Published in: Artificial Organs (2006), vol. 30, iss.7, pp. 560-564.
Status: Postprint (Author's version)

Large-Pore Membrane Hemofiltration Increases Cytokine Clearance and Improves Right Ventricular Vascular Coupling During Endotoxic Shock in Pigs[1]

*†Bernard Lambermont, ‡Pierre Delanaye, †§Jean-Michel Dogné, *†Alexandre Ghuysen, ¶Nathalie Janssen, ‡Bernard Dubois, †Thomas Desaive, †¶Philippe Kolh, *†Vincent D'Orio, and ‡Jean-Marie Krzesinski

**Department of Medicine, University of Liège; †Hemodynamic Research Laboratory, HemoLiège, University of Liège; ‡Department of Nephrology, University of Liège; §Department of Pharmacy, University of Namur, Namur; ¶Department of Surgery, University of Liège, Liège, Belgium*

Abstract

Hemodynamic improvement in patients suffering from both septic shock and renal failure who received hemofiltration suggested that an extrarenal epuration technique could be of interest in patients with septic shock alone. However, most of the studies using continuous veno-venous hemofiltration (CVVH) in this setting evidenced neither cytokine clearance nor significant reduction in their plasma level. Lack of significant clearance was explained in part by the small size of the membrane pores. Therefore, we investigated the effects of large-pore membrane hemofiltration (LPHF) during endotoxic shock in pigs on interleukin 6 (IL-6) and interleukin 10 (IL-10) clearances, and on right ventricular (RV)-vascular coupling. Thirteen anesthetized healthy pigs weighing 20-30 kg were divided into two groups. In the Endo group (n = 6), the pigs received a 0.5-mg/kg endotoxin infusion over a period of 30 mins from TO to T30. In the EndoHF group (n = 7), LPHF (cutoff = 80 kDa) and an ultrafiltration rate of 45 mL/kg/h were started 30 mins after the end of the endotoxin infusion, from T60 to T240. In this model of porcine endotoxic shock, LPHF was responsible for a significant clearance of IL-6 (20 mL/min) and Il-10 (14 mL/min), and for an improvement in RV-vascular coupling.

Keywords: cytokine ; endotoxin ; hemofiltration ; pulmonary circulation ; septic shock.

Hemodynamic improvement in patients suffering from both septic shock and renal failure who received hemofiltration suggested that an extrarenal epuration technique could be of interest in patients with septic shock alone (1). The rationale for such interest derives from knowledge that part of the systemic effects of sepsis may be mediated by endogenous, water-soluble, small-(<0.5 kDa) and medium-sized (>0.5 kDa but <60 kDa) molecules and that their removal from the bloodstream may be of significant benefit.

However, most of the studies using CVVH in this setting, even with a high ultrafiltration rate, evidenced neither cytokine clearance nor significant reduction in their plasmatic level. In best cases, clearance was limited and transient (2-4). Lack of significant clearance was explained in part by the small size of membrane pores. Indeed, the membrane cutoff in most of these studies was around 30 kDa, while molecular weight of the trimeric biologically active form of TNFα is more than 50 kDa, for example.

Large-pore membrane hemofiltration (LPHF) leads (in ex vivo studies) to a cytokine clearance greater than in classic membranes. Membranes with a cutoff of 60 kDa, for example, resulted in a significant clearance of interleukin 6 (IL-6), interleukin 8, and interleukin 1 (5).

According to this better cytokine clearance opportunity, we designed this study to investigate in vivo the effects of LPHF with an 80 kDa cutoff membrane on IL-6 and interleukin 10 (IL-10) clearances, and on right ventricular (RV)-vascular coupling.

Materials and methods

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

The experiments were performed on 13 healthy pure pietran pigs of either sex weighing 20-30 kg. The animals were premedicated and anesthetized as described elsewhere (6). Systemic arterial pressure, pulmonary arterial pressure (PAP), and flow and (RV) pressure-volume loops were recorded and analyzed as described previously

[1] Presented in part at the 34th Congress of the Société de Réanimation de Langue Française held January 18-20, 2006, in Paris, France.

(6). To assess RV-vascular coupling, we examined the Ees/Ea ratio. Ees represents the end-systolic RV elastance and Ea represents the pulmonary arterial elastance.

In both groups, the animals received a 0.5-mg/kg endotoxin infusion (lipopolysaccharide from *Escherichia coli* serotype 0127:B8; Sigma Chemical, St. Louis, MO, U.S.A.) over 30 mins (from T0 to T30). They were then randomized into two groups. In the Endo group ($n = 6$), they received no further intervention while in the EndoHF group ($n = 7$), they underwent, from T60 onward, a zero-balance CWH at a rate of 45 mL/kg/h (7). A 0.7-m^2 large-pore (78 Å) membrane with a cutoff of 80 kDa (Sureflux FH 70, Nipro, Osaka, Japan) and a Baxter BM 25-BM 14 hemofiltration device (Baxter Health Care, Munich, Germany) were used. Ultrafiltrate was replaced in the postdilution mode by a bicarbonate-buffered hemofiltration fluid (Na^+: 150 mM; K^+: 3 mM; bicarbonate: 30 mM) at a temperature of 37°C. Anticoagulation of the extracorporeal circuit was achieved using a loading dose of 5000 IU of heparin followed by an anticoagulation regimen based on the activated clotting time (100-200 s). Rectal temperature was monitored every 30 min.

Blood (before and after the membrane) and ultrafiltrate samples were taken at T0, T60, T120, and T240 to measure IL-6 (28 kDa) and IL-10 (19 kDa) concentrations using an ELISA test (Biosource, Paris, France). Clearance and sieving coefficients were calculated at T120 and T240 using the following formula:

$$\text{Sieving coefficient} = 2.\ [UF]/([A] + [V])$$

$$\text{Clearance (mL/min)} = \text{Sieving coefficient.} \frac{\text{Ultrafiltration rate (mL/min)}}{}$$

where [UF] is the concentration in ultrafiltrate, [A] is the concentration in inlet supernatant, and [V] is the concentration in outlet supernatant.

Plasma albumin concentration was measured at T60 and T240.

Statistical analysis

Data were presented as mean ± standard error of the mean. A two-way analysis of variance for repeated measurements before (from T0 to T60) and after (from T90 to T240) the start of LPHF gave *P* values for time and group effects (Statistica, Statsoft, Inc., Tulsa, OK, U.S.A.). $P < 0.05$ was considered statistically significant.

Results

The mean systemic arterial pressure, heart rate, and cardiac output did not differ between Endo and EndoHF groups from T0 to T240. The mean PAP reached its first maximum at T30 in the Endo and EndoHF groups. After the start of LPHF (from T90 to T240), the mean PAP in the EndoHF group remained lower than the mean PAP in the Endo group ($P < 0.05$) (Fig. 1).

After the start of LPHF (from T90 to T240) pulmonary Ea values in the EndoHF group remained lower than Endo group values ($P < 0.05$), while the time evolution of Ees remained similar in the two groups (Fig. 2). Consequently, the Ees/Ea ratio decreased slightly from TO to T30 in both groups. From T90 to T240, the Ees/Ea ratio in the EndoHF group remained greater than 2, while in the Endo group, the Ees/Ea ratio decreased progressively (Fig. 2). This difference between the two groups was significant ($P < 0.05$).

No significant differences in rectal temperature occurred between groups from T0 to T240.

At T120 and T240, the IL-6 clearance was 22 ± 7 and 15 ± 3 mL/min, respectively. The IL-6 sieving coefficient was 0.84. At T120 and T240, the IL-10 clearance was 14 ± 4 and 10 ± 7 mL/min, respectively. The sieving coefficient was 0.55. At T60 and T240, the plasmatic albumin concentration was 24 ± 4 and 23 ± 4 g/L, respectively ($P = 0.13$).

Discussion

The major findings of our study are that LPHF is responsible for a significant clearance of IL-6 and IL-10, and prevents the RV-vascular uncoupling that habitually occurs during the late phase of endotoxic shock. The difference between the two groups during the second phase of endotoxic shock resulted from a difference in pulmonary vascular resistance as all the other factors contributing to the Ea value remained similar.

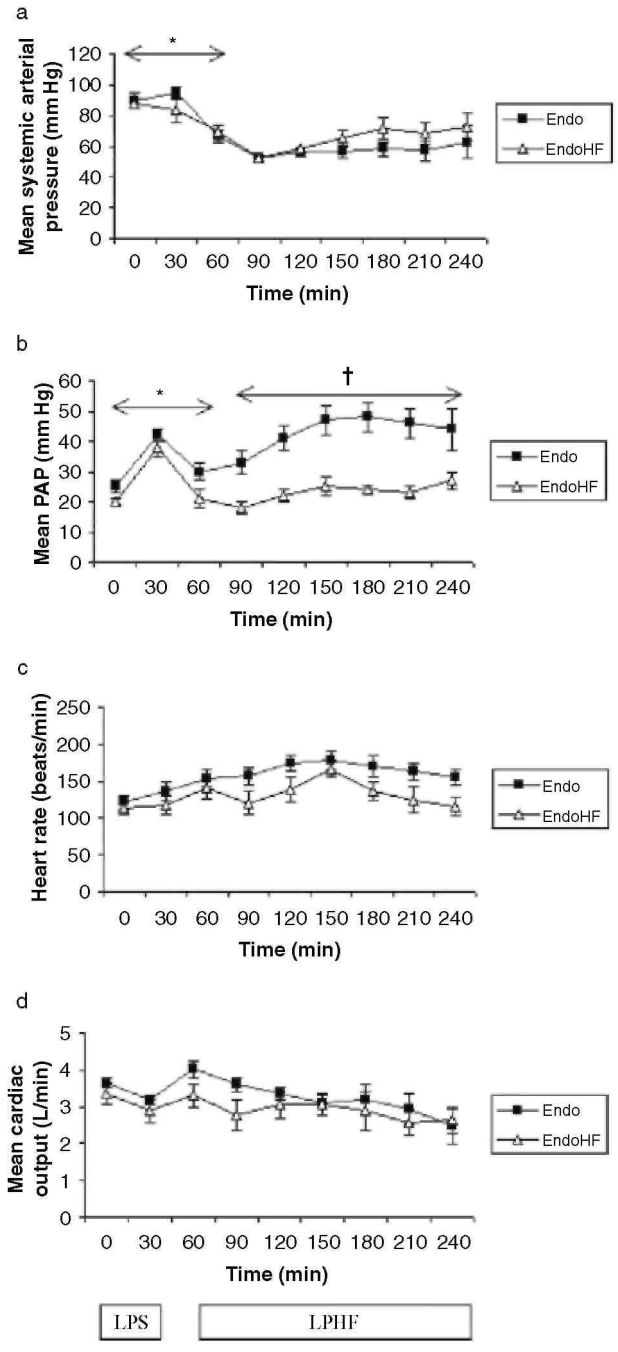
The effects of LPHF on RV-vascular coupling were never tested previously. At the best, Grooten-dorst et al. tested the effects of CWH with an ultra-filtration rate of 6 L/h on RV ejection fraction in pigs with endotoxin-induced shock. In their model, the authors concluded that high-volume hemofiltration improved RV ejection fraction and cardiac performance by the removal of vasoactive mediators responsible for myocardial depression (8). LPHF did not improve the mean systemic arterial pressure and cardiac output, which remained similar in both groups. These results are in accordance with those of Stein et al. (9), but they are in opposition with those of Grootendorst, who showed an increase in the mean arterial pressure and cardiac output in endotoxic pigs

Published in: Artificial Organs (2006), vol. 30, iss.7, pp. 560-564.
Status: Postprint (Author's version)

receiving continuous high-volume hemofiltration (8,10). Lee et al. found no significant increase in the mean arterial pressure in endotoxic pigs receiving LPHF (100 kDa pore size membrane), although it increased survival rate (11).

Clearance values and sieving coefficients obtained in the present study are largely superior to those obtained with standard hemofiltration membranes. Heering et al. measured an IL-6 sieving coefficient of 0.04, and IL-10 was undetectable in the ultrafiltrate (4). In the study of Kellum et al., the IL-10 concentration in ultrafiltrate was very low and the IL-6 clearance was 3.3 mL/min (3).

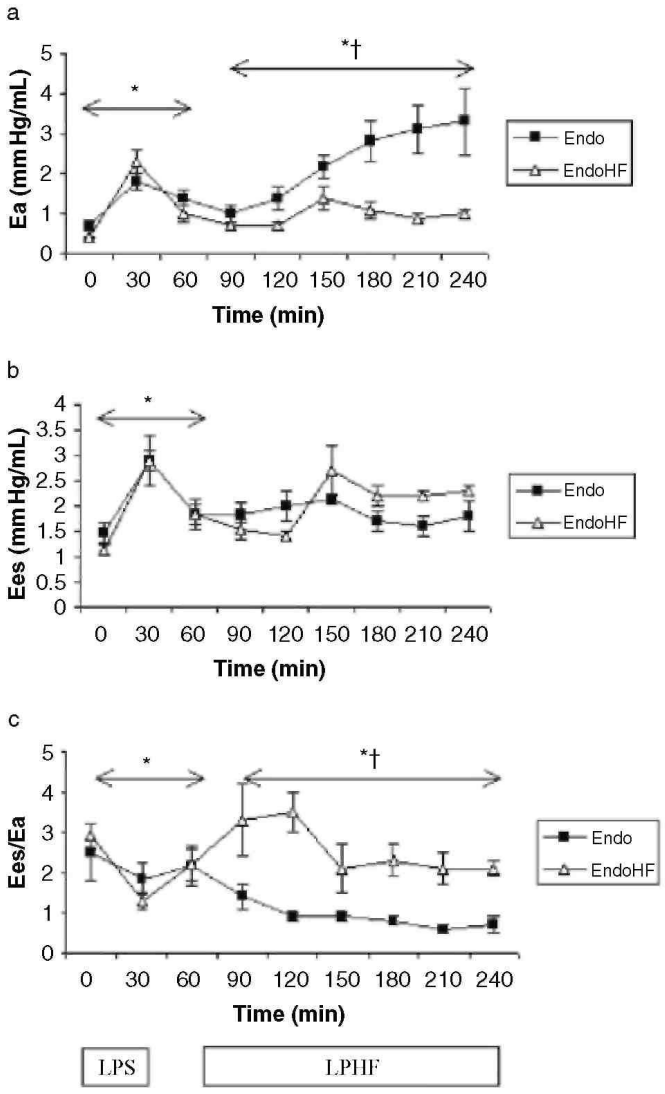
FIG. 1. *Evolution of (a) mean systemic arterial pressure, (b) mean PAP, (c) heart rate, and (d) mean cardiac output during endotoxic shock in Endo and EndoHF groups. The Endo group received an endotoxin infusion (LPS) over 30 min from T0 to T30. The EndoHF group received an endotoxin infusion over 30 min from T0 to T30 and an LPHF from T60 to T240. *P < 0.05 for time effect; †P < 0.05 for group effect. Data are presented as mean ± SEM.*



Published in: Artificial Organs (2006), vol. 30, iss.7, pp. 560-564.
Status: Postprint (Author's version)

Cytokines are considered to be important mediators in the pathophysiology of septic shock, and it was suggested that the nonspecific and continuous removal of pro- and anti-inflammatory soluble mediators may be the most logical and adequate approach to a complex and long-running process such as sepsis (12). Several studies demonstrated significant removal rates of inflammatory cytokines from the circulation of septic patients by CWH (4,13,14). As one of the mechanisms of cytokine removal is convective clearance (3), increasing convective clearance should increase blood purification effectiveness. One way to achieve this goal is the use of larger-pore membranes (15). In vitro, large-pore membranes can lead to cytokine clearance comparable to that currently achieved for urea during standard continuous renal replacement therapy (5). In vivo, Morgera et al. found that a 60-kDa cutoff membrane can lead to IL-6 clearance similar to that of our study (16). In the present work, an 80-kDa cutoff membrane leads to significant cytokine clearances for IL6 and IL-10, without significant protein loss as demonstrated by albumin concentration values at T60 and T240.

FIG. 2. *Evolution of (a) Ea, (b) Ees, and (c) Ees/Ea during endotoxic shock in Endo and EndoHF groups. The Endo group received an endotoxin infusion (LPS) over 30 min from T0 to T30. The EndoHF group received an endotoxin infusion over 30 min from T0 to T30 and an LPHF from T60 to T240. *P < 0.05 for time effect, †P < 0.05 for group effect. Data are presented as mean ± SEM*



Conclusion

The use of LPHF in this model of porcine endotoxic shock was responsible for a significant clearance of IL-6 and IL-10, and an improvement in RV-vascular coupling by preventing increase in pulmonary Ea.

Published in: Artificial Organs (2006), vol. 30, iss.7, pp. 560-564.
Status: Postprint (Author's version)

Acknowledgments

The authors would like to thank Philippe Devel, Michèle Focan, and Marie-Antoinette Graceffa for their technical assistance. This work is supported by a grant from the Nipro Europe, Zaventem, Belgium. Philippe Kolh is a recipient of a postdoctoral grant from the Fonds National de la Recherche Scientifique.

References

1. 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.

2. De Vriese AS, Vanholder RC, Pascual M, Lameire NH, Colardyn FA. Can inflammatory cytokines be removed efficiently by continuous renal replacement therapies? *Intensive Care Med* 1999;25:903-10.

3. Kellum JA, Johnson JP, Kramer D, Palevsky P, Brady JJ, Pinsky MR. Diffusive versus convective therapy: effects on mediators of inflammation in patient with severe systemic inflammatory response syndrome. *Crit Care Med* 1998;26: 1995-2000.

4. Heering P, Morgera S, Schmitz FJ, et al. Cytokine removal and cardiovascular hemodynamics in septic patients with continuous venovenous hemofiltration. *Intensive Care Med* 1997;23:288-96.

5. Uchino S, Bellomo R, Goldsmith D, et al. Super high flux hemofiltration: a new technique for cytokine removal. *Intensive Care Med* 2002;28:651-5.

6. Lambermont B, Ghuysen A, Kolh P, et al. Effects of endotoxic shock on right ventricular systolic function and mechanical efficiency. *Cardiovasc Res* 2003;59:412-8.

7. Ronco C, Bellomo R, Homel P, et al. Effects of different doses in continuous veno-venous haemofiltration on outcomes of acute renal failure: a prospective randomised trial. *Lancet* 2000;356:26-30.

8. Grootendorst AF, van Bommel EFHB, 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.

9. Stein B, Pfenninger E, Grunert A, Schmitz JE, Hudde M. Influence of continuous haemofiltration on haemodynamics and central blood volume in experimental endotoxic shock. *Intensive Care Med* 1990;16:494-9.

10. Grootendorst AF, van Bommel EF, van Leengoed LA, Nabuurs M, Bouman CS, Groeneveld AB. High volume hemofiltration improves hemodynamics and survival of pigs exposed to gut ischemia and reperfusion. *Shock* 1994;2:72-8.

11. Lee PA, Weger GW, Pryor RW, Matson JR. Effects of filter pore size on efficacy of continuous arteriovenous hemofiltration therapy for *Staphylococcus aureus-in*duced septicemia in immature swine. *Crit Care Med* 1998;26:730-7.

12. Ratanarat R, Brendolan A, Piccinni P, et al. Pulse high-volume haemofiltration for treatment of severe sepsis: effects on hemodynamics and survival. *Crit Care* 2005;9:R294-R302.

13. Bellomo R, Tipping P, Boyce N. Continuous veno-venous hemofiltration with dialysis removes cytokines from the circulation of septic patients. *Crit Care Med* 1993;21:522-6.

14. Sander A, Armbruster W, Sander B, Daul AE, Lange R, Peters J. Hemofiltration increases IL-6 clearance in early systemic inflammatory response syndrome but does not alter IL-6 and TNF alpha plasma concentrations. *Intensive Care Med* 1997;23:878-84.

15. van Deuren M, van der Meer JW. Hemofiltration in septic patients is not able to alter the plasma concentration of cytokines therapeutically. *Intensive Care Med* 2000;26:1176-8.

16. Morgera S, Slowinski T, Melzer C, et al. Renal replacement therapy with high-cutoff hemofilters: impact of convection and diffusion on cytokine clearances and protein status. *Am I Kidney Dis* 2004;43:444-53.