Confirmation of high cytokine clearance by hemofiltration with a cellulose triacetate membrane with large pores: an in vivo study

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Abstract:

Objective: To confirm in vivo the hypothesis that hemofiltration with a large pore membrane can achieve significant cytokine clearance. Method: We used a well-known animal model of endotoxemic shock (0.5 mg/kg of lipopolysaccharide from *Escherichia Coli* over a period of 30 mins). Six pigs were hemofiltrated for 3 hours with a large pore membrane (78 Å pore, 80 kDa cut off) (Sureflux FH 70, Nipro, Osaka, Japan). The ultrafiltration rate was 45 ml/kg/min. Samples were taken from arterial, venous line and in the ultrafiltrate at T120 and T240. We measured concentrations of interleukin 6, interleukin 10 and albumin. Results: At T120 and T240, the IL-6 clearances were 22 ± 7 and 15 ± 3 ml/min, respectively. The IL-6 sieving coefficients were 0.97 and 0.7 at T120 and T240, respectively. At T120 and T240, the IL-10 clearances were 14 ± 4 and 10 ± 7 ml/min, respectively The sieving coefficients were 0.63 and 0.45 at T120 and T240, respectively. The concentrations of IL-6 and IL-10 were the same at T0 and T240. At T60 and T240, the plasmatic albumin concentrations were 24 ± 4 g/L and 23 ± 4 g/L, respectively (p = 0.13). Conclusions: In this animal model of endotoxemic shock, we confirm the high cytokine clearance observed when hemofiltration is applied to a large pore membrane. The loss of albumin seems negligible. The impact of such clearances on hemodynamic stability and survival remains to be proved.

Keywords: cytokine; endotoxin; hemofiltration; clearance; septic shock

Introduction

Despite recent therapeutic progress, severe sepsis-associated mortality remains high (1). Since cytokines play an important pathogenic role in sepsis (2) and their concentration is predictive of the shock gravity and global mortality (3), several authors have suggested that hemofiltration could be of interest by removing diverse pro- and anti-inflammatory mediators from the circulation (4-6). This theory has been called "the peak concentration hypothesis" (6). However, the most frequently used membranes have a cut off between 30 and 40 kDa and induce no sufficient cytokines clearance (7-10). For example, the convective clearance of IL-6, one of the most important pro-inflammatory cytokines (11), is less than 2 ml/min with such membranes and an ultrafiltrate rate of 1 l/h (which corresponds to a sieving coefficient (SC) of 0.12) (9). Moreover, De Vriese et al and Heering et al measured no IL-10, one of the most important anti-inflammatory cytokines (12), in ultrafiltrate during CWH with such membranes (7, 8). A cut off membrane of 40 kDa means a SC of 0.05 or 0.1 for a 40 kDa molecule (13, 14). These results are obtained in vitro and these cut offs could be decreased by up to 40% when they are calculated in vivo (15). The molecular weight of IL-6 and IL-10 (28 and 19 kDa respectively) may explain the low SC and clearance rate of these cytokines with classical membranes.

Some authors have recently proposed the use of membranes with larger pores to increase effective cytokine clearances (16, 17). Uchino *et al* have demonstrated high cytokine clearance in an *ex vivo* model of septic shock using a cellulose triacetate membrane with large pores (78 Å diameter) (17). In our study, using a similar large pore membrane, we measured IL-6 and IL-10 clearance in a porcine model of endotoxemic shock.

Materials and methods

All experimental procedures and protocols used in this investigation were reviewed and approved by the Ethics Committee of the Medical Faculty of the University of Liège. The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

Surgical preparation

Experiments were performed on 6 healthy pure pietran pigs of either sex weighing from 20 to 30 kg. The
animals were premedicated with an intramuscular administration of ketamine (20 mg/kg) and diazepam (1 mg/kg). Anaesthesia was then induced and maintained by a continuous infusion of sufentanil (0.5 µg/kg/h) and pentobarbital (5 mg/kg/h). Spontaneous movements were prevented by pancuronium bromide (0.2 mg/kg). After endotracheal intubation through a cervical tracheostomy, the pigs were connected to a volume-cycled ventilator (Evita 2, Dräger, Lübeck, Germany) set to deliver a tidal volume of 10 ml/kg with a FiO₂ of 0.4 and at a respiratory rate of 20 breaths/min. End-tidal CO₂ (PETCO₂) measurements (Capnomac, Datex, Helsinki, Finland) were used to monitor the adequacy of ventilation. Respiratory settings were adjusted to maintain PETCO₂ between 30 and 35 mmHg. A 12F double lumen dialysis catheter (Arrow International, Reading, PA, USA) was inserted through a left femoral venotomy.

Experimental protocol

The animals received a 0.5 mg/kg endotoxin infusion (Lipopolysaccharide from *Escherichia Coli* serotype 0127:B8; Sigma Chemical, St Louis, MO) over 30 mins (from T0 to T30). This model of endotoxic shock has been previously validated and is reproducible (18). They underwent, from T60 onwards, a zero-balance continuous veno-venous hemofiltration at a rate of 45 ml/kg/h. A 0.7 m², large pore (78 Å) membrane with a cut off of 80 kDa (Sureflux FH 70, Nipro, Osaka, Japan) and a Baxter BM 25-BM 14 hemofiltration device (Baxter Health Care, Munich, Germany) were used. Blood flow through the membrane was calculated with the following equation:

\[
\text{Blood flow (ml/min) = UF (ml/min) x 3.5.}
\]

Ultrafiltrate was replaced in the post-dilution mode by a bicarbonate-buffered hemofiltration fluid (Na⁺: 150 mmol/L, K⁺: 3 mmol/L, bicarbonate: 30 mmol/L) at a temperature of 37°C. Anticoagulation of the extracorporeal circuit was achieved using a loading dose of 5000 IU heparin followed by an anticoagulation regimen based on the activated clotting time (100-200 s). The calcium chloride (100 mg/mL) infusion rate was calculated with the following equation:

\[
\text{CaCl₂ infusion rate (ml/h) = UF (ml/h) x 1.2/1000.}
\]

Cytokine clearance

Blood (before and after the membrane) and ultrafiltrate samples were taken at T0, T60, T120 and T240 to measure IL-6 (28 kDa), and IL-10 (19 kDa) concentrations using an ELISA test (Biosource, Paris, France). We calculated their clearance and SC at T120 and T240 with a classical formula: SC = 2 X [UF]/[A] + [V] and clearance = SC X ultrafiltration rate (ml/min), where [UF] is the concentration in ultrafiltrate, [A] is the concentration in inlet supernatant and [V] the concentration in outlet supernatant. Plasmatic albumin concentration was measured at T60 and T240 (with the bromocresol green method).

Data are presented as mean ± standard deviation (SD). The values have been compared with the classical Wilcoxon test (MedCalc Software, Mariakerke, Belgium). P < 0.05 was considered statistically significant.

Results

At T120 and T240, the IL-6 clearances were 22 ± 7 and 15 ± 3 ml/min, respectively. The IL-6 sieving coefficients were 0.97 and 0.7 at T120 and T240, respectively.

At T120 and T240, the IL-10 clearances were 14 ± 4 and 10 ± 7 ml/min, respectively. The sieving coefficients were 0.63 and 0.45 at T120 and T240, respectively. All the results are summarized in Table I.

The concentration of IL-6 and IL-10 were the same at T0 and T240.

At T60 and T240, the plasmatic albumin concentrations were 24 ± 4 g/L and 23 ± 4 g/L, respectively (p = 0.13).

**Table I** - Summary of the clearances and sieving coefficient (SC) of cytokines IL-6 and IL-10 at T120 (after one hour of hemofiltration) and at T240 (after three hours of hemofiltration) using a large pore membrane

<table>
<thead>
<tr>
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<th>T120</th>
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<th>T240</th>
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<tr>
<td></td>
<td>Clearance (ml/min)</td>
<td>SC</td>
<td>Clearance (ml/min)</td>
<td>SC</td>
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<tr>
<td>IL-6</td>
<td>22 ± 7</td>
<td>0.97</td>
<td>15 ± 3</td>
<td>0.7</td>
</tr>
<tr>
<td>IL-10</td>
<td>14 ± 4</td>
<td>0.63</td>
<td>10 ± 7</td>
<td>0.45</td>
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</table>
Discussion

Our results show a high SC and a high clearance rate for IL-6 and IL-10 with a large pore membrane and confirm, in vivo, those recently published by Uchino et al in an ex vivo study (17). IL-6 SC and clearances are 5 or 6 times higher than those obtained with classical membranes (7-9). Our results with IL-10 are also of interest because other authors have described a SC of 0 with a classical membrane (7). Despite a higher molecular weight, SC values of IL-6 are superior to those of IL-10. Actually, the molecular weight is not the only factor which explains crossing through the membrane. For example, it is well known that, despite a low molecular weight (8 kDa), interleukin 8 does not cross through the classical membranes because it binds the heparin present in the circuit (19).

In septic patients, Morgera et al have recently described high SC and clearance for IL-6 (SC of 0.48 and clearance, at 8 ml/min at an ultrafiltration rate of 1 l/h, after 4 hours) but they used another synthetic membrane (Polyflux, Gambro) (20). Morgera et al and Uchino et al have also shown that the cytokine clearance increases when the ultrafiltration rate increases (even if the SC decreases) (17, 20). Others have studied membranes with higher cut off values (around 100 kDa). Their results show high SC and clearances, but albumin loss becomes significant (13, 14, 16, 21-23). With the membrane used in our study, albumin loss is timely and quantitatively limited (17, 20). We have not noted any decrease in albumin concentration.

Our study has several limitations. We did not study the adsorption of the membrane. However, it is well known that adsorption is very limited with ail membranes composed of cellulose triacetate due to their specific physico-chemical properties (24-26). This seems also the case with cellulose triacetate and large pore membranes, as illustrated by Uchino (17). Secondly, we have measured the concentration, clearance and SC of only two cytokines. Although IL-6 and IL-10 are two of the most important inflammatory mediators, other mediators, like TNFα, could have been studied. Due to its relatively high molecular weight (active, trimeric, TNFα has a molecular weight of 51 kDa (27)), the TNFα SC should be theoretically low. Uchino et al have found a SC value of 0.34 for TNFα and Morgera et al, with another large pore membrane, have found a SC value of 0 for TNFα (17, 20). Moreover, it should be stressed that TNFα is a very early and transient inflammatory marker in severe sepsis, making it an undesirable therapeutic target (9, 20).

Although we have confirmed the high cytokine clearances obtained with large pore membranes, its beneficial effects on hemodynamics and survival remains to be demonstrated in severe sepsis. Indeed, the "peak concentration hypothesis" is not the only hypothesis to explain hemodynamic improvement and better survival observed with hemofiltration (especially with high volume hemofiltration) (28, 29). Until now, little data has been published on large pore membranes. Nevertheless, Lee et al have shown a better survival of swine with severe sepsis (Staphylococcus aureus induced septicaemia) after continuous arteriovenous hemofiltration with a large pore membrane (cut off of 100 kDa). However, these authors did not measure cytokine clearances (30).

While using cellulose triacetate membrane with large pores induces a significant cytokine clearance by hemofiltration, animal and human protocols studying the impact of such membrane on hemodynamic stability and on survival are still awaited.

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