adsorption experiments with plasma derived from donated citrate anticoagulated whole blood were performed at 37°C. 50 mg adsorbents were incubated with 1 ml plasma spiked with various amounts of *Escherichia coli* endotoxin (BioWhittaker, Inc., Walkersville, Md., USA). After 2 h incuba-

Table 1. Patients' baseline characteristics

Patient	Age/sex	Blood cultures	Source	Cirrhosis	APACHE II	SOFA	SAPS3	Survived
1	53/m	Gram-negative rods	pneumonia	yes	29	14	74	yes
2	69/f	*E. coli*	peritonitis	yes	46	17	72	no
3	44/m	*E. coli*	liver abscess	liver transplant	18	4	60	yes
4	68/m	*E. coli*	pneumonia	no	26	19	94	no
5	49/m	Negative	SBP	yes	28	15	83	no
6	53/m	*Klebsiella pneumoniae*	perineal cellulitis	no	32	19	88	yes
7	68/m	*Enterococcus*	line infection	no	40	16	121	no
8	38/m	*E. coli*	SBP	yes	22	17	66	yes
9	59/m	*E. coli*	pneumonia	no	26	14	98	yes
10	61/f	*E. coli*	peritonitis	no	22	10	75	yes

SBP = Spontaneous bacterial peritonitis; *E. coli* = *Escherichia coli*; APACHE II = acute physiology and chronic health evaluation score; SOFA = sequential organ failure assessment score; SAPS3 = simplified acute physiology score.

tion, equilibrium endotoxin concentrations were measured in the supernatant using the chromogenic *Limulus* amebocyte lysate test (Charles River Endosafe, Inc.) as previously described [14]. Samples were heat-inactivated at 75°C for 15 min, and vortexed for 5 min. A 0.03–300-EU/ml standard curve was prepared with certified control standard ET (BioWhittaker, Inc.). This curve resulted in a kDa value of 1.1×10^{-9} M (based on the molecular weight of the LPS monomer) and a maximum adsorption capacity Q_{max} of 372 EU/g indicating the high affinity of the adsorbent for the endotoxin dissolved in human plasma.

Adsorber columns had been tested by perfusion with plasma derived from donated citrate anticoagulated whole blood. Endotoxin (LPS from *E. coli* O55B.5, BioWhittaker, Inc.) was added at a concentration of 10 EU/ml. In this setting, the adsorber was able to completely remove the added endotoxin.

Statistical Analysis

Laboratory parameters before and after treatment were not distributed normally and were compared using a Wilcoxon paired samples test. Hemodynamic parameters were normally distributed and Student's t test was used to compare values before and after treatment sessions.

Results

Baseline Demographics (table 1)

Ten patients were enrolled (8 male, 2 female; mean age 56 ± 11 years). The exclusion of patients receiving activated protein C resulted in a high proportion of patients with cirrhosis of the liver (n = 4). Sepsis was caused by pneumonia (n = 3), spontaneous bacterial peritonitis (n = 2), peritonitis due to colonic perforation (n = 2) and liver abscess, soft tissue infection and line infection in 1 case each. In 8 out of 10 patients, blood cultures grew Gram-negative rods, cultures were sterile in 1 patient with spontaneous bacterial peritonitis (ascitic fluid granulocyte count 1.2 g/l), and *Enterococcus faecium* was found in blood cultures of 1 patient, in whom *Klebsiella oxytoca* was cultured from bronchoalveolar lavage fluid. Median scores (25th–75th percentile) for SAPS3 were 79 (72.5–92.5), for APACHE II 27 [23–31] and for SOFA 15.5 [14–17].

Clinical Course and Outcome

Overall, in 10 patients, 49 treatments were completed. One patient received four treatments only and was transferred to intermediate care after clinical improvement. Three treatments were performed without invasive hemodynamic monitoring, due to technical problems. All patients were mechanically ventilated at the beginning of the study and all had oliguric kidney failure, placement of a dialysis catheter being a prerequisite for enrollment. Seven patients were treated with continuous veno-venous hemodiafiltration after each adsorption treatment. One patient with chronic kidney failure received hemodialysis. In 2 patients, renal function recovered and they could be managed without renal replacement therapy. Of 10 patients enrolled, 4 died within 28 days but none during the 5 days of adsorption treatment.

Safety Profile

There were no catheter-related problems. During two treatment episodes, there were hypertensive reactions (systolic blood pressure >160 mm Hg, diastolic blood pressure >100 mm Hg), one leading to bradycardia at the end of the adsorption period. There were no clinically

Table 2. Hemodynamic parameters before and after adsorption treatment

	Before treatment	After treatment	p
MAP, mm Hg	81 ± 16	88 ± 17	0.001
Norepinephrine, μg/min	1.7 (0.0–5.0)	0.5 (0.0–3.3)	0.002
CVP, mm Hg	11.5 (8–16)	13 (6–20)	0.036
GEDVI, ml/m^2	745 ± 127	784 ± 109	0.002
CPI, mm Hg · l/min/m^2	0.72 ± 0.22	0.81 ± 0.22	0.002
CI, l/min/m^2	4.0 ± 1.0	4.2 ± 0.9	0.062
HR, beats/min	95 ± 22	96 ± 22	0.763
SVRI dyne · s/cm^5/m^2	1,446 ± 534	1,441 ± 535	0.939

Data presented as mean ± SD or median (25th–75th percentile). MAP = Mean arterial pressure; CVP = central venous pressure; GEDVI = global end-diastolic volume index; CPI = cardiac power index; CI = cardiac index; HR = heart rate; SVRI = systemic vascular resistance index.

obvious bleeding episodes, no allergic reactions, and no instances of hemodynamic instability (drop of MAP >10 mm Hg) upon initiation of adsorption treatment.

Hemodynamic Parameters

At inclusion, 8 of 10 patients fulfilled the criteria of septic shock, being dependent on therapy with norepinephrine to maintain a mean arterial pressure of ≥65 mm Hg even after fluid resuscitation. In 2 patients, dobutamine was added because of reduced central venous oxygen saturation and signs of impaired peripheral perfusion. Comparing hemodynamic parameters immediately before and after adsorption treatments, there was a significant increase in MAP despite a significant reduction in norepinephrine doses. Cardiac power index increased, but the rise in CO failed to reach significance and systemic vascular resistance index remained stable. GEDV index (GEDVI) was increased significantly (table 2). These changes were partially reversed in between adsorption treatments, with significant reductions of MAP (88 ± 17 to 82 ± 16 mm Hg; p = 0.035), CPI (0.81 ± 0.22 to 0.74 ± 23 mm Hg · l/min/m^2; p = 0.035) and a concomitant increase in norepinephrine doses (0.5 [0.0–3.3] to 1 [0.0–2.2] μg/min; p = 0.049).

Measurements of Endotoxin

On average, there was no significant change of measured endotoxin levels during adsorption treatment. However, there were large intra- and interindividual variations and changes of plasma values over a wide range between the lower limit of detection and values not described in clinical conditions. Values did not follow any recognizable pattern. Pretreatment values were 0.2 EU (0.1–0.8), range 0.1–14.1, posttreatment values were 0.3 EU (0.1–1.7), range 0.1–9.5. Percent changes of measured endotoxin levels ranged from –67 to +1,250.

Inflammatory and Coagulatory Markers

Clinical chemical and hematological parameters are presented in table 3. There were significant reductions in the levels of coagulation factors after each treatment, values recovered, however, and pre-adsorption values remained unchanged over the treatment period. C-reactive protein (CRP) and procalcitonin were significantly decreased after adsorption therapy, whereas IL-1ra and IL-6 remained constant.

Markers of Oxidative Stress and Pro-Apoptotic Activity on U937 Monocytic Cells in vitro (table 4)

At the beginning of therapy, levels of AOPPs and RCOs were strikingly elevated compared to values found in healthy subjects. We observed a significant reduction of AOPPs from the beginning to the end of adsorption treatments, whereas there was no change in the levels of RCOs. Levels of AOPPs before treatment were significantly correlated with the respective SOFA scores (r = 0.497, p = 0.007) and CPI (r = 0.497, p = 0.014).

Compared with apoptosis of U937 cells exposed to plasma drawn before adsorption treatments, there was a significant decline in apoptosis of U937 cells exposed to plasma collected at the end of adsorption treatments at 72 h and a trend that barely missed the level of significance at 96 h. Changes of pro-apoptotic activity of plasma during adsorption treatment were correlated with changes in systemic vascular resistance (r = 0.562, p = 0.006 at 72 h).

Table 3. Clinical chemical and hematological parameters

	Before treatment	After treatment	p
Creatinine, mmol/l	209 (146–285)	203 (147–285)	0.293
Bilirubin, mmol/l	57 (27–115)	52 (25–104)	<0.001
CRP, mg/l	67 (35–128)	37 (19–87)	<0.001
Coagulation factor II, %	32 (25–43)	18 (12–24)	<0.001
Coagulation factor V, %	26 (20–47)	12 (10–32)	<0.001
Coagulation factor VII, %	27 (11–55)	12 (9–32)	<0.001
Fibrinogen, mg/dl	254 (198–351)	213 (166–288)	<0.001
Activated protein C, %	32 (19–53)	18 (13–36)	<0.001
Procalcitonin, ng/ml	1.7 (0.0–5.0)	0.5 (0.0–3.3)	<0.001
White blood cells, g/l	14.5 (6.5–29)	17.4 (7.7–31.7)	0.010
Platelets, g/l	32 (15–70)	32 (14–69)	0.121
Il-1ra, pg/ml	256 (86–803)	248 (94–876)	0.700
Il-6, pg/ml	45 (13–170)	48 (16–164)	0.958

CRP = C-reactive protein; IL-1ra = interleukin-1 receptor antagonist; IL-6 = interleukin 6. Values presented as median (25th–75th percentile).

Table 4. Markers of oxidative stress and in vitro pro-apoptotic activity of plasma on U937 culture

	Before treatment	After treatment	p
AOPP, μmol/l	152 (89–232)	123 (67–184)	<0.001
RCO, nmol/mg protein	1.00 (0.72–1.60)	1.33 (0.68–1.61)	0.614
Apoptosis at 72 h, %	21 (16–37)	16 (13–22)	0.001
Apoptosis at 96 h, %	36 (22–48)	25 (17–37)	0.059

AOPP = Advanced oxidation protein products; RCO = plasma carbonyl residues. Values presented as median (25th–75th percentile).

Discussion

In this safety and feasibility study we observed only minor complications of the combined plasma separation and adsorption treatment with an arginine-coated adsorber. The most pronounced treatment effect was an improvement of circulatory function. While catecholamine doses could be significantly reduced, peripheral vascular resistance remained stable, suggesting a decrease in vasodilatory factors. With no change in vascular resistance, there still was a marked increase in MAP, obviously caused by an improved cardiac function. This may have been partly due to an increase in GEDVI, which could have improved cardiac preload by the starling mechanism. The adsorption column was prefilled with 300 ml of saline being transfused to the patient at the beginning of plasmapheresis, but this amount of fluid seems too small to cause a relevant increase in GEDVI [15]. Likewise, fluid boli were not administered during adsorption treatments and patients only received parenteral fluids at a combined rate of <100 ml/h. Thus, the increase in GEDVI may have been caused by an increased venous return and reduced blood pooling due to the attenuation of septic vasodilation or by an increased afterload due to a higher peripheral vascular resistance.

The increase in CPI noted in our study is suggestive for an improvement in myocardial contractility. Myocardial depression has long been known to contribute to circulatory failure in sepsis syndrome [16, 17]. Recently, endotoxin has been found to depress cardiac myocyte shortening via TNF-α produced by infiltrating monocytes and neutrophils [18]. Therefore, the improvement in myocardial dysfunction seen in our study could be due to either a global decrease of the inflammatory response or a reduced production of TNF-α after endotoxin removal.

The fact that the hemodynamic effects observed during plasma separation and adsorption treatment were partially reversed in between treatment sessions suggests

a causal relation between adsorption treatment and hemodynamic effects.

However, measurements of endotoxin in plasma drawn at the beginning and end of each treatment did not reveal any change in LPS plasma levels. This may be due to problems with the endotoxin assay used, changes in the compartmentalization of endotoxin or intermittent endotoxemia. The chaotic pattern of measurement results suggests technical problems with the assay. Also, preclinical tests evaluated adsorber efficacy only for endotoxin from *E. coli*. As the conformation of the lipid A moiety is different for lipopolysaccharide from various Gram-negative bacteria [19], this may have impaired endotoxin adsorption. However, in the majority of patients, infection with *E. coli* was documented and it is unlikely that the lack of detectable effects of adsorption on endotoxin levels is due to diverse species of endotoxin.

Adsorption of inflammatory mediators may occur during renal replacement therapy. We specifically addressed serum levels of IL-6 and IL-1ra. IL-6 is both a mediator and a marker of sepsis and has a longer half-life than other inflammatory cytokines such as TNF-α or IL-1. IL-1ra is an important part of the counter-inflammatory response. Both markers are associated with mortality in sepsis [20, 21]. We could not detect any changes in the levels of both cytokines, arguing against direct adsorption by the adsorber column as well as indirect changes to the cytokine profile caused by adsorption treatment. Significant reductions in serum levels of CRP and procalcitonin suggest a reduced inflammatory response, but, without measurements of plasma levels before and after the adsorber column, we cannot exclude elimination by the adsorber.

In the post-hoc analysis of parameters associated with oxidative stress and apoptosis, we found a significant reduction of AOPPs and of pro-apoptotic activity of plasma after adsorption treatments. In sepsis there are several potential sources of reactive oxygen species (ROS) [22]. Granulocytes and other phagocytes produce O_2^- as a cytotoxic agent after activation by complement or endotoxin and an increase of ROS in macrophages and lymphocytes after LPS challenge has been demonstrated in various models of septic shock. Recently a correlation of ROS production with patient survival could be demonstrated [23]. Accumulation of ROS leads to accumulation of AOPP and advanced glycation end-products (AGE). AOPP as well as AGE have been shown to induce monocyte activation and lead to enhanced production of pro-inflammatory cytokines and respiratory burst products [12]. Furthermore, recent data describe a key role for the receptor of AGE (RAGE) in animal models of septic shock. This again underlines the importance of AOPP and AGE and gives a rational for our observation that initial levels of AOPPs were correlated with pretreatment SOFA scores, and that the relative reduction during each treatment was correlated with the observed hemodynamic improvement. These data, and the reduced pro-apoptotic activity of plasma, suggest that the circulatory changes observed in our patients are part of a general reduction of the inflammatory response. However, an influence of adsorption treatment on mortality could not be assessed in this small number of patients. The observed mortality of 40% compares favorably with the literature, given the severity of disease evidenced by the high APACHE II, SAPS3 and SOFA scores of our patients. Secondary ischemic changes may amplify sepsis-related activation of coagulation and inflammation [24, 25] and early hemodynamic resuscitation aiming at restoration of an adequate tissue perfusion has been shown to reduce mortality in sepsis [26]. The rapid circulatory improvement noted in our study thus may be relevant in the treatment of septic shock.

In conclusion, plasma separation and treatment with an arginine-based adsorber is safe and resulted in improved hemodynamics and a reduction of AOPP and pro-apoptotic activity of plasma. Particularly the hemodynamic improvement may be relevant, as it has been shown that early circulatory stabilization has a large impact on patient survival in severe sepsis and septic shock [26]. A reduction of endotoxin levels could not be proved, and we cannot exclude the possibility that the clinical effects observed were due to other effects of plasma separation and adsorption treatment. Given the prevalence of severe sepsis and its high mortality, further in vitro and in vivo studies should be performed to elucidate the mode of action of this arginine-based adsorber column.

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