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Elimination of LPS or/and TNFα from human plasma by adsorption-apheresis

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SUMMARY

We report on the in-vitro elimination of lipopolysaccharide (LPS, Endotoxin) or/and tumor necrosis factor α (TNFα) from human plasma using a novel apheresis hardware (DIAPACT®, B. Braun-Carex, Mirandola, Italy) equipped with an appropriate hollow fiber plasma separator and two cartridges placed in series for the selective adsorption of the target compounds. LPS originating under clinical conditions from gram-negative bacteria is removed by use of an anion-exchange cartridge packed with rolled fiber mats of DEAE-modified cellulose (H.E.L.P.-Heparin Adsorber, B. Braun Melsungen AG, Melsungen, Germany). Removal of TNFα from plasma is achieved by perfusion of dextransulfate-modified cellulose beads (Liposorber LA-15®, Kanegafuchi Chemical Industry, Osaka, Japan).

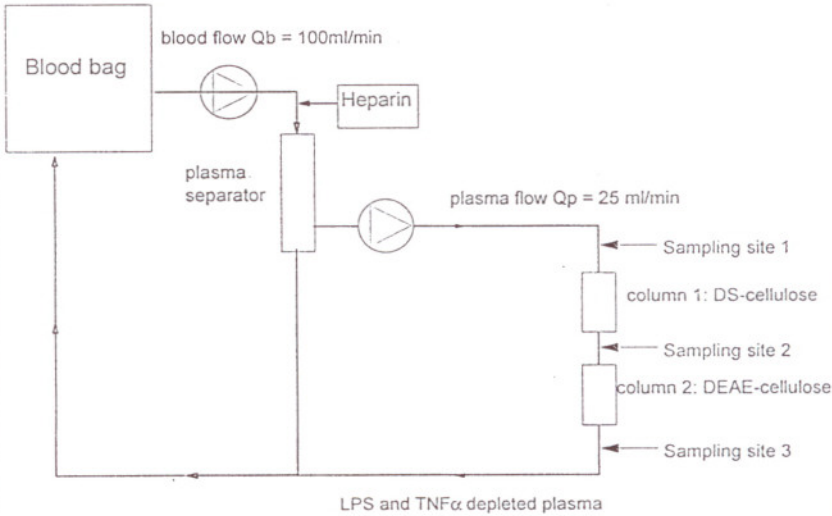
INTRODUCTION

For the treatment of sepsis originating from gram-negative bacteria different extracorporeal blood/plasma treatment procedures have been described. These are 1) unselective plasma exchange, i.e. substitution of patient plasma with donor plasma [1,2]; 2) hemofiltration, which removes low-molecular mediators (e.g. IL1β and IL 8) but not TNFα and LPS [3]; 3) plasma perfusion or hemoperfusion

using polymyxin B modified hollow fibers for selective removal of LPS [4]. More recently, patients suffering from refractory septic shock have been treated with the H.E.L.P. (Heparin-induced Extracorporeal Lipoprotein-fibrinogen Precipitation) system [5]. H.E.L.P. initially has been developed for treatment of severe familial hypercholesterolemia and more than 50,000 applications have been performed during the last decade [6,7]. When reevaluating the H.E.L.P. procedure we found that TNFα is precipitated in the presence of an excess of heparin at pH 5.12 and LPS is adsorbed to DEAE-modified cellulose [8]. H.E.L.P. treatment of septic patients (average processed plasma volume, 3.4 L) resulted in a reduction of 25 % for TNFα and 50 % for LPS [5] .The interaction of heparin with TNFα prompted us to use the synthetic polyanion dextransulfate being immobilized on cellulose beads (Liposorber LA-15®) for adsorptive removal of TNFα from plasma. Additionally we found, that the DEAE-cellulose adsorber (H.E.L.P. Heparin Adsorber, B.Braun Melsungen AG, Melsungen, Germany) used in the H.E.L.P. system also binds LPS with a high affinity and capacity at physiological pH from plasma. Here we report on our first in vitro experiments using both adsorber cartridges in an extracorporeal circulation for the removal of TNFα or/and LPS from human plasma.

MATERIALS AND METHODS

The newly developed apheresis equipment (DIAPACT®, B. Braun Carex, Mirandola, Italy) was used (Fig 1) to separate plasma (Haemoselect® 0.2 m^2, B. Braun Melsungen AG, Melsungen, Germany) under recirculating conditions from 2100 mL anticoagulated (heparin 5 U/mL) human blood (flow rate 100 ml/min) and to pump the plasma at a flow rate of 25 mL/min through the TNFα adsorber (Liposorber LA-15®,bed volume,150 mL) as well as the series-connected LPS adsorber (DEAE-cellulose, bed volume,500 mL).The whole adsorption-apheresis

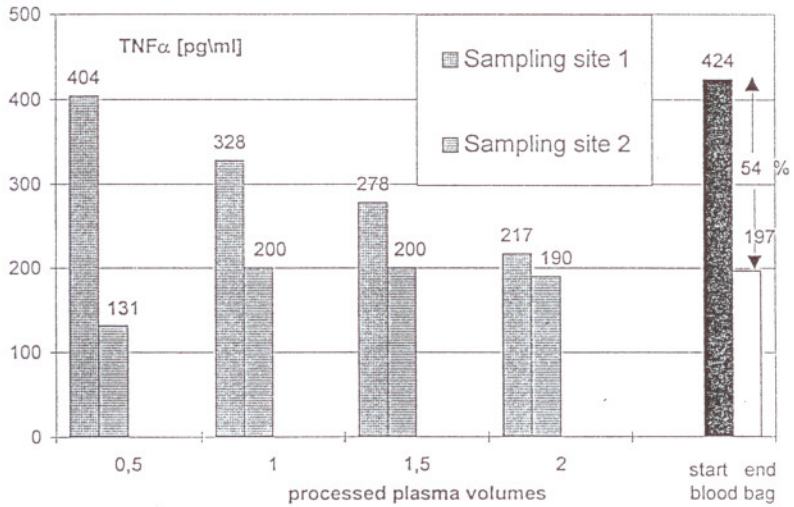


[Fig.1] Experimental set-up of the DIAPACT® adsorption-apheresis system. For details see text.

system was rinsed with 6 L of a Ringer solution. Prior to its use, the donor blood (2,100mL from four healthy persons) first was spiked with TNFα (rhTNFα, Boehringer Ingelheim, Germany) and after the processing of two plasma volumes (2400mL) additionally with LPS (E.coli 055: B5, Sigma Chemical Co, St Louis, USA). Samples were taken from the bag and sites indicated in Fig.1. LPS was measured using a kinetic Limulus Amoebocytes Lysate assay (LAL-QLC, BioWhittacker, Wakersville, USA). TNFα was assayed using an immunoenzymetric kit (EAISA, Medgenix Diagnostics, Fleurus, Belgium). All other parameters were determined with routine laboratory techniques.

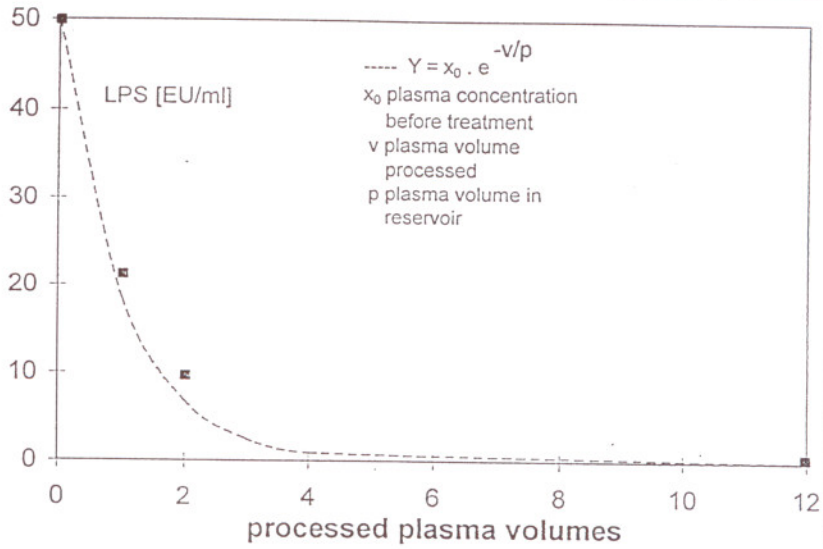
RESULTS AND CONCLUSIONS

Perfusion of plasma (TNFα, 424 pg/mL ; Fig.2) separated from TNFα–spiked donor blood resulted in a reduction of 54 % with respect to TNFα in the blood reservoir after the processing of two plasma volumes, i.e. 2400mL under recirculating conditions. The Liposorber LA-15© material was saturated with TNFα (Fig.2) after processing two plasma volumes. The saturation kinetics and binding capacity for TNFα strongly depend on the initial plasma level of fibrinogen (in-vitro experiment: 244 mg/dL) and low-density-lipoproteins (LDL; in-vitro experiment: LDL-cholesterol, 81 mg/dL) as this cation-exchanger primarily has been developed to adsorb these plasma proteins.



[Fig.2] Tumor Necrosis Factor α (TNFα) binding characteristics of the Liposorber LA-15© and reduction rate in the blood reservoir under recirculating conditions.

Fig.3 illustrate the reduction of the LPS concentration during the perfusion of 12 plasma volumes, i.e.14 400 ml under recirculating conditions. A comparison of these data with the theoretical elimination curve (dashed line in Fig.3) shows, that the DEAE-cellulose anion-exchanger removes LPS with nearly 100 % efficiency from the perfused plasma.



[Fig.3] Reduction profile of lipolysaccharide (LPS) in the blood reservoir during recirculating adsorption apheresis. Dashed line depicts 100 % elimination efficiency.

During adsorption-apheresis plasma levels of only a few constituents were affected, even after the perfusion of 6 plasma volumes, i.e. 7200mL. Elimination of fibrinogen, low-density-lipoproteins (LDL) and cholesterol, respectively was in accordance with the well-known binding of these proteins to the Liposorber LA-15®. IgM was reduced by approx. 41 % and IgA by 26 % after the perfusion of 2 plasma volumes. However, there was no further significant change when processing more plasma.

The DIAPACT®-based adsorption-apheresis system is very simple to install and to operate. According to our in-vitro results it is possible to treat at least 14 L of plasma which equal 4 plasma volumes of a patient. The extracorporeal processing of such a large plasma volume would lead to a very effective reduction of the target pathogens (Fig.2,3). On the other hand, the H.E.L.P. procedure has a higher efficiency with respect to elimination of fibrinogen. However, hypofibrinogenemia inherently limits the plasma volume that can be processed by H.E.L.P. Thus, both procedures could add to each other, especially in situations, where septic patients have very high fibrinogen levels (>1000mg/dL). Initial reduction of fibrinogen and LDL by H.E.L.P. also results in a higher binding capacity of the cation-exchanger for TNFα. In addition, the patient should benefit, as it was shown, that elimination of fibrinogen improves microcirculation [9] and hence supports tissue oxygenation.

In conclusion, single or combined removal of TNFα and LPS from plasma by adsorption-apheresis offers an attractive new approach for the treatment of septic patients. However, the ideal time to initiate this extracorporeal treatment as well as the optimal treatment period and intervals still have to be defined.

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