Anesthesiology 2001; 95:428–36

Continuous Venovenous Hemofiltration Improves Arterial Oxygenation in Endotoxin-induced Lung Injury in Pigs

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Background: **Hypoxemia is common in septic acute lung failure. Therapy is mainly supportive, and most trials using specific inhibitors of key inflammatory mediators (*i.e.*, tumor necrosis factor α, interleukin 1) have failed to prove beneficial. The authors investigated if a nonspecific blood purification technique, using zero-balanced high-volume continuous venovenous hemofiltration (CVVH), might improve arterial oxygenation in a fluid-resuscitated porcine model of endotoxin-induced acute lung injury.**

Methods: **Piglets of both sexes weighing 25–30 kg were anesthetized and mechanically ventilated. After baseline measurements, animals received an intravenous infusion of 0.5 mg/kg endotoxin (*Escherichia coli* lipopolysaccharide). One hour after endotoxin, animals were randomly assigned to either treatment with CVVH (endotoxin + hemofiltration, n = 6) or spontaneous course (endotoxin, n = 6). At 4 h after randomization, animals were killed. Hemofiltration was performed from femoral vein to femoral vein using a standard circuit with an EF60 polysulphone hemofilter.**

Results: **Endotoxin challenge induced arterial hypoxemia, an increase in peak inspiratory pressure, pulmonary hypertension, and systemic hypotension. Treatment with CVVH did not improve systemic or pulmonary hemodynamics. However, arterial oxygenation was increased in endotoxin-challenged animals at 5 h after completion of endotoxin infusion, as compared with animals not receiving CVVH (arterial oxygen tension, 268 ± 33 *vs.* 176 ± 67 mmHg, respectively, $P < 0.01$). In addition, treatment with CVVH attenuated the endotoxin-induced increase in peak inspiratory pressure and increased lung compliance.**

Conclusion: **These results suggest that nonspecific blood purification with high-volume CVVH improves arterial oxygenation and lung function in endotoxin-induced acute lung injury in pigs, independent of improved hemodynamics, fluid removal, or body temperature.**

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Received from the Department of Anesthesiology and General Critical Care Medicine, Vienna General Hospital, University of Vienna, Vienna, Austria; Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, Vienna, Austria; and the Department of Anesthesia, Massachusetts General Hospital, Boston, Massachusetts. Submitted for publication February 17, 2000. Accepted for publication February 14, 2001. Supported by an educational grant of the Jubilaeumsfond der Oesterreichischen Nationalbank, Austrian National Bank, Vienna, Austria. Presented in part at the 12th Annual Congress of the European Society of Intensive Care Medicine, Berlin, Germany, October 3–6, 1999.

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SEPSIS is the major cause of death in intensive care units.[1] Arterial hypoxemia is common in sepsis and frequently occurs without a direct inflammatory process present within the lungs. Infusion of endotoxin or tumor necrosis factor mimics the pathophysiologic changes observed in sepsis and has been shown to result in arterial hypoxemia and acute lung injury in experimental animals.[2,3] Patients with sepsis demonstrate elevated plasma concentrations of endotoxin and other proinflammatory mediators, thus suggesting that systemic mediator release is associated with the indirect organ injury observed in sepsis.[4,5]

The identification of key inflammatory mediators released during sepsis and inflammation has encouraged the development of specific therapeutic strategies directed against these mediators.[1,6] However, most large clinical trials using inhibitors of cytokines or endotoxin have failed to prove beneficial.[7]

These discouraging results have strengthened new interest in nonspecific blood purification techniques, such as hemofiltration. Early clinical reports suggested that the use of arteriovenous hemofiltration for nonrenal indication improved hemodynamics, cardiac contractility, and arterial oxygenation in patients with acute respiratory failure after cardiac surgery[8] or sepsis.[9–11] Gotloib *et al.*[10] reported that treatment with intermittent hemofiltration improved gas exchange and hemodynamics in 24 patients with nonoliguric septic acute respiratory distress syndrome. Although clinical data suggest a beneficial effect for treating septic acute respiratory distress syndrome with hemofiltration,[10–12] most of these studies were either uncontrolled or nonrandomized cohort studies. Moreover, in the only prospective, randomized clinical trial investigating the effect of hemofiltration on respiratory parameters and hemodynamics in septic acute respiratory distress syndrome, Cosentino *et al.*[13] reported that arteriovenous hemofiltration did not improve gas exchange, although it did improve survival.

In addition, experimental animal studies investigating the use of hemofiltration for nonrenal application as a treatment of sepsis focused mainly on hemodynamics, cardiac performance, or survival.[14,15] Thus, it remains unknown whether treatment with hemofiltration exerts a specific effect on the lungs in sepsis.

We investigated the impact of treatment with high-volume continuous venovenous hemofiltration (CVVH) on oxygenation and lung function in endotoxin-induced acute lung injury in pigs. To avoid the confounding

effect of hypodynamic septic shock, a protocol of fluid resuscitation was applied to maintain adequate filling pressures. We used high-volume zero-balanced CVVH because this regimen has been shown to result in increased removal of inflammatory mediators.[2,16] To determine if treatment with CVVH effectively removed solute mediators from the circulation, plasma concentrations of nitrate–nitrite and interleukin-1 receptor antagonist (IL-1ra) were measured before and after treatment with CVVH.

Methods

Experimental Preparation

The Committee on Animal Experiments of Vienna (Vienna, Austria) approved the experimental protocol, and all experiments were performed during conditions described in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. We studied piglets of both sexes aged of 2–5 months and weighing 25–30 kg. Animals were anesthetized by intravenous injection of azaperone (7 mg/kg) and alloferin (0.2 mg/kg), their tracheas were intubated, and ventilation was started at a tidal volume of 10 ml/kg, a respiratory rate between 20 and 25 breaths/min, and a positive end-expiratory pressure level of 2–3 cm H_2O. Respirator settings were adjusted to maintain blood gas values within the physiologic range and were kept constant thereafter. Anesthesia was maintained by adding 0.6–0.8% halothane to the inspiratory gas mixture (50% O_2 in nitrous oxide). Alloferin (0.2 $\mu g \cdot kg^{-1} \cdot min^{-1}$) was added to produce muscle relaxation. Polyethylene catheters were placed in the right carotid artery and right femoral vein. A 7.5-French Swan-Ganz flow-directed thermodilution tip catheter (93A-431H7.5; Baxter Healthcare Corp., Irvine, CA) was positioned in the proximal pulmonary artery *via* the right jugular vein. A 10.5-French double-lumen catheter (Quinton Mahurkar; Quinton Instruments, Bothall, WA) was inserted into the left femoral vein and served as hemofiltration access. A 14-French silastic catheter was inserted suprapubically into the bladder. All catheters were placed by direct cutdown and the wounds closed surgically. Electrical heart activity (electrocardiogram) was derived from six-lead needle electrodes and body temperature from a rectal thermistor. All catheters were flushed with heparinized saline to prevent clotting.

Continuous Venovenous Hemofiltration

The extracorporeal circuit consisted of a roller pump with air detector, bubble trap, and pressure limiter (Gambro, Lundt, Sweden), blood pump lines, and a hemofilter (EF60; Fresenius, Oberursel, Germany). The EF60 is a polysulphone hollow fiber filter with a cutoff point of less than 50,000 Da. Hemofiltration was performed from femoral vein to femoral vein by use of a double-lumen catheter. The speed of the roller pump was adjusted to achieve an ultrafiltration rate of approximately 4,500 ml/h. The ultrafiltered volume was continuously weighed with a balance (BS 1; Gambro) and replaced before the filter with warmed acetate-buffered electrolyte substitution fluid with an osmolarity of 293 mOsm/l (HAEMFL6; Fresenius, Bad Homburg, Germany). The balance computer automatically compensated for variance in fluid filtration and substitution.

Experimental Protocol

After baseline measurements, animals received an intravenous infusion of 0.5 mg/kg endotoxin (*E. coli* lipopolysaccharide 026:B6; Difco Laboratories, Detroit, MI) over 2 h. One hour after the end of endotoxin infusion, animals were randomly assigned to either treatment with high-volume CVVH (endotoxin plus hemofiltration, n = 6) or spontaneous course (endotoxin, n = 6). An additional four endotoxin-challenged animals were subjected to sham hemofiltration with the ultrafiltration line clamped. Systemic anticoagulation was started with a bolus dose of 100 IU/kg intravenous heparin sulfate followed by an intravenous infusion of 10 IU $\cdot mg^{-1} \cdot kg^{-1} \cdot h^{-1}$ in all animals, including those not receiving CVVH. The animals were monitored thereafter for 4 h and then killed with a bolus injection of 10 ml KCl. Measurements of hemodynamics, blood gases, hemoglobin, and metabolic variables were obtained every hour. All other measurements were taken at baseline, at the start of CVVH, and at the end of CVVH. Ringer's lactate was started at a rate of 7 ml $\cdot kg^{-1} \cdot h^{-1}$ intravenously. To achieve adequate fluid resuscitation, the infusion rate was increased to 10 ml $\cdot kg^{-1} \cdot h^{-1}$ at mean arterial pressure less than 70 mmHg and to 15 ml $\cdot kg^{-1} \cdot h^{-1}$ at mean arterial pressure less than 50 mmHg. If pulmonary artery wedge pressure exceeded 10 mmHg, the rate of infusion was lowered to the previous level. No colloids were applied during the experiment. Body temperature was kept constant around 38–39°C using a heating blanket.

Measurements of Hemodynamics and Lung Mechanics

Mean arterial pressure, mean pulmonary artery pressure, central venous pressure, and intratracheal pressure were continuously monitored using biomedical amplifiers. Heart rate was derived from the electrocardiograph. The expiratory tidal volume (V_{Texp}) was measured with a flowmeter, and static lung compliance was calculated as $P_{PLATEAU} - P_{PEEP}/V_{Texp}$, where $P_{PLATEAU}$ is inspiratory plateau pressure and P_{PEEP} is positive end-expiratory pressure. The length of the plateau period was 3 s. All measured signals were transferred to an analog-to-digital converter, displayed on a computer screen, and recorded using a data acquisition system. All monitoring equipment was calibrated before each experiment. Car-

diac output was determined by thermodilution technique (REF-1, Baxter Healthcare Corp.). The average of three measurements taken at end expiration was accepted as the value of each period.

Measurements of Arterial Blood Gas, Hemoglobin, Lactate, and Blood Cell Count

Blood samples were immediately analyzed for arterial and mixed-venous *p*H, arterial oxygen tension (Pa_{O_2}), arterial carbon dioxide tension, hemoglobin, hemoglobin saturation, electrolytes, and lactate concentrations using an automated blood gas analyzing system (ABL 625; Radiometer, Copenhagen, Denmark). Oxygenation parameters were corrected for animal body temperature and alveolar-to-arterial oxygen difference was calculated. Blood samples for leukocyte and erythrocyte counts were collected on EDTA and analyzed with a blood cell counter (Cell Dyn 1300; Abbott, Vienna, Austria).

Measurements of Plasma Endotoxin Concentrations

Heparinized plasma sample aliquots were analyzed for endotoxin concentrations using a limulus amebocyte lysate test as described previously.[17] The method has a detection limit of 0.75 pg/ml in 1:10 plasma dilution.

Measurements of Plasma Nitrate–Nitrite, Interleukin-1 Receptor Antagonist, Bilirubin, and Creatinine Concentrations

Nitrate–nitrite was determined in heparin plasma with a modification of the method by Green *et al.*,[18] including an automated injection high-performance liquid chromatography analysis technique with postcolumn derivatization. After protein precipitation with acetonitrile, samples were injected onto a Cd–Cu column to reduce nitrate to nitrite, detected by diazotization of sulfanilamide, and subsequently coupled with a chromophore, according to the method of Griess.[18] Sample aliquots of plasma were subjected to specific enzyme-linked immunosorbent assay for IL-1ra, using an antibody raised against IL-1ra, according to the manufacturer's instructions (R&D Systems Europe, Abingdon, United Kingdom). Serum concentrations of bilirubin and creatinine were measured using commercially available standard kits (Roche, Basel, Switzerland).

Lung Morphometry

At the end of the experiment, the right middle lobe was excised and placed into 10% buffered formaline. Using standard techniques, paraffin and semithin sections were obtained and stained with hematoxylin-eosin stain and toluidin blue, respectively. Lung sections were examined by light microscopy by an experienced pathologist unaware of group or treatment assignment. Severity of alveolar congestion, bleeding, atelectasis, leukostasis, and perivascular edema was estimated, respectively, using a four-grade scoring system, where 0 = absence ($<$ 25% of maximum pathology), 1 = mild ($<$ 50%), 2 = moderate (50–75%) and 3 = severe ($>$ 75%), *i.e.*, complete collapse of all alveoli within the visible field (maximum pathology for atelectasis) would give a score of 3 for presence of atelectasis. A total of three slides from each lung sample was randomly screened, and the mean was taken as the representative values of the sample.

Statistical Analysis

All data are expressed as mean ± SD. Within-group comparisons were analyzed using one-way analysis of variance for repeated measures followed by *post hoc* comparison with the Student-Newman-Keuls test. Treatment effect of CVVH was assessed at 4 h after start of treatment using the Student *t* test for independent samples (Statistica, StatSoft Inc., Tulsa, OK). $P < 0.05$ indicated a significant difference.

Results

Effect of Endotoxin Challenge

Endotoxin challenge induced sustained and progressive derangement of systemic and pulmonary hemodynamics, including tachycardia, systemic hypotension, and pulmonary hypertension. Cardiac output was maintained, and mean arterial pressure increased with fluid substitution but did not return to baseline values (table 1). In addition to these hemodynamic changes, pulmonary function was impaired as assessed by an increase in peak inspiratory pressure and a decrease in pulmonary static compliance, despite recruitment maneuvers and application of positive end-expiratory pressure (table 2). This endotoxin-induced impairment of pulmonary function was associated with a worsening of pulmonary gas exchange. The Pa_{O_2} decreased significantly 1 h after completion of endotoxin infusion and remained below baseline for the remainder of the experiment (fig. 1). Moreover, endotoxin induced a widening of the alveolar-to-arterial oxygen difference at 1 h after endotoxin, with further deterioration present the end of the experiment (table 2).

Endotoxin-induced increases in creatinine and bilirubin concentrations did not recover spontaneously at 5 h after completion of endotoxin infusion (creatinine, 139 ± 40 mg/dl; bilirubin, 1.29 ± 1.09 mg/dl; $P < 0.05$ *vs.* baseline). Animals challenged with endotoxin demonstrated a minor metabolic acidosis at 5 h after completion of endotoxin infusion (pH, 7.29 ± 0.07; base excess, −3.3 ± 2.7; lactate, 2.0 ± 0.9 mM), together with leukopenia and thrombopenia (table 3).

Effect of High-volume Continuous Venovenous Hemofiltration on Endotoxin Response

Treatment with CVVH did not alter systemic or pulmonary hemodynamics (table 1). In addition, there was no

Table 1. Pulmonary and Systemic Hemodynamics

	Cardiac Index ($l \cdot min^{-1} \cdot m^{-2}$)	Mean Systemic Arterial Pressure (mmHg)	Systemic Vascular Resistance Index ($dyne \cdot s \cdot cm^{-5} \cdot m^{-2}$)	Mean Pulmonary Arterial Pressure (mmHg)	Pulmonary Vascular Resistance Index ($dyne \cdot s \cdot cm^{-5} \cdot m^{-2}$)	Pulmonary Capillary Wedge Pressure (mmHg)
Baseline						
Endotoxin	5.2 ± 0.9	97 ± 6	1,461 ± 277	14 ± 2	86 ± 20	8 ± 2
Endotoxin + hemofiltration	5.0 ± 0.5	98 ± 7	1,532 ± 60	17 ± 2	153 ± 16	8 ± 2
Hemofiltration-start (1 h after endotoxin)						
Endotoxin	4.1 ± 0.8	64 ± 15*	1,188 ± 390*	37 ± 6*	566 ± 234*	10 ± 3
Endotoxin + hemofiltration	4.5 ± 1.4	72 ± 19*	1,312 ± 209*	38 ± 5*	556 ± 210*	10 ± 3
Hemofiltration-end (5 h after endotoxin)						
Endotoxin	4.1 ± 0.7	58 ± 19*	1,062 ± 482*	29 ± 4*	385 ± 153*	10 ± 3
Endotoxin + hemofiltration	4.5 ± 0.4	69 ± 5*	1,115 ± 83*	35 ± 7*	445 ± 122*	10 ± 1

Values are expressed as mean ± SD.

* $P < 0.05$ compares with baseline within group.

difference in cardiac index, systemic and pulmonary vascular resistance indexes between endotoxin-treated animals receiving CVVH and those not treated with hemofiltration (table 1). However, treatment with CVVH induced an early and sustained increase in Pa_{O_2}, restoring arterial oxygenation to baseline values at 5 h after completion of endotoxin infusion (fig. 1). In contrast, Pa_{O_2} further deteriorated in endotoxin-challenged animals not receiving CVVH. The endotoxin-induced widening in alveolar-to-arterial oxygen difference, another indicator of impaired gas exchange, decreased during treatment with CVVH but increased in animals not receiving CVVH (table 2).

These beneficial effects on gas exchange were associated with an improvement in lung mechanics (table 2). Treatment with CVVH attenuated the increase in peak inspiratory pressure and the impairment of static compliance induced by endotoxin challenge (table 2).

Treatment with CVVH for 4 h reduced elevated plasma concentrations of creatinine (115 ± 34 *vs.* 139 ± 40 mg/dl, $P < 0.05$, endotoxin plus hemofiltration *vs.* endotoxin) and bilirubin (0.54 ± 0.54 *vs.* 1.29 ± 1.09 mg/dl, $P < 0.05$, endotoxin plus hemofiltration *vs.* endotoxin). However, it did not alter the mild metabolic acidosis after endotoxin challenge (pH, 7.34 ± 0.13 *vs.* 7.28 ± 0.07, nonsignificant), nor did it affect blood cell count (table 3).

Table 2. Effect of Hemofiltration on Oxygenation, Respiratory Parameters, and Temperature

	PIP (cm H_2O)	Compliance (ml/cm H_2O)	Pa_{O_2} (mmHg)	Pv_{O_2} (mmHg)	Aa_{DO_2} (mmHg)	Ca_{O_2} (ml/l)	Cv_{O_2} (ml/l)	Shunt (%)	Temperature (°C)
Baseline									
Endotoxin	17 ± 1	31 ± 4	287 ± 13	45 ± 2	22 ± 14	12.6 ± 0.8	8.8 ± 0.6	6 ± 1	38.1 ± 0.3
Endotoxin + hemofiltration	17 ± 1	30 ± 5	275 ± 18	51 ± 9	32 ± 15	11.9 ± 1.4	8.0 ± 0.9	7 ± 1	38.2 ± 0.1
Hemofiltration-start (1 h after endotoxin)									
Endotoxin	24 ± 3*	20 ± 4*	200 ± 61*	48 ± 5	105 ± 59*	13.5 ± 1.1	8.6 ± 0.9	11 ± 6	38.5 ± 0.4
Endotoxin + hemofiltration	23 ± 3*	21 ± 5*	189 ± 50*	49 ± 6	114 ± 50*	13.1 ± 1.4	8.0 ± 1.2	10 ± 2	38.6 ± 0.2
Hemofiltration-end (5 h after endotoxin)									
Endotoxin	26 ± 3*	18 ± 4*	176 ± 67*	49 ± 4	119 ± 62*	12.8 ± 1.5	7.1 ± 0.3	10 ± 4	39.1 ± 0.5
Endotoxin + hemofiltration	22 ± 4*†	21 ± 5*†	268 ± 33†	47 ± 7	41 ± 25†	12.9 ± 1.4	6.7 ± 0.8	6 ± 2†	38.3 ± 0.2

Values are expressed as mean ± SD.

* $P < 0.05$ compares with baseline within group. † $P < 0.05$ compares endotoxin *versus* endotoxin + hemofiltration group at 4 h of treatment (5 h after endotoxin).

PIP = peak inspiratory pressure; Pa_{O_2} = arterial oxygen tension; Pv_{O_2} = mixed venous oxygen tension; Aa_{DO_2} = alveolar-to-arterial oxygen difference; Ca_{O_2} = arterial oxygen content; Cv_{O_2} = mixed venous oxygen content.

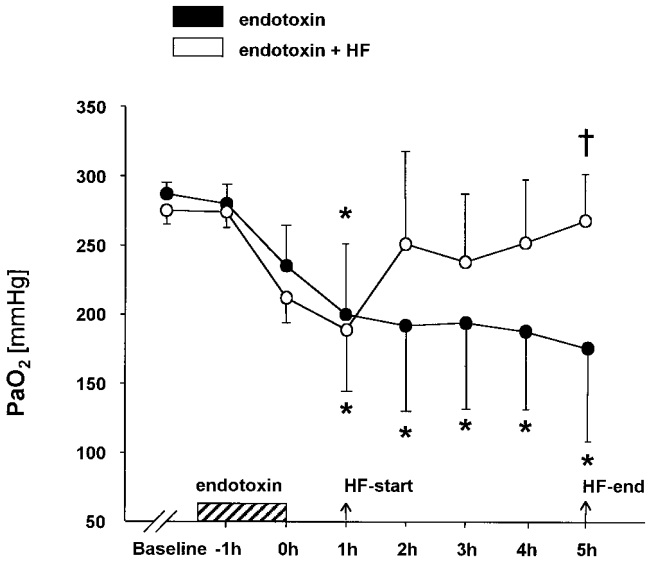

Fig. 1. Effect of high-volume continuos venovenous hemofiltration (CVVH) on endotoxin-induced arterial hypoxemia in pigs. Changes in partial pressure of arterial oxygen (Pao_2), induced by an intravenous infusion of 500 μg/kg endotoxin (*E. coli* lipopolysaccharide) over 2 h (endotoxin). In one group of animals (endotoxin plus hemofiltration [HF], n = 6), treatment with CVVH was initiated 1 h after end of endotoxin infusion (start of hemofiltration) and performed for 4 h (end of hemofiltration). The other group (endotoxin alone, n = 6) served as control. CVVH was zero-balanced, and the ultrafiltration rate was set at 4,500 ml/h. Values are expressed as mean ± SD. *P < 0.05 compared with baseline within group; †P < 0.05, endotoxin *versus* endotoxin-plus-hemofiltration group at 4 h of treatment (5 h after endotoxin challenge). Note that treatment with CVVH improved endotoxin-induced arterial hypoxemia as early as 1 h after the start of hemofiltration.

Effect of Sham Hemofiltration on Endotoxin Response

Endotoxin-challenged animals connected to the extracorporeal circuit at 1 h after endotoxin with the ultrafiltration line clamped (sham hemofiltration) and treated for 4 h thereafter did not differ from animals not receiving CVVH (Pao_2, 169 ± 81 mmHg; alveolar-to-arterial oxygen difference, 134 ± 75; mean arterial pressure, 73 ± 2 mmHg; pulmonary artery pressure, 35 ± 5 mmHg; cardiac index, 4.7 ± 0.3 $l \cdot min^{-1} \cdot m^{-2}$; nonsignificant compared with control animals). These results suggest that systemic anticoagulation and recirculation of venous blood through the extracorporeal circuit without ultrafiltration does not alter the oxygenation and hemodynamic response to an endotoxin challenge in pigs.

Effect of High-volume Continuous Venovenous Hemofiltration on Plasma Nitrate–Nitrite, Interleukin-1 Receptor Antagonist, and Endotoxin Concentrations

To determine whether CVVH treatment resulted in removal of inflammatory markers released in response to endotoxin challenge, we measured plasma concentrations of nitrate–nitrite, a marker of nitric oxide production, and IL-1ra, an antiinflammatory molecule,[19] at baseline, at the start of hemofiltration, and 5 h after completion of endotoxin infusion. At the start of CVVH treatment, 1 h after endotoxin challenge, plasma nitrate–nitrite concentrations did not differ between groups. However, plasma nitrate–nitrite concentrations increased in endotoxin-challenge animals not being treated with CVVH, but were decreased in animals receiving CVVH at 5 h after completion of endotoxin infusion (149 ± 59 *vs.* 72 ± 21% of baseline values, $P <$ 0.05, endotoxin plus hemofiltration *vs.* endotoxin; fig. 2).

Interleukin-1 receptor antagonist was not detectable at baseline but increased in response to endotoxin challenge at 1 h after endotoxin in both groups, with and without CVVH (6,641 ± 707 and 6,220 ± 428 pg/ml, respectively, $P < 0.05$ *vs.* baseline for both groups; fig. 3). After endotoxin challenge, at 5 h after completion of endotoxin infusion, IL-1ra remained elevated in untreated animals as compared with a decrease in animals receiving

Table 3. Effect of Hemofiltration on Blood Count

	Leukocyte Count (10^6/ml)	Erythrocyte Count (10^6/ml)	Platelet Count (10^6/ml)	Hemoglobin (g/dl)
Baseline				
Endotoxin	13.2 ± 3.3	5.5 ± 0.3	489 ± 170	9.3 ± 0.5
Endotoxin + hemofiltration	16.1 ± 4.5	5.2 ± 0.4	554 ± 141	9.0 ± 0.9
Hemofiltration-start (1 h after endotoxin)				
Endotoxin	1.3 ± 0.4*	5.7 ± 0.4	210 ± 114*	9.2 ± 0.7
Endotoxin + hemofiltration	1.8 ± 0.8*	5.3 ± 0.8	238 ± 91*	8.9 ± 1.5
Hemofiltration-end (5 h after endotoxin)				
Endotoxin	3.0 ± 2.0*	5.4 ± 0.6	154 ± 83*	8.6 ± 0.9*
Endotoxin + hemofiltration	4.8 ± 2.1*	4.7 ± 0.8	202 ± 82*	7.5 ± 1.2*

Values are expressed as mean ± SD.

* $P < 0.05$ compares with baseline within group.

CVVH (5,942 ± 1,087 *vs.* 4,540 ± 1,484 pg/ml, $P < 0.05$, endotoxin *vs.* endotoxin plus hemofiltration; fig. 3).

Serum endotoxin concentrations were not detectable at baseline. At 1 h after endotoxin challenge, there was an abundance of endotoxin observed that gradually declined over the course of the experiment in animals treated with and without CVVH . The decrease in endotoxin concentrations from 1 h after endotoxin to the end of the experiment did not differ between the two groups (Δ = 3,409 ± 1,436 *vs.* 4,537 ± 3,111 pg/ml, nonsignificant, endotoxin *vs.* endotoxin plus hemofiltration).

Fluid Balance

To avoid the confounding effects of hypodynamic shock on organ function and to maintain adequate filling pressures, a standardized protocol of fluid replacement was applied. The total positive fluid balance required to achieve this goal was +3,550 ± 1,355 ml for animals without CVVH and +3,762 ± 1,391 ml for animals with CVVH. Endotoxin-challenged animals with or without CVVH received the same amount of Ringer's lactate and did not differ in total diuresis. In all endotoxin-challenged animals receiving CVVH treatment, an ultrafiltration rate greater than 4,500 ml/h could be achieved. At

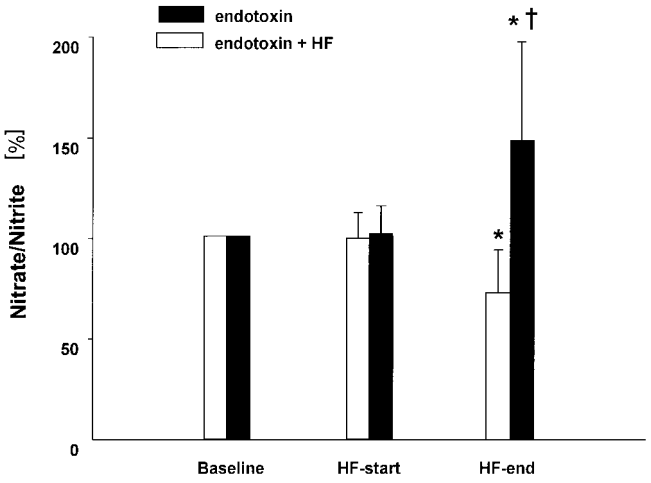

Fig. 2. Effect of high-volume continuos venovenous hemofiltration (CVVH) on endotoxin-induced nitrate–nitrite concentrations, a marker of nitric oxide production. Plasma nitrate–nitrite concentrations were determined 1 and 5 h after an intravenous infusion of 500 μg/kg endotoxin (*E. coli* lipopolysaccharide). In one group of animals (endotoxin plus hemofiltration [HF], n = 6), treatment with CVVH was initiated 1 h after the end of endotoxin infusion (start of hemofiltration) and performed for 4 h (end of hemofiltration). The other group (endotoxin alone, n = 6) served as control. CVVH was zero-balanced, and the ultrafiltration rate was set at 4,500 ml/h. Values are percent of baseline and expressed as mean ± SD. *$P < 0.05$ compared to baseline within group; †$P < 0.05$, endotoxin *versus* endotoxin-plus-hemofiltration group at 4 h of treatment (5 h after endotoxin). Note that after 5 h of endotoxin challenge, there was an increase in plasma nitrate–nitrite concentrations in animals not treated with CVVH (endotoxin alone). Endotoxin-challenged pigs treated with CVVH for 4 h showed a reduction of this endotoxin-induced increase in plasma nitrate–nitrite concentrations.

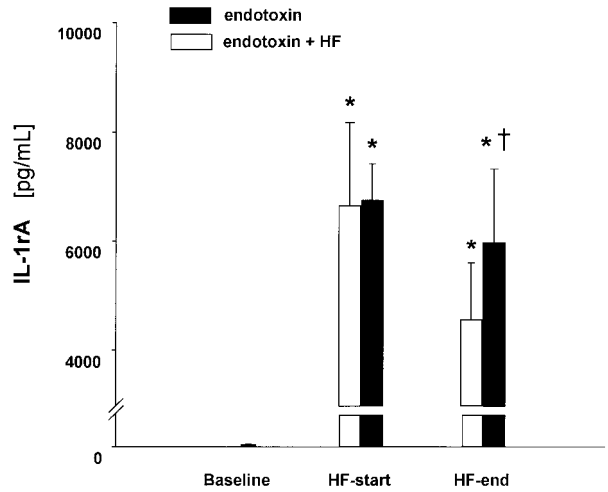

Fig. 3. Effect of high-volume continuos venovenous hemofiltration (CVVH) on endotoxin-induced increase in interleukin-1 receptor antagonist (IL-1ra). IL-1ra concentrations were determined 1 and 5 h after an intravenous infusion of 500 μg/kg endotoxin (*E. coli* lipopolysaccharide). In one group of animals (endotoxin plus hemofiltration [HF], n = 6), treatment with CVVH was initiated 1 h after the end of endotoxin infusion (start of hemofiltration) and performed for 4 h (end of hemofiltration). The other group (endotoxin alone, n = 6) served as control. CVVH was zero-balanced, and the ultrafiltration rate was set at 4,500 ml/h. Values are percent of baseline and expressed as mean ± SD. *$P < 0.05$ compares with baseline within group; †$P < 0.05$, endotoxin *versus* endotoxin-plus-hemofiltration group at 4 h of treatment (5 h after endotoxin). Note that after 1 h of endotoxin challenge, there was an increase in IL-1ra concentrations in both groups. Endotoxin-challenged pigs treated with CVVH for 4 h showed a significant decrease in IL-1ra concentrations.

5 h after completion of endotoxin infusion, lung weight did not differ between animals with or without CVVH (390 ± 102 and 422 ± 127 g, respectively, nonsignificant, endotoxin plus hemofiltration *vs.* endotoxin).

Morphology

Endotoxin induced diffuse alveolar damage represented by alveolar bleeding, atelectasis, leukocyte sequestration, and perivascular edema within the lungs at 5 h after endotoxin challenge. There was no difference in lung morphometry between endotoxin-challenged animals treated with or without CVVH (table 4). Light microscopy of sections obtained from liver, spleen, heart, and kidney demonstrated that endotoxin induced nonspecific minor organ injury in all specimens at 5 h after endotoxin challenge (data not shown).

Discussion

Reports from clinical studies suggested that treatment with hemofiltration when used for nonrenal application might be useful in improving arterial oxygenation and gas exchange in patients with acute respiratory failure.[8–11] However, nonspecific effects of hemofiltration on pulmonary gas exchange may provide an alternative

Table 4. Lung Morphometry at 5 h after Endotoxin in Animals Treated with and without Hemofiltration

	Congestion	Bleeding	Atelectasis	Leukostasis	Perivascular Edema	Median Score
Endotoxin	0 (0–2)	1 (0–1)	1 (0–3)	2 (1–2)	2 (1–3)	1 (0–3)
Endotoxin + hemofiltration	1 (0–2)	1 (0–2)	1 (0–1)	2 (1–2)	2 (0–2)	1 (0–2)

Values are expressed as median values (range). Lung sections were screened for presence of alveolar congestion, bleeding, atelectasis, leucostasis, and perivascular edema and scored using a semiquantitative four-grade scoring system: 0 = absence (< 25% of field), 1 = minimal (< 50% of field), 2 = medium (< 75% of field), 3 = maximum (> 75% of field).

explanation for these beneficial effects. For example, treatment with hemofiltration has been shown to facilitate fluid removal and to restore hemodynamics in sepsis, all of which might importantly contribute to improved pulmonary gas exchange.[20] In addition, until now only few experimental studies have addressed the impact of hemofiltration on sepsis-induced organ injury, and the majority of these studies investigated the hemodynamic effects of hemofiltration in animal models of septic hypodynamic shock.[14,15] Therefore, it remains incompletely understood whether hemofiltration exerts a specific effect on the injured lungs in sepsis.

In the current study we demonstrate that treatment with high-volume CVVH improves arterial oxygenation and lung mechanics in endotoxin-induced acute lung injury in pigs. Endotoxin challenge induced a reduction in Pao_2 and a widening of the alveolar-to-arterial oxygen difference. This endotoxin-induced arterial hypoxemia was sustained and persisted throughout the experiment. Treatment with CVVH increased Pao_2 as early as 1 h after the start of treatment (fig. 1), restoring arterial oxygenation at 5 h after completion of endotoxin infusion. In contrast, endotoxin-challenged animals not receiving hemofiltration demonstrated worsening of gas exchange. This beneficial effect of hemofiltration on endotoxin-induced lung injury was associated with improved lung mechanics (table 2).

One possible explanation for the improved gas exchange in endotoxin-challenged animals treated with hemofiltration is that fluid removal is facilitated and lung water content is decreased by achieving a negative fluid balance.[20] In agreement with this view, clinical and experimental studies have shown that treatment with hemofiltration was associated with improved systemic and pulmonary hemodynamics, facilitated fluid removal, and improved organ function in sepsis.[14,15] In our study, however, all animals received aggressive fluid resuscitation, resulting in a positive fluid balance. Moreover, animals treated with or without CVVH did not differ in their fluid intake, diuresis, fluid balance, or lung wet weights, suggesting that hemofiltration did not facilitate fluid removal from the lungs. Therefore, we believe that the improved arterial oxygenation and lung mechanics observed at 5 h after completion of endotoxin infusion occurred by a mechanism independent of fluid removal or restored hemodynamics. Interestingly, in a study reported by Stein *et al.*,[21] reduction of extravascular lung water content in porcine acute lung injury was not associated with improved gas exchange, suggesting that removal of lung water alone is not sufficient to ameliorate arterial oxygenation in acute septic lung injury in pigs.

In addition to its effect on fluid balance and hemodynamics, treatment with hemofiltration alters body temperature and corrects electrolyte and acid-base disturbances. Differences in temperature and pH in-

Table 5. Metabolic Parameters, Urine Output and Fluid Balance

	pH	BE	Anion Gap	Urine Output (ml)	Fluid Balance (ml)
Baseline					
Endotoxin	7.52 ± 0.02	7.8 ± 0.9	9 ± 2	—	—
Endotoxin + hemofiltration	7.48 ± 0.04	5.6 ± 1.9	8 ± 5	—	—
Hemofiltration-start (1 h after endotoxin)					
Endotoxin	7.39 ± 0.05*	−0.1 ± 2.0*	10 ± 1	396 ± 166	+2,015 ± 1,162
Endotoxin + hemofiltration	7.37 ± 0.09*	−1.1 ± 4.7*	11 ± 2	340 ± 141	+2,466 ± 2,160
Hemofiltration-end (5 h after endotoxin)					
Endotoxin	7.29 ± 0.07*	−3.3 ± 2.7*	10 ± 2	444 ± 234	+3,550 ± 1,355
Endotoxin + hemofiltration	7.34 ± 0.12*	−0.7 ± 4.2*	9 ± 3	624 ± 343	+3,763 ± 1,391

Values are expressed as mean ± SD.

* $P < 0.05$ compares with baseline within group.

BE = base excess.

fluence measurements of pulmonary gas exchange and alter the lung's response to physiologic and pathologic stimuli. For example, hypoxic pulmonary vasoconstriction is severely impaired in sepsis and endotoxemia, thus contributing to arterial hypoxemia. Changes in temperature and pH modify the pulmonary vasoconstrictor response to hypoxia and might contribute to improved arterial oxygenation during hemofiltration treatment. However, we did not observe any differences in electrolyte balance, pH, or temperature in endotoxin-challenged animals treated with or without CVVH (table 5), suggesting that improvement in pulmonary gas exchange by hemofiltration was not caused by correction of metabolic acidosis or changes in body temperature.

In contrast to other reports,[2,21,22] we could not observe any improvement in hemodynamics or cardiac performance by treatment with CVVH (table 1). One possible explanation for this difference is that we used a fluid-resuscitated model of sepsis, thereby avoiding hypodynamic shock. Presence or absence of septic hypodynamic shock importantly alters the response to treatment with hemofiltration in animal models of sepsis. For example, it has been demonstrated that hemofiltration exerts a profound effect on systemic hemodynamics in animals with septic hypodynamic shock,[2,21,22] whereas it had no effect on fluid-resuscitated dogs challenged with live bacteria.[23]

Furthermore, differences in experimental setup may have a major impact on results from animal studies investigating septic lung injury. One important point is the mode of respiratory support. Application of small tidal volumes has been shown to protect the lungs of experimental animals from ventilator-induced lung injury.[24] As many studies investigating the effect of hemofiltration on endotoxin-induced organ injury applied large tidal volumes, we ventilated the animals with 10 ml/kg to facilitate comparison of our results with that of others. Several studies suggested that a substantial degree of mediator removal is achieved not by ultrafiltration but by adsorption to the membrane.[9,11] Hemofiltration membranes vary considerably in their adsorption and filtration characteristics, which might, in turn, influence the results of experiments. The EF60 membrane used in our studies is a very biocompatible, high porosity but low-adsorption membrane, capable of achieving the high ultrafiltration rates required for a high-volume CVVH experimental protocol. When endotoxin-challenged animals were connected to the extracorporeal circuit with the ultrafiltration line clamped (sham hemofiltration) they did not differ from endotoxin-treated animals not receiving hemofiltration. Thus, potential adsorption of mediators to the membrane was not attributable for the beneficial effect of CVVH on arterial oxygenation. In addition, these results suggest that systemic anticoagulation with heparin during the extracorporeal treatment did not alter the course of endotoxin-induced lung injury in our studies.

A wealth of data exists demonstrating the presence of various inflammatory mediators (cytokines, prostanoids, and complement factors) in the ultrafiltrate of patients with sepsis.[15,20] However, there is substantial disagreement whether the beneficial clinical effects observed during treatment with hemofiltration can be attributed to removal of specific inflammatory mediators. To date, no specific inflammatory mediators solely responsible for endotoxin-induced organ injury have been identified. Moreover, as hemofiltration is a nonspecific blood purification technique, antiinflammatory and beneficial mediators may be removed as well. Because of these methodologic difficulties, we did not intend to investigate the hypothesis that removal of specific inflammatory mediators is beneficial in experimental sepsis.

We measured plasma concentrations of IL-1ra and nitrate, antiinflammatory and proinflammatory molecules, respectively, to assess whether our hemofiltration protocol was effectively reducing elevated plasma concentrations of circulating markers. IL-1ra and nitrate are both produced in large amounts after an endotoxin challenge, and we observed that treatment with CVVH greatly reduced the increased concentrations of IL-1ra and nitrate that followed an endotoxin challenge in pigs. However, as we used these measurements mainly to demonstrate effective clearance by CVVH of substances involved in the systemic response to an endotoxin challenge, no inference can be drawn from these data to a potential beneficial effect of removal of inflammatory mediators on the course of septic lung injury.

Furthermore, an alternative explanation for the observed reduction of endotoxin-induced increases in IL-1ra and nitrate concentrations might be that early treatment with CVVH attenuated the entire inflammatory response, including decreases in tumor necrosis factor α and IL-1 concentrations, which, in turn, might have reduced the production of IL-1ra. In agreement with this view, Journois *et al.*[25] recently reported that treatment with high-volume, zero-balanced hemofiltration during cardiopulmonary bypass in children attenuated the inflammatory response to the extracorporeal circuit by removal of tumor necrosis factor α and IL-1, thus reducing the release of late cytokines such as IL-1, IL-6, IL-8, and myeloperoxidase.

Studies of experimental sepsis bear inherent limitations, including species differences in the response to endotoxin challenge and a short-term experimental setup that contrasts with the long-term course of clinical sepsis. Therefore, potential clinical implications of our findings must be cautiously interpreted, and further studies are warranted to examine the impact of CVVH on the course of septic lung injury in long-term use and in the clinical setting.

In summary, in this study we report a specific and beneficial effect of treatment with high-volume CVVH on gas exchange and lung mechanics in an experimental animal model of septic lung injury. This beneficial effect of hemofiltration on arterial oxygenation and pulmonary function was not attributable to fluid removal, changes in body temperature, correction of acidosis, or improved systemic hemodynamics. Nonspecific blood purification by CVVH might prove beneficial in septic lung injury.

The authors thank Karl Kropik, B.S. (Staff Technician), Christine Kober (Medical Technical Assistant), Eva Toegel, B.S. (Medical Technical Assistant), Anna Schiesser (Medical Technical Assistant), Mohammed Jafarmadar, B.S. (Staff Technician), and Martin Thurnher, Vet.M.D. (Staff Veterinarian) (all at Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, Vienna, Austria) for excellent technical support in the conduct of these studies; and Yu Chiao Chang, Ph.D.(Stat.) (Statistician, Harvard Medical School, Boston, Massachusetts), and Alexandra Kaider, Ph.D. (Statistician, Institute of Medical Computer Sciences, University of Vienna, Vienna, Austria), for statistical advice.

References

1. Bone RC: Sepsis and its complications: The clinical problem. Crit Care Med 1994; 22:S8–11

2. Grootendorst AF, van Bommel EF, van der Hoven B, van Leengoed LA, van Osta AL: High volume hemofiltration improves right ventricular function in endotoxin-induced shock in the pig. Intensive Care Med 1992; 18:235–40

3. Leeper-Woodford SK, Carey PD, Byrne K, Jenkins JK, Fisher BJ, Blocher C, Sugerman HJ, Fowler AA: Tumor necrosis factor: Alpha and beta subtypes appear in circulation during onset of sepsis-induced lung injury. Am Rev Respir Dis 1991; 143:1076–82

4. de Groote MA, Martin MA, Densen P, Pfaller MA, Wenzel RP: Plasma tumor necrosis factor levels in patients with presumed sepsis: Results in those treated with antilipid A antibody vs placebo. JAMA 1989; 262:249–51

5. Hyers TM, Tricomi SM, Dettenmeier PA, Fowler AA: Tumor necrosis factor levels in serum and bronchoalveolar lavage fluid of patients with the adult respiratory distress syndrome. Am Rev Respir Dis 1991; 144:268–271

6. Windsor AC, Walsh CJ, Mullen PG, Cook DJ, Fisher BJ, Blocher CR, Leeper-Woodford SK, Sugerman HJ, Fowler AA: Tumor necrosis factor-alpha blockade prevents neutrophil CD18 receptor upregulation and attenuates acute lung injury in porcine sepsis without inhibition of neutrophil oxygen radical generation. J Clin Invest 1993; 91:1459–68

7. Grau GE, Maennel DN: TNF inhibition and sepsis: Sounding a cautionary note. Nat Med 1997; 3:1193–5

8. Coraim FJ, Coraim HP, Ebermann R, Stellwag FM: Acute respiratory failure after cardiac surgery: Clinical experience with the application of continuous arteriovenous hemofiltration. Crit Care Med 1986; 14:714–8

9. Bellomo R: Continuous hemofiltration as blood purification in sepsis. New Horiz 1995; 3:732–7

10. Gotloib L, Barzilay E, Shustak A, Lev A: Sequential hemofiltration in nonoliguric high capillary permeability pulmonary edema of severe sepsis: Preliminary report. Crit Care Med 1984; 12:997–1000

11. Grootendorst AF: The potential role of hemofiltration in the treatment of patients with septic shock and multiple organ dysfunction syndrome. Adv Ren Replace Ther 1994; 1:176–84

12. Koperna T, Vogl SE, Poschl GP, Hamilton G, Roder G, Germann P: Cytokine patterns in patients who undergo hemofiltration for treatment of multiple organ failure. World J Surg 1998; 22:443–7

13. Cosentino F, Paganini E, Lockrem J, Stoller J, Wiedemann H: Continuous arteriovenous hemofiltration in the adult respiratory distress syndrome: A randomized trial. Contrib Nephrol 1991; 93:94–7

14. Grootendorst AF, van Bommel EF: The role of hemofiltration in the critically-ill intensive care unit patient: Present and future. Blood Purif 1993; 11:209–23

15. Silvester W: Mediator removal with CRRT: Complement and cytokines. Am J Kidney Dis 1997; 30:S38–43

16. Kellum JA, Kramer D, Palevsky P, Brady JJ, Pinsky MR: Diffusive versus convective therapy: Effects on mediators of inflammation in patients with severe systemic inflammatory response syndrome. Crit Care Med 1998; 26:1995–2000

17. Bahrami S, Yao YM, Leichtfried G, Redl H, Schlag G, Di Padova FE: Monoclonal antibody to endotoxin attenuates hemorrhage-induced lung injury and mortality in rats. Crit Care Med 1997; 25:1030–6

18. Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR: Analysis of nitrate, nitrite, and [15N]nitrate in biological fluids. Anal Biochem 1982; 126:131–8

19. Gabay C, Smith MF, Eidlen D, Arend WP: Interleukin 1 receptor antagonist (IL-1Ra) is an acute-phase protein. J Clin Invest 1997; 99:2930–40

20. van Bommel EF: Should continuous renal replacement therapy be used for 'non-renal' indications in critically ill patients with shock? Resuscitation 1997; 33:257–70

21. Stein B, Pfenninger E, Grunert A, Schmitz JE, Deller A, Kocher F: The consequences of continuous haemofiltration on lung mechanics and extravascular lung water in a porcine endotoxic shock model. Intensive Care Med 1991; 17:293–8

22. Gomez A, Wang R, Unruh H, Light RB, Bose D, Chau T, Correa E, Mink S: Hemofiltration reverses left ventricular dysfunction during sepsis in dogs. ANESTHESIOLOGY 1990; 73:671–85

23. Freeman BD, Yatsiv I, Natanson C, Solomon MA, Quezado ZM, Danner RL, Banks SM, Hoffman WD: Continuous arteriovenous hemofiltration does not improve survival in a canine model of septic shock. J Am Coll Surg 1995; 180:286–92

24. Dreyfuss D, Saumon G: Role of tidal volume, FRC, and end-inspiratory volume in the development of pulmonary edema following mechanical ventilation. Am Rev Respir Dis 1993; 148:1194–203

25. Journois D, Israel-Biet D, Pouard P, Rolland B, Silvester W, Vouhe P, Safran D: High-volume, zero-balanced hemofiltration to reduce delayed inflammatory response to cardiopulmonary bypass in children. ANESTHESIOLOGY 1996; 85:965–76