Comparison of polyacrylonitrile (AN69) and polysulphone membrane during hemofiltration in canine endotoxic shock

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Objective: This study was designed to compare the effects of continuous venovenous hemofiltration (CVVH) with two different membranes, polysulphone and polyacrylonitrile (AN69), on global and regional hemodynamics, plasma lactate, tumor necrosis factor- α levels, and plasma nitrite/nitrate during endotoxic shock in dogs.

Methods: Fifteen pentobarbital anesthetized and mechanically ventilated dogs were randomized into three groups of five dogs each. One group served as an endotoxin alone, time matching group and, 1 hr after endotoxin administration, the two other groups received CVVH at 3 L/hr for 270 mins, with either a polysulphone membrane or an polyacrylonitrile membrane.

Results: At 90 mins after endotoxin administration, dogs receiving CVVH with polyacrylonitrile membranes had a higher cardiac output, stroke volume, and left-ventricular stroke work index than the endotoxin alone and the polysulphone groups. CVVH with either polyacrylonitrile or polysulphone membranes prevented the rise in pulmonary artery pressure and pulmonary

vascular resistance compared with the endotoxin alone group. Plasma lactate levels were not significantly altered, but the fall in bicarbonate seen in the endotoxin alone group did not occur in the two CVVH groups. Tumor necrosis factor levels in the plasma were not significantly altered by CVVH and remained very low (<50 pg/mL) in the ultrafiltrate fluid.

Conclusion: In this acute endotoxic shock model, CVVH with the polyacrylonitrile membrane improved cardiac performance when compared with the polysulphone membrane. These effects could be caused by a more effective adsorption of inflammatory mediators other than tumor necrosis factor. Whether the polyacrylonitrile membrane should be preferred over the polysulphone membrane for CVVH in severe sepsis warrants further experimental and clinical study. (Crit Care Med 2003; 31:1219–1225)

KEY WORDS: hemofiltration; septic shock; endotoxin; cytokines; polysulphone and polyacrylonitrile membranes; hemodynamics; renal failure

Despite modern intensive care unit treatment, septic shock remains a leading cause of death in critically ill patients (1). The pathogenesis of septic shock begins with the release of bacterial products such as lipopolysaccharide (or endotoxin), which induce the release of endogenous mediators, including cytokines, arachidonic acid metabolites, coagulation factors, complement, nitric oxide, endorphins, kinins, and others that can lead to multiple organ failure and death (2). Effective treatment consists of appropriate antibiotic treatment and surgical removal of the septic source, but attempts to de-

velop new sepsis therapies have, in large, been disappointing.

By modulating the immune response, hemofiltration is potentially an effective approach in severe sepsis (3–5). Many factors can influence the effects of hemofiltration in septic conditions, including the ultrafiltration rate, the type of filter, and the buffer solutions used. Several studies have evaluated the effects of hemofiltration on hemodynamics during septic shock in animals. In general, low-flow (≤ 1 L/hr) hemofiltration did not significantly influence hemodynamics or survival (6–8), but high flow (6 L/hr) hemofiltration markedly increased arterial pressure and cardiac index and improved outcome (9–11). Recently, we (12) compared the effects of high-volume (6 L/hr) and moderate-volume (3 L/hr) hemofiltration and found that the higher flow rate increased arterial pressure, cardiac output, left-ventricular stroke work, and regional blood flow and also decreased blood lactate levels when compared with the lower flow rate.

Many of these experimental studies were performed with polysulphone mem-

branes, but polyacrylonitrile (AN69) membranes are also commonly used in critically ill patients with acute renal failure. A filter's ability to remove solutes is dependent on many factors, including the material, pore size, wall thickness, electrical charge, physical structure, and surface area. *In vitro*, AN69 membranes have good adsorptive capacities and may be more effective in removing tumor necrosis factor (TNF), interleukin (IL)-6, and IL-8 (13). We sought to determine whether using different membranes during continuous venovenous hemofiltration (CVVH) could have an impact on hemodynamics during hemofiltration in acute endotoxic shock. As a secondary issue, we also compared the effects on regional blood flow, TNF (an important proinflammatory cytokine) levels, and nitrite/nitrate levels (reflecting the production of nitric oxide, a well-known vasodilating agent in septic shock).

MATERIALS AND METHODS

The study was approved by the Ethical Committee of Animal Research of the Erasme University Hospital, and care and handling of

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DOI: 10.1097/01.CCM.0000060446.45080.C6

the animals was in agreement with National Institute of Health guidelines.

Surgical Preparation. Fifteen mongrel dogs of either sex, weighing 30.2 ± 5.5 kg, were anesthetized with a bolus of 30 mg/kg intravenous pentobarbital sodium, followed by a constant infusion of $4 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{hr}^{-1}$ (Infusomat II infusion pump, Braun, Melsungen, Germany). The trachea was intubated with a No. 9 cuffed endotracheal tube, and each animal was ventilated with room air; no additional oxygen was administered during the experiment (Servo ventilator 900B, Siemens-Elma, Solna, Sweden). Controlled ventilation was facilitated with pancuronium bromide (0.15 mg/kg initially, with supplementation at $0.075 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{hr}^{-1}$ thereafter). Respiratory frequency was set at 12 breaths/min, and tidal volume was adapted (47210A Capnometer, Hewlett-Packard, Waltham, MA) to obtain an initial end-tidal CO_2 tension (PetCO_2) between

25 and 35 mm Hg. The right forepaw vein was catheterized for intravenous administration of pentobarbital and the left forelimb vein for intravenous fluid infusion. The right femoral artery was catheterized for monitoring of arterial blood pressure and withdrawal of arterial blood samples.

Through the right external jugular vein, a balloon-tipped pulmonary artery catheter (93A-131-7 Fr, Baxter Healthcare, Irvine, CA) was placed under guidance of pressure waves (monitor Sirecust 302A, Siemens, Erlangen, Germany). Single-lumen catheters (JO-KATH, CP-1175, Hechingen, Germany) were placed in the right femoral vein to take blood from the animal to the hemofiltration machine and in the left jugular vein to return blood to the animal.

A splenectomy was performed after maximal splenic contraction through a midline laparotomy to prevent autotransfusion of erythrocytes during hypotension. Ultrasonic-flow probes were placed around the common hepatic artery (3 mm), portal vein (10–12 mm), left renal (4 mm), and left femoral (4–6 mm) arteries for simultaneous measurement of blood flow in these vessels. The probes were connected to a blood flowmeter (T208, Transonic Systems, Ithaca, NY), which was calibrated before each experiment.

Hemofiltration Setting. The hemofiltration device consisted of a roller pump, air detector, and pressure feedback system (BM 11 BM 14, Baxter). Filter sizes were chosen to correspond with the animals' body weight. A 0.71 m^2 polysulphone hollow-fiber filter

(Renaflor II Hemofilter PSHF 700G, Baxter) and a 0.85 m^2 AN69 filter (Multiflow 100, Hospal, France) were used. Other structural characteristics of the membranes were similar (Table 1). Before the experiment, the filters were rinsed with 1 L of normal saline containing 5000 units of heparin. Zero-balance hemofiltration was achieved using a balance installed in the device. This was fully automated, resulting in constant ultrafiltration rates. The buffer solution containing 30 mM/L bicarbonate, 2.9 mM/L lactate, and other electrolyte ions (HF 32 BIC, Sifra, Italy) was warmed up to 37°C and infused after the filter. A heparin infusion of 1000 units/hr was administered during 270 mins of hemofiltration.

Experimental Protocol. After surgical preparation (time = 0), the animals were allowed to stabilize for at least 30 mins and randomized into three groups. Group 1 (endotoxin, $n = 5$) received a slow bolus of 2 mg/kg *Escherichia coli* endotoxin (055:B5, control 3120-10-7, Difco, Detroit, MI) for 2 mins through the pulmonary artery catheter. Thirty minutes later, normal saline was infused and continued throughout the study to restore and maintain pulmonary artery occlusion pressure at baseline levels. All groups received the same amount of fluid (5 L during the entire the experiment). Group 2 (CVVH polysulphone, $n = 5$) was submitted to the same protocol but underwent CVVH, starting 30 mins after the onset of fluid resuscitation, at a rate of 3 L/hr for 270 mins, using a polysulphone membrane. Group 3 (CVVH AN69, $n = 5$) underwent the same procedure

Table 1. Physical characteristics of the two hemofiltration membranes

	Polysulphone	AN69
Surface (m^2)	0.71	0.85
Convection	Yes	Yes
Adsorption	No	Yes
Sieving coefficient		
Myoglobin	0.51	0.55
Albumin	0.02	0.01
Fiber diameter, μm	200	215
Fiber wall thickness, μm	40	50
Length, cm	25.3	27

Table 2. Selected variables

	Group	CVVH				
		Baseline 0	Endotoxin 30	Fluid 60	90	120
Temperature, $^\circ\text{C}$	1	37.7 ± 0.6	37.8 ± 0.7	37.3 ± 0.5	37.3 ± 0.7	37.3 ± 0.7
	2	37.2 ± 0.5	37.3 ± 0.5	36.8 ± 0.5	36.5 ± 0.9	36.3 ± 1.1
	3	36.8 ± 1.1	36.9 ± 1.4	36.4 ± 1.3	35.7 ± 1.5	35.4 ± 1.5
Heart rate, beats/min	1	154 ± 15	152 ± 14	153 ± 11	156 ± 9	153 ± 12
	2	119 ± 15^a	131 ± 11	135 ± 12	148 ± 15	149 ± 18
	3	147 ± 24	144 ± 23	153 ± 13	148 ± 7	143 ± 5
PAOP, mm Hg	1	3.4 ± 0.9	1.8 ± 1.8	3.0 ± 1.2	3.0 ± 1.6	2.8 ± 2.4
	2	6.0 ± 3.2	4.7 ± 2.5	5.5 ± 2.3	4.5 ± 1.5	5.0 ± 1.7
	3	4.2 ± 1.6	1.8 ± 1.1	3.4 ± 2.3	3.0 ± 3.1	3.0 ± 1.9
Right atrial pressure, mm Hg	1	3.4 ± 0.9	1.6 ± 1.1	2.0 ± 1.4	2.2 ± 1.8	3.0 ± 2.1
	2	4.7 ± 2.6	2.8 ± 2.1	4.3 ± 2.5	3.3 ± 2.0	4.0 ± 1.8
	3	2.8 ± 2.3	0.8 ± 0.8	2.4 ± 1.8	2.2 ± 2.8	2.2 ± 1.6
Oxygen delivery, mL/min/kg	1	38.1 ± 12.0	19.1 ± 2.4	37.7 ± 14.5	29.6 ± 5.8	29.3 ± 8.2
	2	24.9 ± 5.6	12.1 ± 7.0	24.5 ± 8.0	20.9 ± 10.0	22.2 ± 7.2^c
	3	45.1 ± 20.5	21.8 ± 9.7	43.9 ± 18.3	46.2 ± 18.3	40.3 ± 9.0
Oxygen uptake, mL/min/kg	1	6.0 ± 0.4	5.8 ± 0.6	6.2 ± 0.6	6.1 ± 0.4	6.0 ± 0.5
	2	4.9 ± 1.2	4.7 ± 1.2	5.1 ± 1.2	4.9 ± 1.3	5.0 ± 1.2
	3	5.0 ± 1.4	4.9 ± 1.4	5.1 ± 1.3	4.8 ± 1.2	4.7 ± 1.0
Oxygen extraction ratio, %	1	15.5 ± 4.5	30.3 ± 4.5	16.4 ± 8.1	20.6 ± 4.4	20.5 ± 5.4
	2	19.7 ± 2.9	38.8 ± 11.2	20.8 ± 3.3	23.4 ± 5.2	22.5 ± 2.2
	3	11.1 ± 2.1	22.5 ± 12.8	11.6 ± 5.1	10.4 ± 2.4^a	11.7 ± 2.7^a

as group 2, but an AN69 membrane was used. At the end of the study, all animals were killed with an overdose of pentobarbital sodium.

Measurements and Calculations. Pressures from femoral arterial and pulmonary arterial catheters were monitored continuously using pressure transducers (series 966020-01, Baxter Healthcare) with amplifiers (Heilige Servomed, Freiburg, Germany) and a pen recorder (model 2600 S, Gould Instruments, Cleveland, OH). All pressures were determined at end expiration. Cardiac output (in liters per minute) was measured by the thermodilution technique (COM-2, Baxter Healthcare), using three to five 5-mL bolus injections of cold 5% dextrose in ice water at end expiration. Stroke volume, left-ventricular stroke work index, systemic vascular resistance, and pulmonary vascular resistance were calculated according to the standard formulas.

Exhaled gases were directed through a mixing chamber for sampling of expired oxygen fraction (FE_{O_2}) and end-tidal CO_2 ($PetCO_2$). The gas analyzers for expired oxygen (P. K. Morgan, Chatham, Kent, UK) and CO_2 (47210A capnometer, Hewlett-Packard) were calibrated before the experiment. Expired minute volume ($\dot{V}_E$) was measured with a spirometer (Haloscale Wright Respirometer, Edrington, UK) for a 2-min period. Arterial and mixed venous blood samples were simultaneously withdrawn for immediate determination of blood gases (ABL 2, Radiometer, Copenhagen, Denmark), hemoglobin, and oxygen saturation (OSM 3 hemoximeter calibrated for dog blood, Radiometer, Copenhagen, Denmark).

Systemic oxygen consumption ($\dot{V}_{O_2}$) was determined from analysis of expired gases by the following formula: $\dot{V}_{O_2}$ ($mL \cdot kg^{-1} \cdot min^{-1}$) = $\dot{V}_E / wt$ (kg) $\times [(1 - FE_{O_2} - FE_{CO_2} / 1 - FE_{CO_2}) \times F_{IO_2} - FE_{O_2}]$, where FE_{CO_2} represents the expired CO_2 fraction.

Systemic oxygen delivery ($\dot{D}_{O_2}$) was calculated as the product of arterial oxygen content and cardiac output, and related to the animal's weight. Oxygen extraction ratio was derived from the ratio of $\dot{V}_{O_2} / \dot{D}_{O_2}$. Blood lactate concentrations were determined by a glucose-lactate analyzer (2300 Stat Plus, Yellow Springs Instruments, Yellow Springs, OH).

Plasma and ultrafiltrate TNF concentrations were measured every 60 mins by ELISA technique, using specific polyclonal rabbit antibodies to recombinant human TNF. Immuno-maxisorp 96 well plates (Nunc, Roskilde, Denmark) were coated with monoclonal anti-human TNF. Nonattached sites were blocked with 1% human serum albumin in phosphate buffered saline. Goat polyclonal rabbit antibodies, along with peroxidase, were used as detecting antibodies. Developments were carried out with 3,3',5,5'-tetramethylbenzidine, and the reaction was stopped with H_2SO_4 . The activity was measured at 450 nm spectrophotometrically (Molecular Devices, Menlo Park, CA).

Nitric oxide release was determined spectrophotometrically every 60 mins by measuring the accumulation of both nitrite and nitrate (the latter is reduced to nitrite) in both plasma and ultrafiltrate. Nitrate was stoichiometrically reduced to nitrite by incubation of a sample (100 μL) for 2 hr at 37°C in the pres-

ence of 0.1 units/mL nitrate reductase (NADPH: nitrate oxidoreductase, EC 1.6.6.2; *Aspergillus* spp., Sigma, St Louis, MO), 120 μM NADPH, and 5 μM FAD (Sigma) in a final volume of 103 μL . After nitrate had been reduced to nitrite, NADPH, which interfered with the subsequent nitrite determination, was oxidized with 10 units/mL L-lactic dehydrogenase (EC 1.1.1.27; type XI, from rabbit muscle, Sigma) and 10 mM sodium pyruvate for 30 mins at 37°C in a final volume of 114 μL . Sodium nitrate was used as a standard. Nitrite concentration was assayed by a standard Griess reaction. Briefly, 100 μL of plasma or ultrafiltrate was incubated with an equal volume of Griess reagent (1% sulfanilamide, 0.1% N-(1-naphthyl)ethylenediamine dihydrochloride, 2.5% H_3PO_4) at room temperature for 10 mins. The absorbance of the chromophore formed was determined at 540 nm using a microtiter plate reader (Molecular Devices). Sodium nitrite was used as a standard, with control baseline plasma as a blank.

Statistical Methodology and End Points. A two-way analysis of variance, followed by Newman-Keuls test, was used for statistical analysis. A p value of $<.05$ was considered as statistically significant.

RESULTS

Data are shown in Table 2.

Effects of Endotoxin and Fluid Administration. Endotoxin administration was followed by a fall in cardiac output, stroke volume, left-ventricular stroke work in-

Table 2. (Continued)

CVVH						
150	180	210	240	270	300	330 (mins)
37.3 $\pm$ 0.8	37.3 $\pm$ 0.9	37.6 $\pm$ 0.6	37.5 $\pm$ 0.7	37.7 $\pm$ 0.7	37.7 $\pm$ 0.7	37.7 $\pm$ 0.7
36.4 $\pm$ 1.3	36.7 $\pm$ 1.0	36.6 $\pm$ 0.8	36.8 $\pm$ 0.6	36.9 $\pm$ 0.7	36.8 $\pm$ 0.8	36.7 $\pm$ 0.8
35.4 $\pm$ 1.6	35.5 $\pm$ 1.7	35.5 $\pm$ 1.8	35.4 $\pm$ 1.9	35.6 $\pm$ 2.1	35.1 $\pm$ 2.2	34.9 $\pm$ 2.7 ^a
146 $\pm$ 13	141 $\pm$ 12	139 $\pm$ 12	138 $\pm$ 12	136 $\pm$ 12	133 $\pm$ 9	132 $\pm$ 7
144 $\pm$ 15	142 $\pm$ 13	143 $\pm$ 14	141 $\pm$ 12	135 $\pm$ 10	129 $\pm$ 13	123 $\pm$ 16
139 $\pm$ 7	136 $\pm$ 7	134 $\pm$ 9	132 $\pm$ 9	129 $\pm$ 11	124 $\pm$ 9	124 $\pm$ 7
3.6 $\pm$ 2.3	3.4 $\pm$ 1.8	3.3 $\pm$ 2.2	5.0 $\pm$ 2.2	5.4 $\pm$ 2.8	6.3 $\pm$ 3.2	7.1 $\pm$ 3.1
5.2 $\pm$ 1.3	5.2 $\pm$ 2.1	5.3 $\pm$ 2.0	4.5 $\pm$ 1.9	4.9 $\pm$ 1.7	5.6 $\pm$ 2.3	6.8 $\pm$ 3.1
2.6 $\pm$ 1.1	3.0 $\pm$ 1.4	3.2 $\pm$ 1.6	3.2 $\pm$ 1.6	3.6 $\pm$ 2.6	3.0 $\pm$ 2.4	2.0 $\pm$ 1.7
3.0 $\pm$ 2.0	3.2 $\pm$ 1.8	3.0 $\pm$ 2.3	4.4 $\pm$ 1.6	5.3 $\pm$ 1.6	6.1 $\pm$ 2.0	7.1 $\pm$ 2.1
4.2 $\pm$ 2.1	4.2 $\pm$ 1.8	4.3 $\pm$ 2.0	4.0 $\pm$ 1.8	4.5 $\pm$ 1.8	5.0 $\pm$ 2.3	5.8 $\pm$ 3.3
1.8 $\pm$ 0.8	2.0 $\pm$ 1.2	2.0 $\pm$ 1.0	1.8 $\pm$ 0.8	2.6 $\pm$ 1.7	1.8 $\pm$ 1.0 ^a	1.7 $\pm$ 1.1 ^b
24.2 $\pm$ 4.4	22.1 $\pm$ 5.4	18.5 $\pm$ 6.5	17.0 $\pm$ 6.4	13.5 $\pm$ 6.9	12.2 $\pm$ 6.9	10.0 $\pm$ 6.6
18.0 $\pm$ 4.5	18.3 $\pm$ 5.2	17.2 $\pm$ 6.2	15.5 $\pm$ 6.5	13.6 $\pm$ 5.4	12.1 $\pm$ 4.6	11.0 $\pm$ 5.4
37.7 $\pm$ 10.7 ^a	35.1 $\pm$ 11.4 ^a	32.0 $\pm$ 11.6 ^a	31.5 $\pm$ 11.8 ^a	25.9 $\pm$ 9.3	23.5 $\pm$ 9.6	18.0 $\pm$ 5.7
6.0 $\pm$ 0.5	5.8 $\pm$ 0.5	5.9 $\pm$ 0.5	5.5 $\pm$ 0.6	5.3 $\pm$ 0.6	5.2 $\pm$ 0.7	4.6 $\pm$ 0.8
4.8 $\pm$ 1.1	4.8 $\pm$ 1.1	4.8 $\pm$ 1.1	4.7 $\pm$ 1.2	4.6 $\pm$ 1.2	4.5 $\pm$ 1.2	4.4 $\pm$ 1.2
4.6 $\pm$ 0.9	4.6 $\pm$ 0.9	4.5 $\pm$ 1.1	4.4 $\pm$ 1.0	4.3 $\pm$ 0.9	4.4 $\pm$ 0.8	4.3 $\pm$ 1.0
24.8 $\pm$ 5.8	26.2 $\pm$ 6.9	31.9 $\pm$ 9.6	32.4 $\pm$ 10.7	39.3 $\pm$ 16.2	42.6 $\pm$ 21.0	46.0 $\pm$ 20.8
26.7 $\pm$ 2.2	26.2 $\pm$ 2.3	27.9 $\pm$ 5.1	30.3 $\pm$ 5.7	33.8 $\pm$ 7.9	37.2 $\pm$ 7.4	40.0 $\pm$ 18.4
12.2 $\pm$ 2.9 ^a	13.1 $\pm$ 3.0 ^a	14.1 $\pm$ 3.2 ^a	14.0 $\pm$ 4.1 ^a	16.6 $\pm$ 4.1 ^b	18.7 $\pm$ 4.8	23.9 $\pm$ 3.1

CVVH, continuous venovenous hemofiltration; Group 1, endotoxin; Group 2, CVVH polysulphone; Group 3, CVVH PAN; PAOP, pulmonary artery occlusion pressure.

Data are shown as mean $\pm$ SD.

^a $p < .05$ vs. two other groups; ^b $p < .05$ vs. endotoxin; ^c $p < .05$ vs. group 3.

Figure 1. Time course of cardiac output (CO), stroke volume (SV), and left ventricular stroke work index (LVSWI) after endotoxin administration in the three groups of animals. Data presented as mean $\pm$ SD. * $p < .05$ vs. endotoxin; † $p < .05$ vs. the other two groups.

dex (Fig. 1), and mean arterial blood pressure and a significant rise in pulmonary vascular resistance (Fig. 2). Regional blood flow decreased in the portal vein, the femoral artery, and to a lesser extent, in the hepatic artery (Fig. 3). After fluid resuscitation, cardiac output, stroke volume, left-ventricular stroke work index (Figs. 1 and 2), and regional blood flow (Fig. 3) were restored to or above baseline, but blood pressure and systemic vascular resistance (Fig. 2) remained lower than baseline, indicating a hyperdynamic state. Pulmonary vascular resistance progressively increased. Plasma lactate levels increased after endotoxin administration and remained elevated, despite fluid administration (Fig. 4). With time, cardiac output, stroke volume, left-ventricular stroke work index (Fig. 1), and regional blood flow (Fig. 3) decreased progressively, and systemic vascular resistance, pulmonary vascular resistance (Fig. 2), and plasma lactate levels (Fig. 4) in-

Figure 2. Time course of mean arterial pressure (MAP), mean pulmonary artery pressure (MPAP), systemic vascular resistance (SVR), and pulmonary vascular resistance (PVR) after endotoxin administration in the three groups of animals. * $p < .05$ vs. endotoxin; † $p < .05$ vs. the other two groups.

Figure 3. Time course of regional blood flow after endotoxin administration in the three groups of animals. * $p < .05$ vs. endotoxin; † $p < .05$ vs. the other two groups.

creased. At baseline (time = 0), levels of both TNF and nitrate/nitrite were already elevated because this was after the surgical procedure. Plasma TNF levels reached a peak 1 hr after endotoxin and declined during the next 2 hrs (Fig. 5). Levels of nitrite/nitrate remained high (Fig. 5).

Effects of CVVH with Polysulphone Membrane. CVVH with the polysulphone

membrane had no significant effect on cardiac output, stroke volume, and left-ventricular stroke work index (Fig. 1), but it prevented pulmonary vasoconstriction (Fig. 2) and decreased systemic vascular resistance (Fig. 2). Hepatic and femoral blood flows tended to remain higher than in the endotoxin alone group (Fig. 3), although differences were not

Figure 4. Time course of arterial pH, lactate, and bicarbonate after endotoxin administration in the three groups of animals. * $p < .05$ vs. endotoxin; † $p < .05$ vs. the other two groups; § $p < .05$ vs. polysulphone group.

statistically significant. Plasma lactate levels were not altered, but the fall in bicarbonate seen in the endotoxin alone group did not occur (Fig. 4). TNF levels were not significantly altered in the plasma (Fig. 5) and remained very low (<50 pg/mL) in the ultrafiltrate fluid.

Effects of CVVH with AN69 Membrane. At 90 mins after endotoxin (30 mins after CVVH was started), dogs treated with CVVH with the AN69 membrane had a significantly higher cardiac output, stroke volume, and left-ventricular stroke work index than the endotoxin alone and the polysulphone groups (Fig. 1). At 5 hrs after endotoxin, these differences tended to disappear (Fig. 1). CVVH with the AN69 membrane prevented the rise in mean pulmonary artery pressure and in pulmonary vascular resistance observed in the endotoxin alone group (Fig. 3). Arterial blood pressure remained sta-

Figure 5. Time course of tumor necrosis factor and of nitrites/nitrate after endotoxin administration in the three groups of animals. * $p < .05$ vs. endotoxin; † $p < .05$ vs. the other two groups; § $p < .05$ vs. polysulphone group.

ble over time and, at the end of the study, became significantly greater than in the endotoxin alone group. Systemic vascular resistance decreased after CVVH was started and remained lower than in the endotoxin alone group (Fig. 2). As with CVVH with the polysulphone membrane, regional blood flows tended to remain higher than in the endotoxin alone group, although these differences were not statistically significant (Fig. 3). Plasma lactate levels were not altered, but the fall in bicarbonate seen in the endotoxin alone group did not occur (Fig. 4). TNF levels were not significantly altered in the plasma (Fig. 5) and remained very low (<50 pg/mL) in the ultrafiltrate fluid. Plasma nitrate/nitrite levels were significantly lower than in the endotoxin alone group (Fig. 5).

DISCUSSION

After initial fluid resuscitation, human septic shock is characterized by a hyperdynamic state, with high cardiac output, low arterial blood pressure, and low systemic vascular resistance. In our dog model, this situation was well reproduced by the administration of endotoxin and followed by generous fluid administration (14, 15). There were no differences in the amount of fluid given to the different groups.

The major finding of our study was that CVVH with AN69 membranes, but not with polysulphone membranes, can significantly increase cardiac output and cardiac function in these endotoxic shock conditions. The lack of beneficial effects with CVVH using a polysulphone membrane and an ultrafiltration rate of 3 L/hr is in agreement with our previous observations (12). In a previous study (12), we found that CVVH with a polysulphone membrane had beneficial effects at 6 L/hr, an extremely high flow rate. Hence, we sought to define whether another membrane could exert protective effects at a more acceptable rate of 3L/hr. We used the AN69 membrane because it is one of the most commonly used membranes for renal replacement therapy in critically ill patients. Although there was a small (14%) difference in the size of the filter surfaces used, other variables of the filters were similar. In particular, the sieving coefficient for myoglobin, which has a molecular weight close to that of TNF- α , is similar. From *in vitro* studies, we know that polysulphone membranes do not have good adsorptive properties; thus, increasing their surface would not alter much, especially at a flow rate of $100 \text{ mL} \cdot \text{kg}^{-1} \cdot \text{hr}^{-1}$. We therefore believe that the difference in surface area of the two membranes could not account for the different hemodynamic effects.

A number of investigators have studied the effects of low-volume hemofiltration, using a polysulphone or polyamide membrane and a lactate based buffer, and found no, or only moderate, hemodynamic improvement in septic animals (6–8, 16). Other authors demonstrated significant hemodynamic improvement with a polysulphone membrane, but they used much higher flow rates (9–11). Interestingly, the effects in our study were seen immediately after starting CVVH and tended to become less pronounced 5 hrs after endotoxin, suggesting an adsorptive mechanism. Adsorption is a phenomenon that takes place within minutes. Convection, however, takes much more time to remove the same amount of substance. Mean arterial blood pressure only increased later in the course of the experiment.

The beneficial effects of CVVH are likely caused by the removal of inflammatory substances by hemofiltration (17). Among the various mediators involved in the development of septic shock, TNF is a major myocardial depressant substance (18). In the present study and in our

Polyacrylonitrile membranes have greater beneficial hemodynamic effects in the initial phase of continuous venovenous hemofiltration in endotoxemic dogs than polysulphone membranes.

previous study (12), CVVH had no effect on plasma TNF levels, and only very low levels of TNF were found in the ultrafiltrate, indicating no considerable convective removal by CVVH. Similar results have been found in rats (19) and pigs (20), using polysulphone membranes, but no *in vivo* animal studies are available using the AN69 membrane. *In vitro* data reveal adsorption of TNF with AN69 membranes and no, or minimal, convective removal with either AN69 or polysulphone membranes (21, 22). In clinical studies of critically ill patients (23–29), TNF adsorption occurred with AN69 but not with other membranes. Only minimal amounts of TNF could be detected in the ultrafiltrate in our study, suggesting that TNF exists in the circulation either as a trimer (30) or bound to soluble receptors. Our findings suggest that the improvement in cardiac performance during CVVH with AN69 could be due to other mechanisms than TNF attenuation. The immediate hemodynamic improvement in the AN69 group is in favor of an adsorptive mechanism, but other substances than TNF may have been eliminated from the circulating blood. IL-1 may be implicated because AN69 membrane has the highest sieving coefficient for IL-1 *in vitro* (31) and *in vivo* (23). Also, IL-6 can be removed significantly with the AN69 membrane (22, 23). However, in a recent study by Kellum et al. (17), CVVH with the AN69 membrane decreased plasma TNF levels but not IL-6, IL-8, soluble L-selectin, or endotoxin levels.

The adsorption capacity of AN69 membranes seems larger than that of other synthetic membranes, presumably because of its negatively charged surface and because of its symmetrical structure

(32–34). The AN69 membrane has been shown to efficiently adsorb anaphylatoxins (C3a, C5a), bradykinin, endotoxins, and cytokines (IL-1, TNF), all factors that play a role in the pathophysiology of sepsis (35–38). CVVH can also remove eicosanoids like thromboxane B2 and myocardial depressing substances (19, 39, 40). The less severe pulmonary hypertension in CVVH-treated animals is perhaps related to removal of thromboxane A2 and prostaglandin I₂ (19).

Nitrite/nitrate levels were taken after the surgical procedure and are therefore not true baseline levels. These levels are extremely sensitive to stress, including surgical procedures (laparotomy). After a few hours of CVVH, there was a trend toward reduced nitrite/nitrate levels in both hemofiltration groups compared with the endotoxin alone group. In our study, the decrease in plasma nitrite/nitrate levels with CVVH argues against the involvement of nitric oxide in the decrease in pulmonary and systemic vascular resistance seen during CVVH. As in other experiments using the endotoxin shock model, we also found that hemofiltration mainly improved cardiac function (6, 10, 12, 16), explaining the decrease in SVR during hemofiltration.

Other factors should be considered to explain the beneficial hemodynamic effects. Body temperature decreased during CVVH in some clinical studies (41), but this was carefully prevented in our study (Table 1). Furthermore, a lower body temperature should rather decrease cardiac output. The correction of acidosis may be beneficial because a low pH may have deleterious effects on cardiac performance and attenuates the response to catecholamines (42). In our study, CVVH prevented the fall in bicarbonate seen with endotoxin, and the differences in arterial pH between the AN69 and the polysulphone group were quite limited.

In conclusion, AN69 membranes have greater beneficial hemodynamic effects in the initial phase of CVVH in endotoxemic dogs than polysulphone membranes. These differences seem to be related to removal of mediators other than TNF. Whether AN69 membranes should be preferred over polysulphone membranes for CVVH in severe sepsis warrants further experimental and clinical study.

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