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HELP Apheresis in the Treatment of Sepsis

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Abstract: Heparin induced extracorporeal lipoprotein fibrinogen precipitation (HELP) is an established procedure for removal of low-density lipoprotein (LDL) cholesterol, lipoprotein (a), and fibrinogen in patients with severe hypercholesterolemia. In vitro studies revealed that HELP also removes endotoxin, tumor necrosis factor α (TNF-α) and C-reactive protein (CRP). With the intention to treat, we applied this procedure to 4 patients with severe gram-negative sepsis with highly elevated endotoxin blood levels. Nine treatments were performed, 6 using the standard HELP precipitating buffer and 3 without addition of heparin to the precipitating buffer. Heparin was omitted from the precipitating buffer to avoid fibrinogen depletion in patients at risk (low fibrinogen, postoperative). The average processed plasma volume was 3,386 ml in the standard and 2,963 ml in the modified treatment. Mean reductions (%) in plasma solute concentrations were (standard/modified procedure) as follows: endotoxin, 50/57; TNF-α, 25/5; CRP, 49/55; fibrinogen, 49/6; total cholesterol, 38/5; and apolipoprotein B (Apo B), 41/2. Both treatment modalities were equally effective in removing endotoxin and CRP. With the modified precipitation buffer, fibrinogen was not removed. To further simplify the extracorporeal treatment, we have designed a closed-loop circuit with 2 adsorbers in series, one for removal of TNF-α (dextran sulfate modified cellulose) and the other for removal of endotoxin (DEAE-cellulose). In vitro evaluation confirmed very efficient endotoxin and TNF-α removal from plasma. This system is very simple, operates at physiological pH, and uses adsorbers already in clinical use for other purposes. **Key Words:** Apheresis—Sepsis—Endotoxin removal.

Despite all therapeutic attempts, gram-negative sepsis is still a major cause of death in intensive care units in developed countries (1). Therefore, there is an ongoing interest in alternative treatment options. New therapies include administration of immune globulins (2–4) and binding and/or neutralization of endotoxin (lipopolysaccharides [LPS]) (5–7), tumor necrosis factor α (TNF-α) (8,9) and interleukin-1β (IL-1β) (10). None of these therapies has improved the overall prognosis of sepsis. Because endotoxin and proinflammatory cytokines initiate the sepsis cascade, extracorporeal therapies aimed at removing these substances have been incorporated into the management of sepsis and septic shock. Such procedures include continuous hemofiltration (11,12), unselective plasma exchange (13,14), and more recently selective procedures such as perfusion of polymyxin B coated hollow fibers (15–17), the microsphere based detoxification system (MDS) (18), and the heparin induced extracorporeal lipoprotein-fibrinogen precipitation (HELP) procedure (19). Here we report our initial findings when applying the standard and a modified HELP procedure clinically in patients with gram-negative, refractory septic shock. Additionally, we describe in vitro results with a very simple extracorporeal circuit for removing endotoxin and/or TNF-α.

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MATERIAL AND METHODS

The Plasmat Secura system (B. Braun Melsungen, Melsungen, Germany) was used for all clinical HELP treatments. The HELP procedure is an established extracorporeal therapy for the removal of low-density lipoprotein (LDL) cholesterol, lipoprotein (a), and fibrinogen from the blood of patients with severe, otherwise refractory hypercholesterolemia (20).

A total of 9 procedures was performed in 4 patients suffering from gram-negative refractory septic shock. All patients had elevated plasma levels of

LPS and TNF-α before the first treatment (LPS: mean, 97; range, 62–155 pg/ml and TNF-α: mean, 46; range, 18–71 pg/ml). Six treatments were performed under standard HELP operating conditions (heparin buffer concentration, 100,000 IU/L). With the goal of removing LPS but not fibrinogen, in 3 treatments no heparin was added to the buffer (modified treatment). Shaldon type catheters placed in a central vein were used as vascular access, allowing blood and plasma flow rates of 60–200 and 15–45 ml/min, respectively. On average, 3,245 (range, 2,741–4,008) ml of plasma were processed.

For more selective adsorption of LPS and TNF-α, we developed a simple circuit and tested it under in vitro conditions. The circuit consisted of adsorption devices already in use for other extracorporeal treatments. To test the system, 2.1 L of freshly drawn anticoagulated (heparin, 5 U/ml) blood with a hematocrit of 0.43 was used in a recirculation circuit. Blood and plasma flow rates were controlled using newly developed hardware (DIAPACT, B. Braun Carex, Mirandola, Italy). For plasma separation, a hollow-fiber filter (Hemoselect, 0.2 m^2, B. Braun Melsungen AG, Melsungen, Germany) was used. Plasma at a rate of 25 ml/min was first passed through a TNF-α adsorber (Liposorber LA-15; bed volume, 150 ml; Kaneka, Osaka, Japan) and then through an endotoxin adsorber (DEAE-cellulose; bed volume, 500 ml; B. Braun Melsungen AG). In the first part of the experiment, TNF-α (rhTNFα, Boehringer, Ingelheim, Germany; starting concentration, 424 pg/ml) was added to the blood. After processing 2 plasma volumes (corresponding to 2,400 ml), LPS (*E. coli* 055; B5, Sigma Chemical Co., St. Louis, MO, U.S.A.; starting concentration, 50 EU/ml) was added, and another 10 plasma volumes (12,000 ml) were processed under recirculating conditions. Samples were drawn at appropriate perfusion volumes to quantify the extraction of TNF-α and LPS from the blood reservoir during the first and second parts of the experiment, respectively.

LPS was measured using a kinetic limulus amoebocytes lysate assay (LAL-QCL, BioWhittacker, Wakersville, Maryland, U.S.A.). TNF-α was assayed using an immunoenzymetric kit (EAISA, Medgenix Diagnostics, Fleurus, Belgium). All other parameters were determined using routine laboratory techniques.

RESULTS

All 9 clinical HELP treatments performed using either the standard or the modified mode were tolerated very well. In the 1 patient who survived, the improvement of pulmonary gas exchange, the reduction of an initially raised cardiac index, and an increase in peripheral vascular resistance during the first HELP treatment were impressive (details are presented elsewhere [19]). Despite a significant reduction in plasma LPS concentrations and continuing supportive care, the other 3 patients died 5, 16, and 33 days after the last HELP treatment.

Table 1 compares the parameters that were used to determine the efficiency of both treatment modalities. The volume of plasma processed was some 10% higher in the standard mode. While average reduction rates for LPS and C-reactive protein were similar in both treatment modes, the decreases in concentrations of TNF-α, fibrinogen, total cholesterol, and apolipoprotein B were negligible in the modified procedure.

The in vitro evaluation performed to characterize the new LPS/TNF-α adsorption system confirmed an efficient extraction of TNF-α by the Liposorber LA-15 cartridge in the first part of the experiment. However, after perfusion with 2,400 ml of plasma, the cartridge was nearly saturated with TNF-α as indicated by the minimal inlet to outlet concentration difference (Fig. 1). The concentration of TNF-α in the blood reservoir had decreased by 54% after perfusion of 2 plasma volumes. The absolute amount of TNF-α adsorbed during this experiment was calculated to be about 272,000 pg.

TABLE 1. *Average percent reduction of LPS, TNF-α, C-reactive protein, fibrinogen, total cholesterol, and apo B in the standard and modified HELP treatment modes during clinical apheresis*

	Standard treatment (n = 6)	Modified treatment (n = 3)
Processed volume	3,386 ml Mean % reduction	2,963 ml Mean % reduction
LPS	50 ± 3	57
TNF-α	25 ± 10	5
C-reactive protein	49 ± 4	55
Fibrinogen	49 ± 3	6
Total cholesterol	38 ± 12	5
Apo B	41 ± 7	2

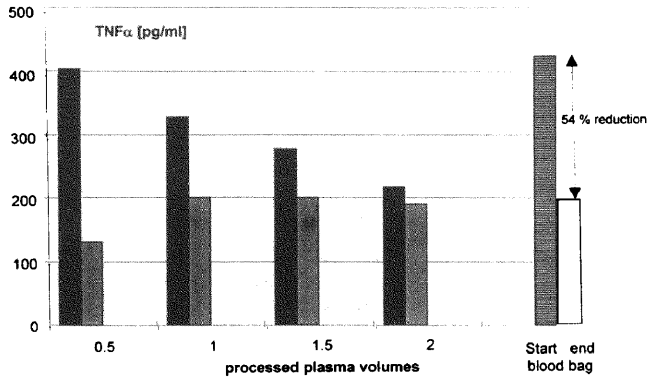

FIG. 1. The graph shows a comparison of inlet and outlet concentrations for TNF-α after perfusions of 600, 1,200, 1,800 and 2,400 ml of plasma representing 0.5, 1, 1.5, and 2 plasma volumes of the plasma pool, respectively. Additionally, the starting concentration and the concentration of TNF-α in the blood bag after perfusion of 2.4 L are indicated by the columns on the right. A 54% reduction of the initial concentration of TNF-α was observed.

In the second part of the experiment, the adsorption of LPS by the DEAE-cellulose cartridge was analyzed. The initial blood concentration of LPS was about 50 EU/ml (about 5,000 pg/ml). The decrease in plasma concentration during perfusion closely followed the theoretical decrease expected if LPS is completely removed by the adsorber (Fig. 2). After perfusion of 2 plasma volumes, the LPS concentration had already decreased to less than 20% of the initial level.

Regarding other plasma proteins, this new system removes LDL and very low-density lipoprotein (VLDL) cholesterol, apolipoprotein B, triglycerides, and lipoprotein (a), which are adsorbed by the Liposorber LA-15 as expected. It also removes ceruloplasmin, α1 acid glycoprotein, and retinol binding protein, which are adsorbed by the DEAE-cellulose (21).

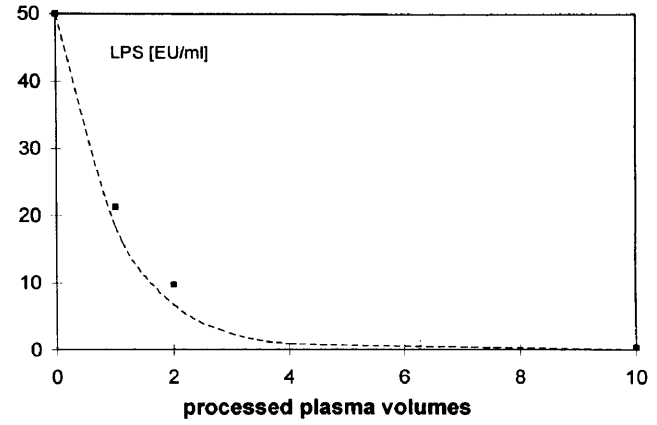

FIG. 2. Plotted is the reduction of the LPS concentration in the blood bag at the beginning and after perfusion of 1, 2, and 10 plasma volumes, respectively. The dashed line depicts the expected concentration course if the adsorber extracts LPS completely from the perfused plasma (Boos, 1997).

DISCUSSION

None of the novel pharmaceutical interventions designed to interrupt the induction phase of gram-negative sepsis published during the last years (2–9) has been able to demonstrate a real reduction in sepsis associated mortality. Therefore alternative strategies such as hemofiltration, unselective plasma exchange, or more recently selective adsorption of LPS and proinflammatory cytokines have engendered interest as experimental therapies in refractory gram-negative sepsis. One of these extracorporeal procedures is HELP apheresis, which was originally designed to remove LDL cholesterol, lipoprotein (a), and fibrinogen from blood for treatment of severe hypercholesterolemia. We recently demonstrated that HELP apheresis also eliminates LPS and TNF-α (19). Therefore, we applied the HELP procedure in patients with severe gram-negative sepsis not responding to standard treatment. With an intention to treat, a total of 9 HELP treatments were applied to 4 such patients. The extracorporeal treatment itself was tolerated very well in all cases. Fibrinogen elimination with the standard procedure is indicated in patients with very high fibrinogen levels and disturbed microcirculation. However, lowering fibrinogen levels may limit the repeated clinical application of HELP in critically ill patients. With the use of a modified buffer, we were able to remove LPS without decreasing fibrinogen or LDL (19). This modified procedure can safely be performed in all patients with low pretreatment fibrinogen levels (below 150 mg/dl) for whom a further decrease in fibrinogen should be avoided.

After characterization of the HELP system and its components with regard to LPS and cytokine removal, we designed a simple extracorporeal circuit consisting of a plasma separator and 2 adsorbers to remove LPS and the primary proinflammatory cytokine, TNF-α, more selectively. Under in vitro conditions, this circuit removed both LPS and TNF-α effectively (21). The LPS concentration decreased from 50 EU/ml to 10 EU/ml (5,000 to 1,000 pg/ml) after perfusion of 2.4 L of plasma. The total amount of LPS removed during this experiment by adsorption by the DEAE-cellulose cartridge amounted to 4,800,000 pg. In comparison, our clinical patients with gram-negative sepsis treated with the HELP system had a mean pretreatment LPS level of about 100 pg/ml. Therefore, in the clinical setting the capacity of our circuit is nearly unlimited and would

allow clearance of more than 48 L of plasma in a typical patient. For TNF-α we calculated a total binding of 272,000 pg to the LA-15 cartridge. In a typical patient with a TNF-α starting level of about 50 pg/ml, this cartridge would allow extracting of TNF-α from more than 6 L of plasma.

This new procedure offers the unique possibility of removing very effectively LPS and TNF-α, especially during the hyperinflammatory phase of sepsis. The use of only 1 adsorber (e.g., DEAE-cellulose alone) would allow an individualized treatment dependent on the clinical requirements.

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