Proinflammatory Cytokines and Hemofiltration Membranes[1]

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ABSTRACT

To determine whether the clinical improvement noted in some septic patients undergoing hemofiltration is in part due to the removal of proinflammatory mediators, *in vitro* hemofiltration of a 1% albumin solution containing recombinant human tumor necrosis factor alpha and interleukin-1 was performed through a variety of hemofilters. Observed sieving coefficients of these cytokines was much higher (up to 0.35) than expected, considering their molecular weights of 17 kd. In addition, binding of up to 32% of the total mass to selected membranes (polyamide and AN69) was noted. These data are consistent with the concept that either the convective or adsorptive removal of proinflammatory cytokines may play a role in the clinical efficacy of hemofiltration in sepsis.

Key Words: *Tumor necrosis factor, interleukin-1, hemofiltration, hemoadsorption, sepsis*

Signs and symptoms of septic shock may be physiologic responses to the release of large amounts of tumor necrosis factor alpha (TNF-α) and/or interleukin-1 (IL-1) from monocytes as part of the immune response. In sepsis, the first significant elevation in TNF-α levels corresponds to the onset of chills, headache, myalgia, nausea, fever, tachycardia, and increased circulating levels of stress hormones (1). Serum TNF levels measured on the day of admission into the intensive care unit are significantly higher in patients with septic shock (80 pg/mL) compared with levels seen in patients without septic shock. Extremely high levels (400 pg/mL) were noted in patients who died within 24 h (2). Thus, proinflammatory cytokines appear to play a significant role in the sepsis syndrome.

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The association between these cytokines and survival has prompted us and others to determine if the beneficial effect of hemofiltration in septic shock might be, in part, due to their convective or adsorptive removal (3–6). However, human TNF is a 17-kd, 157-amino-acid cytokine that circulates as a nonglycosylated, noncovalently bound trimer (7–10). Human IL-1 is a 159-amino-acid, 17-kd protein. On the basis of these structures, it seems unlikely that these molecules could readily sieve through standard hemofiltration membranes in the presence of membrane-fouling proteins such as in plasma. Thus, the purpose of this investigation was to evaluate TNF and IL-1 convective clearance through different artificial hemofiltration membranes and to determine whether these cytokines might be removed from the circulation by adsorption to these same membranes.

MATERIALS AND METHODS

The Extracorporeal Circuit

The system consisted of three reservoirs (arterial, venous, and ultrafiltrate) in a single-pass arrangement. After saline priming the entire circuit, the flow rate of the cytokine test solution through the hemofilter was 100 mL/min for 15 min with an ultrafiltration rate of 30 mL/min. Filtration began immediately after initiation. "MasterFlex" pumps (Cole-Parmer Instrument Co., Chicago, IL) were used to accurately regulate flows and were calibrated before each experiment. The experiments were performed at room temperature. Volumes of the contents of each reservoir were measured by graduated cylinder and used for mass balance studies. Between each filter experiment, the tubings were rinsed with copious amounts of tap water. The same tubings and reservoirs were used for each experiment.

Sampling

Simultaneous, duplicate 0.5-ml samples were obtained for TNF and IL-1 determination from the arterial, venous, and ultrafiltrate lines after 1, 5, and 10 min of flow and from all three reservoirs after saline flushing the system into the venous and ultrafiltrate reservoirs. Specimens were immediately frozen and stored at −70°C. Freezing and thawing only occurred once.

Cytokine Solution

TNF (R&D Systems, Minneapolis, MN) and IL-1 (Endogen Inc, Boston, MA) were diluted in saline to achieve a concentration that was 50-fold higher than the lowest sensitivity level of the assay (see below). One million picograms of TNF and 1.3 million picograms of IL-1 were added together to the 2-L arterial reservoir. These represent concentrations two- to fivefold those seen in sepsis (2). Albumin (human; Baxter Healthcare Hyland Division, Glendale, CA) was added to achieve a 1% concentration. This was done to foul the membranes to reproduce the effects seen by plasma but without the possibility of exposing the cytokines to plasma in which soluble receptors could confound the experiments (11). Phosphate buffer was added to the solution in order to achieve a pH of 7.4. The whole volume of the cytokine solution was 2 L.

At the onset of each experiment, filter priming solution remained in the extracorporeal circuit. We attempted as accurately as possible to discard this priming solution. The effect of the dilution of the cytokine solution by the remnant priming solution is shown below.

Hemofilters

Three identical hemofilters were used for each study. The types of the filters that were studied included: Amicon D-20, polysulfone composition, approximately 0.25 m^2 (W.R. Grace, Danvers, MA), Gambro FH88H, polyamide composition, approximately 2.0 m^2 (Gambro, Lund, Sweden), Fresenius F-40, polysulfone composition, approximately 0.65 m^2 (Fresenius AG, Bad Homburg, Germany), Baxter CA-210, cellulose acetate composition, approximately 2.1 m^2 (Baxter, Round Lake, IL), Hospal Biospal, AN69S composition, approximately 0.5 m^2 (Hospal, Meyzieu, France), Renal System HF250, polysulfone composition, approximately 0.25 m^2 (MinnTech, Minneapolis, MN).

TNF/IL-1 Assays

TNF and IL-1 concentrations were determined in duplicate by ELISA (Endogen Inc., Boston, MA). Both assays are specific for the measurement of the natural and recombinant cytokines and, according to Endogen do not express any cross-reactivity to each other or to IL-2, IL-7, or interferon gamma. The TNF ELISA detects a minimum level of 5 pg/mL of biologically active TNF. The IL-1 ELISA detects a minimum level of 13 pg/mL of biologically active IL-1. The interassay variation was 7.25% for IL-1 and 6.87% for TNF.

Calculations

The mass balance was calculated according to the mass of TNF in each of the three reservoirs

$$M = C * Vol$$

where M is the mass of cytokine in the reservoir, C is the cytokine concentration, and Vol is volume of the reservoir, respectively. The mass in all three reservoirs after the experiment was compared with the mass in the arterial reservoir at the initiation of the experiment. Because all experiments were performed with the same tubing in identical fashion, any loss of mass to the tubing would be systematic and would not vary from experiment to experiment or filter to filter. The mass in the cytokine assay samples was ignored because it represented less than 0.5% of the total mass. The sieving coefficient was determined by:

$$SC = \frac{[UF]}{\frac{1}{2}([A] + [V])}$$

or:

$$SC = \frac{2\,[UF]}{[A] + [V]}$$

where $[UF]$, $[A]$, and $[V]$, respectively, represent: concentration in the ultrafiltrate line, concentration in the arterial line, and concentration in the venous line. The arteriovenous (A-V) concentration difference was expressed in percentages and calculated as:

$$A\text{-}V = \frac{[A] - [V]}{[A]} * 100$$

This mathematical construct reflects the combined processes of adsorption (which would make the A-V difference a positive number, *i.e.*, the arterial concentration is more than the venous concentration) and hemofiltration (which could make the A-V difference a negative number because the large molecule is retained within the blood pathway as water is extracted by filtration, *i.e.*, the arterial concentration is less than the venous concentration).

Filter Volume and Effect of Priming Solution Dilution

To estimate the possible adsorption of the cytokines to the membrane, the venous concentration versus time profile was analyzed. A progressive rise in the venous concentration of the cytokine could be secondary to initial adsorption on the membrane (low venous concentration), followed by hemoconcentration (rising venous concentration) and the ultimate release of bound cytokine (higher venous concentration). An alternative explanation is that there was a dilution of the experimental cytokine solution with

the priming solution within the filter and circuit, causing a delay in the appearance of cytokine in the venous effluent. To evaluate this possibility, we determined the time it took for an albumin solution to completely fill the filter, a function of the filter circuit volume. Only filters with relatively large filling volumes were investigated, *e.g.*, CA-210, FH88H, F-40, and Biospal.

After 5 min of tap water priming, a 0.5% albumin solution was pumped through the circuit at 100 mL/min. Samples from the arterial and venous lines were taken every 15 s for 3 min to evaluate the concentration of albumin. The composition of the circuit and flow rates of the solution and ultrafiltrate was the same as in the cytokine experiments. Albumin concentration was determined by spectrophotometry at 278 nm.

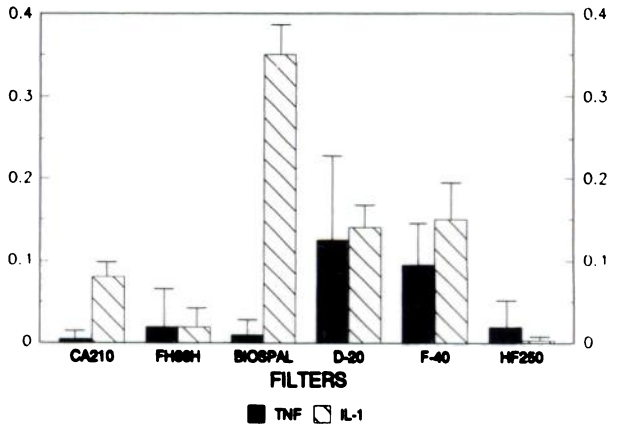

Figure 2. The TNF and IL-1 mean SC from the 10- and 15-min combined data ($N = 6$) with standard deviation bars.

RESULTS

Figure 1 displays the results of the dilution experiment in the four largest filters (by surface area) that we studied. Only the CA-210 required more than 1 min for complete filling of the blood pathway with experimental solution. Therefore, the 1-min data for this device have been disregarded in the results below.

There were no differences in the sieving coefficients (SC) of either cytokine between the 10- and 15-min experiments; thus, we combined these data for Figure 2. The SC of TNF was lowest in Baxter's CA210 cellulose-acetate and Hospal's Biospal AN69 filters and highest in Amicon's D-20 polysulfone filter (Figure 2). For most filters, the SC for IL-1 was slightly higher than that for TNF (Figure 2). The lowest SC of IL-1 was demonstrated by Renal System's HF250 polysulfone and Gambro's FH88H polyamide filters. The highest SC for IL-1 was 0.35, observed in the Biospal.

The FH88H filters demonstrated the greatest A-V difference in the first minute for both TNF and IL-1 (Figures 3 and 4). By the 10th minute, the A-V value was negative for both cytokines, but by the 15th minute, it was positive again. The behavior of the A-V value was directionally similar in the Biospal. Of the three polysulfone filters, the F-40 and HF250 behaved similarly regarding IL-1 in that the A-V was negative at 1 and 10 min and positive at 15 min. The other polysulfone D-20 filter behaved like the other two polysulfone filters, except at 15 min, when the A-V remained negative. For TNF, the three polysulfone filters acted directionally similarly to each other, except that at 15 min, the F-40 demonstrated a pos-

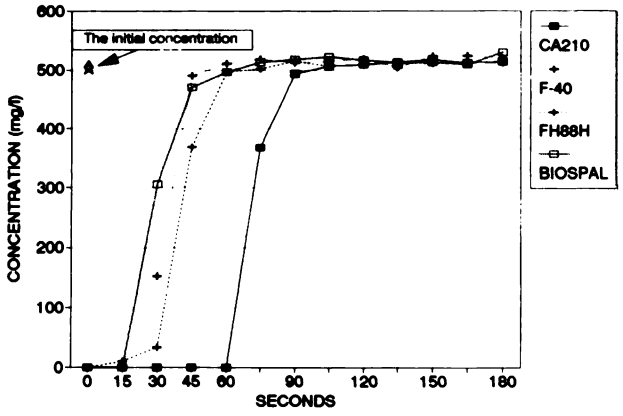

Figure 1. The concentration of albumin in the venous effluent of the dilution experiment. In less than a minute, the albumin concentration in the venous effluent is 100% of the original for each of these large filters *except* for the CA210.

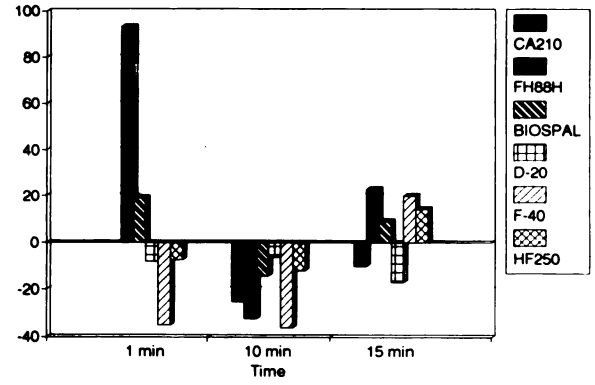

Figure 3. The A-V concentration difference for IL-1 expressed as percentages. Calculations are defined in Methods. A value of −30 would be expected for no binding and no sieving, because the filtration fraction was 30%. A value greater than −30 reflects sieving, binding, or both. A value less than −30 reflects the release of previously bound cytokine. Each bar represents the mean of three observations.

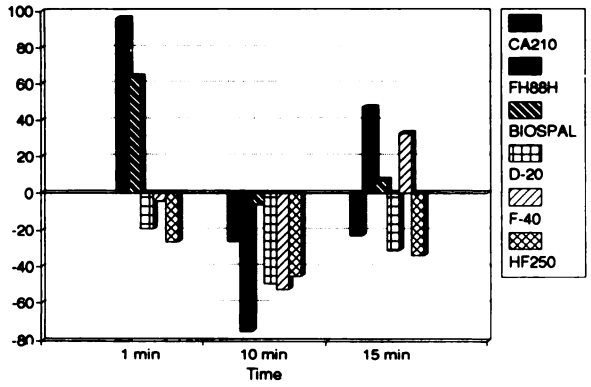

Figure 4. The A-V concentration difference for TNF expressed as percentages. Calculations are defined in Methods. A value of −30 would be expected for no binding and no sieving, because the filtration fraction was 30%. A value greater than −30 reflects sieving, binding, or both. A value less than −30 reflects the release of previously bound cytokine. Each bar represents the mean of three observations.

itive A-V value. For the remaining filter, the cellulose acetate Baxter CA210, the 1-min data were disregarded because the filling volume precluded the adequate delivery of cytokine into the device in only 1 min of flow. At both 10 and 15 min, the A-V values were negative for both cytokines.

Mass loss is displayed in Table 1. Each value is the mean of the three filters studied. There was a loss of ≥30% of the TNF mass during the experiments with the FH88H and Biospal filters.

DISCUSSION

To determine if the beneficial effect of hemofiltration in sepsis is due to the convective or adsorptive removal of proinflammatory cytokines seemed a logical extension of previous investigations in both animals and humans (12–18). Those studies suggested that the signs and symptoms of septic shock and the overall clinical condition improve during hemofiltration. Presumably, this improvement was due to the removal of toxic substances, perhaps TNF and/or IL-1 (3–6).

TABLE 1. Cytokine mass loss

Filter	TNF (%)	IL-1 (%)
CA210	0	3.15
FH88H	30	0
BIOSPAL	32	2
D-20	0	3.3
F-40	0	0
HF250	0	11

Hemodialysis with cuprophan membranes does not alter either TNF or IL-1 plasma concentrations (19). A consistent reduction in plasma levels of both cytokines was noted during hemodiafiltration through PMMA and AN69 (20), but not through Bellco's polysulfone membrane (21).

The SC of TNF and IL-1 that we report here are much higher than one would expect on the basis of their molecular weights (17 kd). The molecular weight cutoffs advertised by hemofilter membrane manufacturers are those obtained in aqueous solutions, not in solutions containing membrane-fouling proteins. Albumin as a 1% solution achieves this fouling effect. Moreover, signal-activating TNF exists as a trimer that has a molecular weight in excess of 50 Kda, as reviewed by Fiers (10). Thus, if these cytokines are removed convectively, one would expect the SC of IL-1 to exceed that of TNF, as we found (Figure 2). An SC of 0.1 or more implies tangible convective removal, which was noted for each cytokine with certain filters.

The various membranes that we studied, including the different polysulfones, differ in their functional behavior (*e.g.*, diffusivity, porosity, and sorbent properties). One would expect the cellulose acetate filter (CA210) to have the lowest SC. This was the case for TNF but not for IL-1. Furthermore, the sieving of IL-1 through AN69 stood out as being surprisingly high.

To evaluate the combined effect of adsorption and convection, we analyzed A-V differences and mass balance during hemofiltration with a filtration fraction of 30%. Under these conditions, if there were no adsorption and no convective losses, the venous concentration should be 30% higher than the arterial concentration. We expressed this as a percentage, as discussed in Methods. An A-V difference percentage greater than −30% (more positive than −30, closer to zero or even greater than zero) means that the venous concentration is less than "expected" and could represent sieving, binding, or both. Very high A-V values almost certainly imply substantial binding, especially because we know the SC is not high. An A-V difference less than −30% (more negative than −30) means that the venous concentration is higher than "expected" and can only mean that previously bound cytokine is now being released into the venous effluent. No cytokine-producing cells were in the test solution. Furthermore, we performed mass balance of cytokines in the arterial, venous, and filtrate reservoirs and could essentially account for most of the cytokine mass involved in the experiment. Lost mass could represent that which remained bound to the membrane at termination. Mass lost to the circuit should be systematic and was calculated to be inconsequential, and thus was ignored.

By virtue of a high A-V difference and a low SC at 1 min, it appears that the polyamide FH88H and

AN69 Biospal devices bind both cytokines. The FH88H is the second largest surface area device that we studied, and the Biospal is middle sized. By the 10th minute, TNF is being released from its binding to the polyamide because the A-V difference is less than −30%. Nonetheless, enough TNF remains bound to the membrane such that 30% of the mass is unaccounted for and presumably bound to the polyamide. However, for the Biospal, there is no release of bound TNF and 32% of the TNF mass was presumably lost to the membrane. This is consistent with high β-2-microglobulin adsorption attributed to this membrane (22). For all other filters, the mass balance loss was less than 11% and no patterns could be discerned. The three different polysulfone filters essentially behaved similarly.

In conclusion, there are appreciable but not large convective losses of these cytokines. In addition, there appears to be substantial binding of TNF to the Biospal AN69 and FH88H polyamide. Whether this is a function of membrane composition and/or surface area is not clear from our experiments. Further investigations will need to address whether these observations can be extrapolated to the whole-blood and *in vivo* situations. If so, then one must determine if the amounts removed are clinically important and if other inflammatory mediators are removed concurrently.

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