H.E.L.P. in Gram-negative, Refractory Septic Shock: First Clinical Experiences.

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Abstract

H.E.L.P. (heparin-induced extracorporeal lipoprotein-fibrinogen precipitation) is an established procedure for extracorporeal treatment of severe hypercholesterolemia. Recently, an in-vitro re-evaluation of this method revealed that not only low-density lipoprotein (LDL), lipoprotein (a) and fibrinogen, but also lipopolysaccharides (LPS; endotoxin), tumor necrosis factor α (TNF-α) and C-reactive protein (CRP) are removed from plasma. As LPS and TNF-α are involved in the pathogenesis and progression of sepsis we performed in a pilot study nine treatments in four cases with LPS-induced refractory septic shock. Extracorporeal treatments were performed in the standard H.E.L.P. mode (N = 6) or in a modified mode without addition of the precipitating agent heparin (N = 3). On average 3.245 L of plasma was processed during each session. All treatments were tolerated very well. Observed percent reduction of plasma constituents were (mean value; standard/modified procedure): LPS, 49.7/57.2; TNF-α, 24.6/4.5; CRP, 49.4/54.8; Fibrinogen, 49.4/5.7; total cholesterol, 37.8/4.7; ApoB, 40.6/2.0. Despite all therapeutic efforts, outcome however, was poor: one patient survived, three patients died 5, 16 and 33 days, respectively, after the last H.E.L.P. treatment.

H.E.L.P. performed in the standard mode removes both LPS and TNF-α without eliminating substances necessary for the endogenous clearance of LPS (e.g., high-density lipoprotein). The advantage of this procedure is that it is aimed not only at a single target of the sepsis cascade. Further clinical evaluation will focus on amount of plasma volume processed, optimal time to initiate treatment as well as treatment intervalls.

Key words: refractory septic shock, sepsis, apheresis, extracorporeal plasma treatment, H.E.L.P., lipopolysaccharides, endotoxin, tumor necrosis factor α

Introduction

Despite a wide range of new therapeutic strategies (1,2,3), septic shock with multiorgan failure still has a poor prognosis and is the leading cause of death in intensive care units. The treatment of septic shock includes the use of antibiotics and the surgical eradication of the nidus of infection, the neutralization (4,5,6) or extracorporeal elimination (7) of microbial lipopolysaccharides (LPS), a modulation of the host response (e.g., 8,9) and finally to provide adequate intensive care life support (2,3). Presently, all new therapeutic approaches are aimed at single targets in the inflammatory cascade either to antagonize microbial toxins or to modulate the host response. Extracorporeal treatment strategies used so far in refractory septic shock include unselective plasma exchange with substitution of fresh plasma (e.g., 10,11), plasma perfusion using a Polymyxin B cartridge (e.g., 7) and more recently continuous hemofiltration (12).

Based on a pilot study we describe a new approach for extracorporeal treatment of

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patients suffering from refractory septic shock according to the definitions of Bone (13). The treatment relies on an established (more than 45,000 applications [14]) apheresis procedure which is known under the acronym H.E.L.P. (heparin-induced extracorporeal lipoprotein-fibrinogen precipitation) and which has been successfully applied for the treatment of severe hypercholesterolemia. When re-evaluating the H.E.L.P. procedure under in vitro conditions, we found that factors involved in the pathogenesis and progression of sepsis, i.e., lipopolysaccharides (LPS; endotoxin) and tumor necrosis factor α (TNF-α) are also removed from plasma (15).

Methods and patients

The H.E.L.P. procedure has been described in detail elsewhere (16). Briefly, plasma is separated from anticoagulated whole blood using a hollow fiber plasma separator. The plasma then is mixed at a 1 : 1 volume ratio with a heparin-acetate buffer (heparin 100,000 U/L, pH 4.85) resulting in a final pH of 5.12. Under these conditions low-density lipoprotein (LDL), lipoprotein (a) and fibrinogen quantitatively precipitate and are removed by filtration. Thereafter, excess heparin is absorbed by an anion-exchanger and pH as well as plasma volume are readjusted to physiological conditions by bicarbonate dialysis and ultrafiltration. Finally, the plasma is recombined with the blood cells and reinfused into the patient. Under standard conditions, the H.E.L.P. procedure allows the extracorporeal processing of one plasma volume (3.0 - 3.5 L) resulting in a 50 - 60 % reduction of the above-mentioned proteins (14,16). TNF-α is removed by precipitation in the presence of excess heparin at pH 5.12, and LPS and CRP are eliminated by adsorption to the DEAE-anion-exchanger (15).

All H.E.L.P. treatments were performed using the Plasmat secura^{R} system (B. Braun Melsungen, Germany). A total of nine treatments have been performed in four patients. All patients suffered from gram-negative, refractory septic shock. Six treatments were run under standard H.E.L.P. conditions (heparin 100,000 U/L); in three treatments no heparin was used in the buffer ("modified" H.E.L.P.). On average, 3,245 (range 2,741 - 4,008) mL of plasma was processed extracorporeally (blood flow rate 60 - 200 mL/minute; resulting plasma flow rate 15 - 45 mL/min). Shaldon type catheters placed in the internal jugular, subclavian or femoral veins served for vascular access. Unfractionated heparin or Orgaran^{R} (only in patient E.M. with presumed type II heparin-induced thrombocytopenia) was used for anticoagulation.

Fibrinogen, total cholesterol, apolipoprotein B (ApoB) and CRP were determined with routine laboratory techniques. LPS was measured using a kinetic Limulus Amoebocytes Lysate assay (LAL-QLC) from BioWhittacker (Walkersville, USA). TNF-α was assayed using an immunoenzymetric kit (EASIA, Medgenix Diagnostics, Fleurus, Belgium).

Four patients with refractory septic shock and highly elevated LPS levels were included in this pilot study with the intention to treat. Details regarding age, pre-treatment LPS levels, total number of treatments, and outcome are given in Table 1. All patients were female. The origin of gram-negative sepsis was fulminant meningococcal infection in patient DM, developed postpartum in patient EM, originated from an infected kidney stone in patient PZ and occurred

several days after a tumor gastrectomy in patient WM.

Table 1
Description of patient characteristics including age at presentation (in years), plasma LPS (pg/ml) and TNF-α (pg/ml) levels before the first H.E.L.P. treatment, number of treatments, and outcome (including intervall between last H.E.L.P. treatment and death).

Patient	Age	LPS	TNF-α	Treat-ments	Outcome, Intervall
DM	16	69	71	2	died, 16 days
EM	33	100	18	3	died, 5 days
PZ	58	155	60	3	survived
WM	58	62	33	1	died, 33 days

Results
So far nine treatments have been performed in a total of four patients. Six treatments were performed in the standard mode and three in the modified one. All treatments were tolerated very well and the cardiovascular status and the pulmonary gas exchange remained stable or improved. Most impressive was the rapid improvement of pulmonary gas exchange, the reduction of increased cardiac index (CI) and the increase of peripheral vascular resistance without changing the dose of catecholamines administered during the first treatment of patient PZ (see table 2). These effects were not so pronounced in subsequent patients.

Table 2
Clinical and laboratory data observed during the first H.E.L.P. treatment in patient PZ (female, 58 years old). This patient suffered from refractory septic shock due to gram-negative sepsis originating from an infected kidney stone. Processed plasma volume, 3.5 L. FIO_2, fraction of inspired oxygen; CI, cardiac index.

Parameter	before H.E.L.P.	after H.E.L.P.
FIO_2	1.0	0.35
CI (L/min)	13.2	10.5
Fibrinogen (mg/dl)	1,014	484
LPS (pg/ml)	155	79
TNF-α (pg/ml)	59.7	37.8
CRP (mg/dl)	29.4	11.5

The reduction of serum or plasma levels of LPS, TNF-α, fibrinogen, CRP, cholesterol and ApoB are summarized in Table 3. A nearly 50 percent reduction of the plasma levels was observed for LPS, CRP and fibrinogen in the standard treatment where an average plasma volume of 3,386 mL was processed. A similar reduction was seen for LPS and CRP in the modified treatment format (mean processed plasma volume, 2,963 mL), while fibrinogen, total cholesterol, ApoB and TNF-α remained nearly unchanged by this procedure. An effective removal of TNF-α is only possible by the standard treatment with a reduction rate of about 25 percent.
Regarding the outcome, patient PZ recovered very quickly with most impressive changes seen during the first treatment session. However, despite all therapeutic efforts, the other three patients died at an intervall of 5, 16, and 33 days, respectively, after the last H.E.L.P. procedure.

Discussion
When performed under standard operating conditions the H.E.L.P. procedure removes both the inducer substance LPS and the early mediator TNF-α of the sepsis cascade after gram-negative infection. The additional elimination of fibrinogen has shown to improve microcirculation (17) and hence supports tissue oxygenation. Thus, septic

patients with very high fibrinogen levels should benefit from fibrinogen removal. Typically, after processing about 3 L of plasma under standard operating conditions, LPS, CRP and fibrinogen are decreased by 50 %, whereas TNF-α is eliminated less efficiently (25 %). In accordance with standard H.E.L.P. treatments in hypercholesterolemic patients, total cholesterol and ApoB plasma levels decreased by about 40 %. When applying the modified H.E.L.P. procedure, the efficiency remained unchanged for LPS and CRP, but declined to less than 6 % for fibrinogen, total cholesterol, ApoB, and TNF-α. This modification was choosen for repeated treatments of patients with normal plasma fibrinogen levels in order to avoid a decrease of fibrinogen below 200 mg/dl.

Table 3
Average percent reduction of LPS, TNF-α, CRP, fibrinogen, total cholesterol and ApoB in the standard and modified H.E.L.P. treatment mode, respectively. Average processed plasma volume was 3,386 mL for the standard and 2,963 mL for the modified procedure.

	Standard treatment	Modified treatment
	Mean % reduction	Mean % reduction
	(N = 6)	(N = 3)
LPS	49.7 +/- 3.2	57.2
TNF-α	24.6 +/- 10.4	4.5
CRP	49.4 +/- 4.0	54.8
Fibrinogen	49.4 +/- 2.5	5.7
Total cholesterol	37.8 +/- 11.5	4.7
ApoB	40.6 +/- 6.5	2.0

H.E.L.P. itself was tolerated very well in both treatment modifications. The simultaneous removal of LPS and TNF-α together with an improved hemorheology offers an attractive new approach for the treatment of septic patients. Extracorporeal treatment procedures available so far are either completely unselective (plasma exchange), are aimed at the specific removal of LPS only (Polymyxin B perfusion [7]) or rely on continuous hemofiltration (12). There are at least three arguments supporting a further evaluation of the H.E.L.P. procedure in sepsis:

1. The standard H.E.L.P. procedure offers the simultaneous removal of LPS and TNF-α. According to animal experiments, these two substances are thought to be mediators responsible for cardiovascular changes found in septic shock (18).
2. High-density lipoprotein known to enhance the endogenous clearance of LPS (2) is not removed by the H.E.L.P. procedure (14,16).
3. Clinically, all treatments were tolerated very well without negatively affecting cardiovascular status or pulmonary gas exchange.

As the outcome of patients with refractory septic shock is still disappointing, our continuing clinical research will focus on the following topics: The ideal time to initiate this extracorporeal treatment and the optimal treatment intervalls have to be defined. Furthermore, we will also consider processing larger plasma volumes.

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