The effect of coupled haemofiltration and adsorption on inflammatory cytokines in an ex vivo model

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Abstract

Background. The objective of this study was to evaluate the ex vivo removal of cytokines with an extracorporeal circuit using coupled large-pore haemofiltration and sorbent adsorption.

Methods. The setting for this study was a laboratory attached to the Intensive Care Unit of a tertiary hospital. Six healthy volunteers donated blood, which was incubated with endotoxin. Control blood was left at room temperature. Treatment blood was recirculated for 6 h through a closed circuit with a large-pore polysulfone haemofilter (average pore size 150 kDa) and an activated charcoal cartridge. Blood and ultrafiltrate were sampled hourly from three sites (pre-haemofilter for the circulating concentration, at cartridge inlet and cartridge outlet) to measure the concentrations of interleukins (IL)-1β, -6, -8 and -10, and tumour necrosis factor (TNF).

Results. Control cytokine concentrations remained the same or increased slightly. Most of the preformed circuit cytokines were removed, with the exception of IL-10. The average sieving coefficients were 0.61 for IL-1β, 1.34 for IL-6, 0.30 for IL-8, and 0.56 for TNF. Average single-pass clearances were 49, 107, 24 and 45 ml/min, respectively. The cartridge adsorbed 90% of IL-1β, 72% of IL-6, 100% of IL-8, and 7% of TNF during each pass.

Conclusion. The combination of a large-pore haemofilter and charcoal cartridge removed several cytokines efficiently under ex vivo conditions. This technique can now be tested for cytokine removal in vivo.

Keywords: adsorption; charcoal cartridge; ex vivo model; haemofiltration; inflammatory cytokines

Introduction

Sepsis and other inflammatory syndromes might represent a non-renal indication for continuous renal replacement therapy, based on the assumption that non-specific removal of several inflammatory mediators would improve outcome in septic shock [1]. However, useful convective removal of mediators from the human septic circulation has not been achieved to date, although many cytokines have a molecular weight below the theoretical cut-off point of the commercial membranes currently used [2].

Adsorption has been identified as a major mechanism for cytokine removal by extracorporeal circuits, both in vitro and in vivo [3,7], but the membranes used are not specifically designed to maximize adsorption, and their pore sizes are too small to allow significant passage of cytokines. Any reduction in plasma concentrations of cytokines has thus been transient [7]. One method of maximizing adsorption is the combination of plasma filtration and sorbent-based adsorption [4]. This combination can be used continuously without the need for donor plasma. However, plasma filters will only tolerate transmembrane pressures up to 120 mmHg, and recommended blood flows are <250 ml/min. A very large-pore haemofilter would tolerate greater transmembrane pressures and blood flows, and might also allow the passage of larger amounts of cytokines, which are then available for adsorption. Greater blood flows might be particularly useful early in severe sepsis if rapid clearance of cytokines is desired.

It is known that uncoated activated charcoal has a high non-selective adsorptive capacity [5], and a commercial uncoated version is now available (Detoxyl 3; Bellco, Mirandola, Italy). This device is used for haemoperfusion and regeneration of ultrafiltrate (paired filtration dialysis technique). It is not known whether uncoated charcoal is useful in removing inflammatory mediators in vivo. More knowledge of
inflammatory mediator binding using an extra-corporeal circuit is required in vitro before an assessment of possible clinical benefit can be made.

The aim of this ex vivo study is to examine the removal of cytokines of varying molecular weights during continuous large-pore haemofiltration coupled with adsorption to an uncoated charcoal cartridge (CHFA).

Subjects and methods

Approval for the study was obtained from the hospital ethics committee.

Study population

Six healthy volunteers donated blood for the experiment. They were not taking regular medication, had no intercurrent infection, and had not used non-steroidal anti-inflammatory drugs for at least 1 week before donation.

Endotoxin preparation

Vials of standard endotoxin (lyophilized powder of lipopolysaccharide from Escherichia Coli serotype 026-86, 10 mg vials; Sigma Aldrich Australia Pty Ltd, Sydney, Australia) were dissolved in sterile water, made up to a volume of 10 ml, and divided into 10 aliquots. The aliquots were stored in sterile silicone Eppendorf tubes (Greiner Interpath Services, Melbourne, Australia) at a concentration of 1 mg/ml, and frozen at –70°C until required.

Blood preparation

Standard sterile blood collection bags (Tuta Laboratories, Sydney, Australia) were pre-heparinized with 10 000 IU heparin (David Bull Laboratories, Melbourne, Australia). Blood (300 ml) was drawn from the volunteers into the bags, and 1 mg of endotoxin was added to each bag. The bags were incubated for 4 h in an agitated water bath at 39°C. The ‘blood’ reservoir was made up with 300 ml donated blood, 250 ml albumin (Albumex 4, 40 g/l; Commonwealth Serum Laboratories, Melbourne, Australia) and 250 ml normal saline (Viaflex 0.9% sodium chloride; Baxter Healthcare, Sydney, Australia) were pre-heparinized with 10 000 IU heparin (David Bull Laboratories, Melbourne, Australia). Blood (300 ml) was drawn from the volunteers into the bags, and 1 mg of endotoxin was added to each bag. The bags were incubated for 4 h in an agitated water bath at 39°C. The ‘blood’ reservoir was made up with 300 ml donated blood, 250 ml albumin (Albumex 4, 40 g/l; Commonwealth Serum Laboratories, Melbourne, Australia) and 250 ml normal saline (Viaflex 0.9% sodium chloride; Baxter Healthcare, Middlesex, UK).

The volunteers subsequently donated 60 ml of blood, which was inserted into blood bags pre-heparinized with 5000 IU. These ‘control’ bags were spiked with 0.2 mg endotoxin, incubated under identical conditions and then diluted with 100 ml normal saline.

Ex vivo conditions

The experimental set-up consisted of the ‘blood’ reservoir, a Baxter BM 11/14 machine (Baxter Healthcare), an experimental polysulfone large-pore hollow-fiber membrane (PS 9002; Fresenius, St Wendel, Germany), an activated charcoal cartridge (Detoxyl 3; Bellco), standard Baxter arterial, venous and filtrate/dialysate tubings, and additional custom-made tubing to connect the charcoal cartridge (Figure 1). The membrane had an estimated mean pore size of 150 kDa in bovine plasma. Its specifications included a sieving coefficient of 1.0 for albumin, 0.5 for gammaglobulins and 0 for fibrinogen in bovine plasma. This membrane was chosen because it tolerated greater blood flows than standard plasma filters without fibre rupture. The charcoal cartridge contained 135 g of medical grade carbon, in the form of homogeneous spherical particles that are very smooth on the surface, hard and resistant to abrasion. Fluid filtered through the cartridge to test for release of particles complied with Pharmacopoeia requirements in Italy and the UK (IX editions) for large volumes of dialysis and infusion solutions.

The BM11 set-up was standard, but the BM14 machine set-up required modification (Figure 1). The ‘blood flow pump’ controlled flow through the circuit, and ‘the filtrate pump’ pulled fluid through the cartridge, which was returned via the ‘blood leak detector’ to the ‘venous bubble trap reservoir’.

The blood flow pump was set at 250 ml/min and the filtrate roller pump was set at 80 ml/min. The circuit, membrane and cartridge were primed with 3 l of heparinized normal saline (Baxter Viaflex with 5000 IU heparin/l) before attaching the ‘blood’ reservoir, with particular effort directed toward prevention of air entry into membrane or cartridge. The haematocrit of the completed circuit was 18% ± 1 (SEM). The blood reservoir was not heated during the 6 h of circulation, because ongoing production of cytokines was not desired for the preliminary experiments. To assess the effect of room temperature on cytokine production, ‘control’ bags were left on the laboratory bench.

Circuit problems and solutions

The machine does not function unless reciprocal changing weights are detected on the filtrate and fluid replacement scales, and therefore a dummy circuit was inserted into the ‘replacement solution pump’ to allow repeated one-way exchange of fluid across the scales. The pump settings were chosen following a study of incremental flows through the
Cytokine measurements

Blood was sampled from the arterial port A (the circulating concentration), and ultrafiltrate was sampled at the cartridge inlet B and the cartridge outlet C (Figure 1). A baseline sample from the arterial port was taken after the circuit had been running for 5 min, and before ultrafiltrate diversion was commenced. After ultrafiltrate diversion was commenced, samples from the three sites were taken simultaneously at 1, 2, 3, 4, 5 and 6 h. The circuit samples were drawn into tubes containing solid heparin, and immediately centrifuged with refrigeration at 3000 r.p.m. for 10 min, and the supernatant removed. Circuit supernatant, cartridge inlet and outlet samples were stored in sterile silicon Eppendorf tubes (Greiner Interpath Services) at −70 °C until assays were done for IL-1β, IL-6, IL-8, IL-10 and TNF.

Control blood was sampled at baseline, 1, 2, 3, 4 and 6 h, and processed the same way.

Cytokine assays

IL-6, IL-8 and IL-10 concentrations were measured in duplicate by commercial enzyme-linked immunosorbent assays (ELISA) after constructing a standard plasma curve according to the manufacturers’ instructions (DuoSET kits, Genzyme Diagnostics, Cambridge, MA, USA, for IL-6 and IL-8; Medgenix, Rungis, France for IL-10). The lower limits for detection were 6–10 pg/ml for IL-6, 3 pg/ml for IL-8, and 5 pg/ml for IL-10. The coefficient of variation for each assay was <10% for concentrations between 15.6 and 1000 pg/ml. TNF and IL-1β were measured by a ‘sandwich technique’. Plates were coated with mouse anti-human TNF-α or IL-1β antibodies (2 μg/ml; Genzyme Diagnostics), which bound the cytokines in the sample. This complex reacted with a combination of polyclonal rabbit anti-human TNF-α or IL-1β antibodies (Genzyme Diagnostics) and their swine anti-rabbit horseradish peroxidase conjugate (DAKO Corporation, Carpinteria, CA, USA). Colour development in plates due to bound peroxidase was then read at 450 nm in a plate reader (Microplate Reader model 550; Bio-Rad Laboratories, Hercules, CA, USA). Assay sensitivities were for IL-1α (250 ml/min) and IL-1β (195.5 pg/ml, and IL-10 and TNF.

where Cinlet is the cytokine concentration at the cartridge inlet (pg/ml), and Coutlet is the cytokine concentration at the cartridge outlet (pg/ml).

The average of the duplicate measurements was used.

Calculations

The sieving coefficient (SC) and clearance (Cl) of each cytokine across the membrane were calculated as follows:

\[
SC = \frac{C_{inlet}}{Q_{uf} \cdot T_{min}}
\]

\[
Cl = \frac{Q_{uf} \cdot T_{min}}{C_{cire}}
\]

where C_cire is the circulating cytokine concentration (pg/ml), C_inlet is the cytokine concentration at the cartridge inlet (pg/ml), and Q_{uf} is the ultrafiltrate flow (80 ml/min).

The percentage adsorption by the charcoal cartridge was calculated as follows:

\[
\text{Percentage adsorption of cytokine} = 100 \times \frac{(C_{inlet} - C_{outlet})}{C_{inlet}}
\]

where C_{inlet} is the cytokine concentration at the cartridge inlet (pg/ml), and C_{outlet} is the cytokine concentration at the cartridge outlet (pg/ml).

Total clearance of each cytokine from the circuit blood solution was calculated as follows:

\[
\text{Total clearance} = Cl \times \% \text{adsorption}
\]

where Cl is the membrane clearance, and % adsorption is the percentage removed by the cartridge.

The average SC and membrane and circuit clearance were calculated for each experiment over the 6 h period, and the average data were then summarized.

Data analysis

Summary text data are expressed as median and interquartile range (IQR), but displayed in parametric form for clarity. Circuit concentrations, sieving coefficients and percentage adsorption of cytokines were analysed for each cytokine with Friedman’s test, to determine if there was a change over time (Statview® package; Abacus Concepts Inc, Berkeley, CA, USA). Post-hoc Wilcoxon signed rank tests identified which time points were associated with significant changes. \( P < 0.05 \) was considered statistically significant.

Results

All samples of CHFA blood produced sufficient initial amounts of IL-1β, IL-6, IL-8 and TNF for analysis, but only three samples produced sufficient initial IL-10. All the membranes tolerated the circuit flow of 250 ml/min without fibre rupture.

Sufficient initial IL-8, IL-10 and TNF for analysis were produced by the control samples. Only three control samples produced IL-1β, and only two control samples produced IL-6, and IL-6 was not analysed in the control group.

IL-1β

The control concentrations of IL-1β did not change when left at room temperature (Figure 2). The CHFA concentration of IL-1β fell from a median of 838.2 pg/ml (683.4 IQR) at baseline to 23.1 pg/ml (43.9) at 6 h (Figure 3A). The first significant Wilcoxon signed rank was between baseline and 2 h (\( P = 0.03 \)).

This was associated with an average SC of 0.61 (0.18) and average clearance of 48.5 ml/min (14.4) for the membrane (Table 1). Occasionally there was minor ‘release’ of IL-1β from the membrane (e.g. experiment 2 at 1 h had a circulating concentration of 142.3 pg/ml and a cartridge inlet concentration of 195.5 pg/ml). The cartridge adsorbed an average of 89.8% (14.8) during each pass, which resulted in an average circuit clearance of 45.0 ml/min (22.9) over the 6 h period (Table 2). The cartridge removal of IL-1β did not seem to reach saturation.
There was no significant change in the SC or cartridge removal during 6 h.

**IL-6**

The CHFA concentration of IL-6 fell from 1170.7 pg/ml (857.1) at baseline to 0 pg/ml (45.6) at 2 h (Figure 3A). The first significant Wilcoxon signed rank test was between baseline and 1 h ($P = 0.03$).

An average SC of 1.34 (0.27) was associated with an average single-pass clearance of 106.8 ml/min (21.6) for the membrane (Table 1). This reflected a frequent increase in concentration of IL-6 across the membrane. At 1 h, for example, subjects 3 and 5 showed increases in concentration across the membrane from 47.2 and 44.0 pg/ml to 92.7 and 92.2 pg/ml respectively. Comparison of the circulating and post-membrane concentrations by Wilcoxon signed rank test suggests that there was a systematic release of IL-6 by the membrane at 1 h ($P = 0.04$), but not at other times (all $P > 0.05$).

Average single-pass adsorption by the cartridge was 72.0% (54.7), and the average circuit single-pass clearance was 98.1 ml/min (75.4) (Table 2). The cartridge removal of IL-6 did not seem to reach saturation uniformly.

There were insufficient data during the latter half of the experiments to determine whether changes in the SC or cartridge removal occurred over time.

**IL-8**

The control concentrations of IL-8 increased when left at room temperature (Figure 2). The first significant Wilcoxon signed rank test was between baseline and 1 h ($P = 0.03$). The CHFA concentration of IL-8 fell from 1735.0 pg/ml (633.7) at baseline to 117.0 pg/ml (160.7) at 3 h (Figure 3B). The first significant Wilcoxon signed rank value was between baseline and 1 h ($P = 0.03$).

An average SC of 0.30 (0.12) was associated with an average membrane clearance of 24.2 ml/min (9.9) (Table 1). The SC decreased from 0.48 (0.07) at 1 h to 0.20 (0.10) at 4 h. The first significant Wilcoxon signed rank test value was between baseline and 3 h ($P = 0.03$). An average single-pass removal by the cartridge of 100.0% (0.9) resulted in average single-pass circuit clearance of 24.7 ml/min (3.6) (Table 2). Removal by the cartridge did not change significantly with time, and did not reach saturation.

**IL-10**

Control concentrations of IL-10 did not increase (Figure 2). Only three CHFA samples produced initial IL-10, and the circulating concentration of IL-10 did not change either (Figure 3C).

Useful conclusions could not be drawn about the behaviour of IL-10 in the circuit. This was partly due to the paucity of data, and partly due to a frequent increase in the concentration of IL-10 across the membrane. The latter point resulted in inflated sieving coefficients [average 1.46 (1.15)] and membrane clearances [average 116.6 ml/min (92.3)] (Table 1). Cartridge adsorption was erratic (range 0–100%), and only low concentrations of IL-10 (<40 pg/ml) were removed efficiently (Table 2). The average circuit single-pass clearance was 91.9 ml/min (168.9).

**TNF**

The control concentrations of TNF did not change (Figure 2). The circulating concentration of TNF fell from 6456.7 pg/ml (1271.8) to 420.4 pg/ml (224.8) at 6 h (Figure 3B). The first significant Wilcoxon signed rank test was between baseline and 1 h ($P = 0.03$). The average SC was 0.56 (0.11), and the average single-pass membrane clearance was 44.9 ml/min (8.9) (Table 1). The SC decreased significantly from 0.63 (0.06) at 1 h to 0.43 (0.08) at 6 h. The first significant Wilcoxon signed rank test was between 1 and 3 h ($P = 0.03$).

The average single-pass removal by the cartridge was 7.4% (20.6), which did not change significantly over time (Table 2). Presumably the cartridge reached saturation, and thus ‘released’ TNF back into the circuit. Poor cartridge removal resulted in an average single-pass circuit clearance of 4.3 ml/min (4.6).

**Discussion**

This ex vivo model successfully produced IL-1β, IL-6, IL-8 and TNF using freshly donated blood from healthy human volunteers. Production of IL-10 was less consistent. When this blood was inserted into a closed haemofiltration/adsorption circuit, it was possible to remove most of the preformed IL-1β, IL-6, IL-8 and TNF, as shown by the reductions in the circulating concentrations of these molecules. Significant removal of IL-10 did not occur, as shown by the lack of reduction of its circulating concentration.
The large-pore membrane enhanced convective transfer of the cytokines, although passage was not uniform. There were insufficient data for IL-10 to determine a consistent pattern of transfer, and the data for IL-10 and IL-6 also showed considerable variation between experiments. The average SCs were 0.30 for IL-8, 0.56 for TNF, 0.61 for IL-1β, and 1.34 for IL-6, and the respective membrane clearances were 24.2, 44.9, 48.5 and 106.8 ml/min. These results are two to 10 times greater than the best reported ex vivo SCs for commercially available membranes with nominal cut-off points of ~30 kDa (0.12 for IL-8 using a polysulfone membrane, 0 for TNF using a variety of membranes, and 0.12 for IL-6 using a cellulose triacetate membrane [6]). Increasing the nominal cut-off point to 35–40 kDa in vivo improved the passage of some cytokines through a polyacrylonitrile membrane [7], but the SCs were still less than those achieved by our membrane (0.16 for TNF, 0.22 for IL-1β, 0.18 for IL-6, and 0 for IL-10). Our SC results are less than those achieved by plasmafiltration (0.70 for IL-8, 1.22 for TNF, 1.48 for IL-1β, and 0.55 for IL-6, calculated from [4]), but the ultimate membrane clearances of cytokines were greater in our study because our membrane achieved greater filtrate flow. The cut-off point of our membrane is less than the cut-off point of a plasma separator, which is probably the most important explanation for the different SCs. In addition, our circuit used a greater filtration fraction and greater transmembrane pressure gradient than the circuit used by Tetta et al [4], which would be expected to reduce our SCs.

Our study suggests that convective transport of IL-8 was less than expected, if molecular weight of IL-8 was the only consideration (8–9 kDa). There are at least two possible explanations for this. Some IL-8 might be unavailable for transport, because it is bound to autoantibodies, red cells or heparin [8,11], or the membrane affinity for IL-8 might be so great that >6 h are required for membrane saturation to occur. The latter explanation is unlikely, because IL-8 does not seem to bind to polysulfone [6]. Moreover, our SCs for IL-8 declined over time, which is contrary to the expected increase in SC associated with a membrane approaching saturation.

Our results for IL-6 show an unexpectedly high SC and membrane clearance. If the assay was reliable, this implies that there might be intermittent isolated release of IL-6 from the membrane, which could be due to net generation of IL-6 at the membrane, or desorption. Polysulfone can trigger the production of IL-1β, particularly in the presence of endotoxin [9], and therefore might trigger the production of IL-6. However, isolated generation and release of IL-6 seems unlikely, as we would expect monocytes to produce a range of cytokines, which would be simultaneously released by the membrane. Simultaneous release of cytokines did not occur. Polysulfone membranes can adsorb modest amounts of IL-6 [6], and would release the molecule after membrane saturation had occurred. If so, membrane saturation must have occurred before 1 h, because SCs were already >1 at 1 h. Other possibilities include variability of immunoassay measurement of IL-6 in different substrates, and an increased localized concentration of IL-6 adjacent to filter pores (compared with midstream concentration) that is available for transport by convection or diffusion (‘solute polarization’ [12]).

The charcoal cartridge adsorbed significant amounts of all cytokines except IL-10, although adsorption under competitive conditions was not uniform. It adsorbed an average of 7% of TNF passing through it, 72% of IL-6, 90% of IL-1β, and 100% of IL-8. The time required to achieve minimum circulating
concentrations was 2 h for IL-6, 3 h for IL-8, and 6 h for IL-1β and TNF, suggesting that there was not a simple relationship between speed of adsorption and the cartridge affinity for each cytokine. Only TNF seemed to reach saturation point under our experimental conditions. The adsorption of IL-10 was erratic, and this was probably not due to failure of initial convective transfer of IL-10 molecules. We assume that most of the decrease in the circulating concentration of cytokines was due to cartridge adsorption, because our small reservoirs of control blood showed no change or small increases in cytokine concentrations at room temperature.

Data on the use of any activated charcoal in experimental models of sepsis are limited. One in vitro study [10] compared the performance of polymer-coated activated charcoals and resins in a roller mixer incubated with lipopolysaccharide and recombinant cytokines, and also found that no single adsorbent uniformly removed cytokines from phosphate-buffered saline or human plasma. Polyhema-coated charcoal removed 17%, 65% and 48% of the initial amount of TNF, IL-1α and IL-6, respectively. Uncoated charcoal should be more effective than this, because it is known that polymer coating of charcoal reduces its adsorptive capacity for molecules >3.5 kDa, and adsorption of molecules >1.5 kDa is limited by the pore structure of the specific semipermeable membrane coating [5].

Another study used combined haemofiltration and adsorption with a Detoxyl 3 cartridge [4]. The filtration of TNF, IL-1β and IL-8 was poor, and only 0.15%, 7.4% and 4.2% of the total mass of each cytokine was cleared by convection, respectively. There was no effective transfer of IL-6, and hence no adsorption. However, 100%, 94% and 100% of the delivered TNF, IL-1β and IL-8 was adsorbed by the Detoxyl cartridge, which suggests that adsorption by the cartridge was limited by initial poor membrane clearance. We achieved comparable adsorption of IL-1β and IL-8, but the adsorption of TNF in our study must have been limited by other factors. For uncoated carbon, the adsorptive rate-limiting step is pore diffusion. Thus, one likely factor would be the effect of increased plasma velocity on the ‘streaming’ of molecules with a relatively large molecular weight, which could lead to a reduction in contact between molecule and cartridge. Total system clearance is a more important outcome than isolated membrane clearance or cartridge adsorption. Our total clearances for the pro-inflammatory cytokines (except TNF) are similar to, or greater than, those required for control of urea and creatinine in acute renal failure. Unfortunately, no studies reporting total system clearances for cytokines are available for comparison.

This study has a number of limitations. Our incubation period was relatively short, which might have precluded adequate IL-10 production. However, an increase in incubation time to 16 h does not result in more IL-10 production with this peripheral blood monocyte model (our unpublished data). Incubation

### Table 1. Sieving coefficients (median and interquartile range)

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Time (h)</th>
<th>Average</th>
<th>Friedman’s test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>IL-1β</td>
<td>0.47 (0.30)</td>
<td>0.70 (0.17)</td>
<td>0.82 (0.26)</td>
</tr>
<tr>
<td>IL-6</td>
<td>1.14 (0.26)</td>
<td>0.96 (0.91)</td>
<td>1.00 (2.00)</td>
</tr>
<tr>
<td>IL-8</td>
<td>0.48 (0.07)</td>
<td>0.40 (0.24)</td>
<td>0.28 (0.20)</td>
</tr>
<tr>
<td>IL-10</td>
<td>1.64 (0.98)</td>
<td>1.33 (1.29)</td>
<td>1.76 (1.24)</td>
</tr>
<tr>
<td>TNF</td>
<td>0.63 (0.06)</td>
<td>0.70 (0.13)</td>
<td>0.52 (0.16)</td>
</tr>
</tbody>
</table>

*The average sieving coefficient was calculated for each experiment, and then the median value obtained.

1For a change in sieving coefficient over time.

### Table 2. Percentage removal by cartridge during a single pass (median and interquartile range)

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Time (h)</th>
<th>Average</th>
<th>Friedman’s test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>IL-1β</td>
<td>100 (37)</td>
<td>92 (25)</td>
<td>85 (25)</td>
</tr>
<tr>
<td>IL-6</td>
<td>49 (42)</td>
<td>68 (46)</td>
<td>100 (0)</td>
</tr>
<tr>
<td>IL-8</td>
<td>100 (6)</td>
<td>100 (0)</td>
<td>100 (7)</td>
</tr>
<tr>
<td>IL-10</td>
<td>6 (67)</td>
<td>0 (78)</td>
<td>100 (86)</td>
</tr>
<tr>
<td>TNF</td>
<td>13 (14)</td>
<td>23 (9)</td>
<td>3 (23)</td>
</tr>
</tbody>
</table>

*The average percentage removal by the cartridge was calculated for each experiment, and then the median value obtained.

1For a change in percentage removal by the cartridge over time.

1A negative result indicates percentage release of TNF by the cartridge.
times might need to be increased to those used in cell culture models (24–48 h) for effective IL-10 production.

We presumed that contact between ultrafiltrate and uncoated charcoal would not result in the undesirable cellular manifestations associated with whole blood–charcoal contact, such as thrombocytopenia and monocyte activation. However, it is reasonable to expect that any ultrafiltrate obtained from septic patients will retain the capacity to induce cytokine release when incubated with normal blood, despite passage through a charcoal cartridge. This expectation is based on data that shows that a charcoal cartridge does not remove this capacity when it is included in an ultrafiltrate regeneration system for ultrafiltrate obtained from patients with ESRF [13].

We did not measure all possible inflammatory mediators, or even the natural antagonists of those pro-inflammatory mediators we did measure (with the exception of IL-10). With respect to other molecules, charcoal can efficiently remove creatinine, uric acid, and small molecular weight, high performance liquid chromatography ‘uremic peaks’, but has no effect on urea, sodium, calcium, phosphate, glucose or bicarbonate [13].

It would have been helpful to compare the effect of a range of blood flows on membrane clearance, and a range of filtrate flows on cartridge adsorption, to assess the requirements for maximum system clearance.

Finally, these laboratory results cannot be extrapolated to in vivo settings, because we had a relatively small volume of distribution of cytokines, without the effect of continual endogenous cytokine production and clearance induced by sepsis, and presumably without major production of cytokine antagonists and binding proteins. However, it is also likely that increases in circuit temperature would result in increased sieving coefficients for in vivo cytokines [14].

In conclusion, our study showed that it is possible to remove, simultaneously, a range of preformed pro-inflammatory cytokines from an extracorporeal circuit with coupled haemofiltration and adsorption, using a haemofilter with a nominal average 150 kDa pore size and an uncoated charcoal cartridge. IL-10 removal was poor. This technique shows some potential for the continuous removal of cytokines, if such a goal is appropriate. It also has the additional advantage that no replacement blood products or additional fluids are required. In vivo investigation into the effects of this technique on the balance of pro- and anti-inflammatory mediators, cellular immune function, and therapeutic and nutritional molecules is now required.

Acknowledgements. Fresenius (St Wendel, Germany) kindly donated membranes and Bellco (Mirandola, Italy) kindly donated charcoal cartridges.

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