Therapeutic Apheresis and Dialysis

Therapeutic Apheresis and Dialysis 15(1):98–104
doi: 10.1111/j.1744-9987.2010.00850.x

Efficacy of Cytokine Removal by Plasmadiafiltration Using a Selective Plasma Separator: In Vitro Sepsis Model

Mingxin Li, Jun Xue, Junfeng Liu, Dingwei Kuang, Yong Gu, and Shanyan Lin

Department of Nephrology, Huashan Hospital, Fudan University, Shanghai, China

Abstract: More effective removal of pro- and anti-inflammatory cytokines may play an important role in the treatment of sepsis. Plasmadiafiltration (PDF) with a larger selective plasma separator was performed to study the cytokine and plasma protein permeability profiles of the membrane in an in vitro sepsis model. The in vitro sepsis model was constructed by exposure of human whole blood to bacterial lipopolysaccharide. EVACURE 2A, a selective plasma separator, was placed in the blood circuit of PDF. Sieving coefficients of cytokines and plasma protein were tested in post-dilution PDF mode at the following operating parameters: blood flow rate 150 mL/min; dialysate flow rate 33.33 mL/min; replacing fluid flow rate 6.67 mL/min; ultrafiltration rate 5 mL/min. An enzyme linked immunoadsorbent assay was used to measure the concentrations of tumor necrosis factor-α (TNF-α), high-mobility group box 1 protein (HMGB1), interleukin-1β (IL-1β), interleukin-1 receptor antagonist (IL-1ra), interleukin-2 (IL-2), interleukin-2 receptor (IL-2r), interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-10 (IL-10) in plasma and ultrafiltrate. Sieving coefficients of different solutes ranged from 0.1 to 1.0 at first, decreased 10%–60% after 1 h of PDF, and then remained stable. Total clearance rates of cytokines ranged from 15 to 80 mL/min. The concentrations of cytokines decreased 20–80% after 1 hour of PDF. The sieving coefficient of albumin was 0.1 at first and then decreased to 0.05 after 1 hour of therapy. Plasmadiafiltration with Evacure 2A plasma separator can effectively remove almost all of the inflammatory mediators with low albumin loss. **Key Words:** Cytokine, Inflammation, Plasmadiafiltration, Sepsis, Sieving coefficient.

Inflammatory mediators spilling into the circulation from local sites of inflammation can cause remote tissue injury and organ dysfunction in sepsis. It is widely accepted today that cytokines play pivotal roles in the course of sepsis (1–3). Both pro-inflammatory [tumor necrosis factor (TNF)-α, interleukin (IL)-1, IL-6] and anti-inflammatory (IL-10, IL-1ra) cytokines are over-expressed even in the early phase of lethal sepsis. Modulation of the inflammatory response has been considered an important way to improve survival in sepsis. However, anti-inflammatory interventions have failed to show any success in phase III clinical trials in the face of the intense activation of the inflammatory mediator network in sepsis despite promising results of anti-inflammatory interventions in animal models (4,5).

Received January 2010; revised May 2010.
Address correspondence and reprint requests to Dr Jun Xue, Associate Professor, No.12 Wulumuqi Road (middle), Shanghai, 200040, China. Email: xuejunxuejun@yahoo.com.cn

Blood purification techniques are applied to treat severe sepsis and septic shock by removing excess humoral mediators and maintaining homeostasis. Blood purification, which can remove cytokines by sieving and or by adsorption, began to show its clinical efficacy in the treatment of septic shock while the cytokine-removing effects of blood purification are still controversial (6–8). The pores of the filtration membrane should be large enough to meet the clinical needs of sufficient removal of inflammatory mediators. But, filtration with a larger pore filter may attain higher clearances of cytokines at the expense of a higher loss of albumin and plasma proteins (9–11).

A newly designed high-cut-off EVAL membrane (Kuraray Medical Inc., Tokyo, Japan) with in vitro albumin sieving coefficient of 0.2–0.3 which was tested by the 10–100 mL/min blood flow, 10–100 mL/min dialysate flow and 0 mL/min ultrafiltration rate (provided by Kuraray Medical), should filter sufficiently a wide range of inflammatory mediators, and

also the membrane requires additional plasma to compensate the albumin loss. This has been used successfully to treat acute liver dysfunction (12,13). At the first step, we performed plasmadiafiltration (PDF) in an in vitro sepsis model to test whether the membrane could provide an acceptable balance between high cytokine and low albumin clearances, meanwhile defining a suitable treatment index for further in vivo sepsis models.

MATERIALS AND METHODS

In vitro sepsis model

Human whole blood was spiked with bacterial lipopolysaccharide (LPS) to establish an in vitro sepsis model (14). Four hundred milliliters of fresh blood, stored in sterile blood standard acid citrate dextrose (ACD)-containing collection bags, were heparinized at a concentration of 62.5 IU/mL (5000 IU heparin, Shanghai No.1 Biochemical Pharmacy, Shanghai, China), and recalcified (calcium chloride 10%, 44 μL/mL blood) and diluted with 400 mL isotonic saline solution (Baxter, Guangzhou, China). One milligram of LPS (from *Pseudomonas aeruginosa* serotype 0; Shanghai Qianchen Biotechnology Company, Shanghai, China) and 40 grams of creatinine (Shanghai Yixin Biotechnology Company, Shanghai, China) were added to the blood. After that, the blood was incubated for 4 h in a 37°C water bath and then overnight at room temperature.

In vitro PDF

Plasmadiafiltration was performed with a selective plasma separator EVACURE EC-2A made of EVAL hollow fibers (inside diameter 175 μm, wall thickness 40 μm, effective surface area 1.0 m^2, volume 80 mL, highest TMP 250 mm Hg, sterilized by γ radiation ray, made by Kuraray Medical), connected to a flow-controlled blood roller pump (BSM22-VPM, Kuraray Medical). The in vitro albumin sieving coefficient of EVACURE EC-2A is 0.2–0.3.

The post-dilution PDF circuit was a closed loop with four sampling sites: arterial site (Ci), before the plasma separator; venous site 1 (Co1), after the plasma separator and before the replacing pump; venous site 2 (Co2), after the replacing pump; and ultrafiltrate site (Cuf), after the ultrafiltration pump (see Fig. 1).

Blood flow rate was maintained at 150 mL/min while the following three parameters were combined. Replacing fluid consisted of isotonic saline solution and plasma at the ratio of 2:1 and was infused in a post-dilution mode at the desired rate of 6.7 mL/min (400 mL/h). Dialysate flow rate was set at 33.3 mL/min (2000 mL/h) and net ultrafiltration rate 5 mL/min (300 mL/h) (which was only turned on at 10, 60 and 120 min, respectively, and was kept stable for 10 min for the taking of samples.). The circuit was anticoagulated with a bolus heparin infusion (1250 IU) in the afferent limb.

Samples were collected in tubes containing ethylenediaminetetraacetate and transported immediately on ice to the laboratory, where they were centrifuged at 3000 rpm for 5 min and the supernatants removed, frozen, and stored at –70°C until assay.

Laboratory assays

Levels of TNF-α, IL-1β, IL-1 ra, IL-2, sIL-2R, IL-6, IL-8, IL-10, and high-mobility group box 1 protein (HMGB1) were measured by an enzyme-linked immunoadsorbent assay (ELISA, Shanghai West-Tang Bio-tech, Shanghai, China) according to the manufacturer's specifications. All samples were tested twice. The lowest detectable value of cytokines in the sample were TNF-α 9.0 pg/mL, IL-1β 5.0 pg/mL, IL-1ra 10 pg/mL, IL-2 15 pg/mL, sIL-2R 15 pg/mL, IL-6 3.0 pg/mL, IL-8 14 pg/mL, IL-10 15 pg/mL, HMGB1 3 ng/mL. Calibration curves were constructed for plasma and ultrafiltrate samples, respectively. The intra- and inter-assay variation of the assays were <6% for all tested cytokines.

Creatinine and albumin were measured in plasma and ultrafiltrate by automatic biochemical detector (Urit-8000, Baiwei Science and Technology, Jiangsu, China). The intra- and inter-assay coefficients of variation were 3.0%.

Parameter calculations

The following formula was used to calculate the sieving coefficient (SC) of each cytokine (14):

$$SC = 2Cuf/(Ci + Co1)$$

where Ci is the concentration in the inlet plasma (pg/mL), Co1 is the concentration in outlet plasma (pg/mL) before infusion of replacing fluid, and Cuf is the concentration in ultrafiltrate (pg/mL).

Total clearance rate (TCR) was calculated by the following formula,

$$TCR = (Ci - Co2) \times 150/Ci$$

where Ci is the concentration in the inlet plasma, Co2 is the concentration in outlet plasma (pg/mL) after infusion of replacing fluid, and 150 mL/min is flow rate of blood.

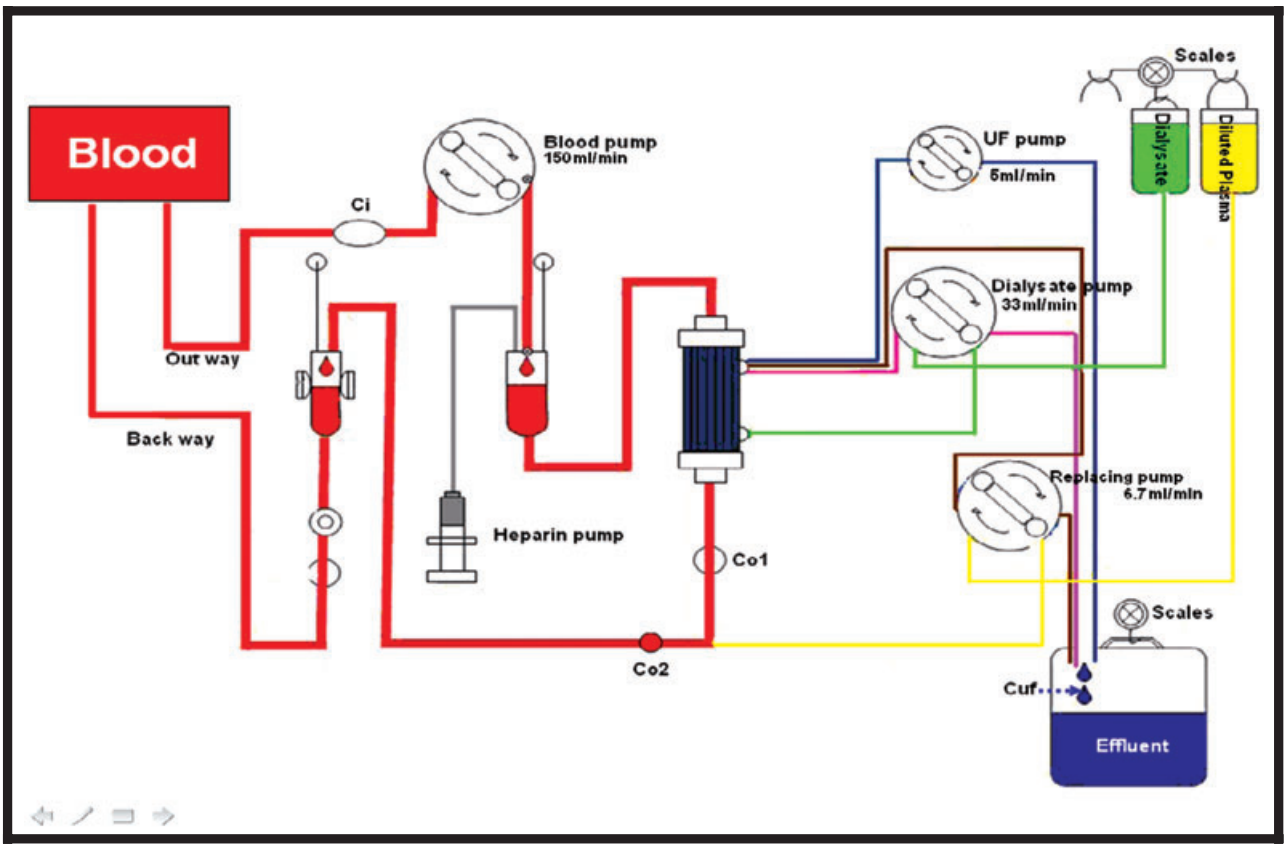

FIG. 1. Schematic diagram of plasmadiafiltration (PDF) circuit.

Clearance rate due to convection and dialysis was calculated by the following formula,

$$CR = Cuf \times F/Ci$$

where CR represents clearance rate due to convection and dialysis, Cuf is the concentration in ultrafiltrate, F is the flow rate of dialysate and replacing fluid, Ci is the concentration in the inlet plasma.

Statistical analysis

The data for each combination of operating parameters and each time point were expressed as mean ± SD. For the evaluation of the operating parameters, one-way repeated measures ANOVA and the Student–Newman–Keuls test for pairwise multiple comparisons were performed. For significance analysis of a variable over time, one-way ANOVA for repeated measures was used. *P*-values of <0.05 were considered statistically significant. All statistical analysis was performed using the SPSS statistical software for windows 12.0 (SPSS Inc, Chicago, IL, USA).

RESULTS

In vitro sepsis model characteristics

The plasma concentrations of cytokines before and after the blood was exposed to LPS are shown in Table 1. After exposure to LPS, each of the cytokines in the blood increased significantly ($P < 0.05$) compared to the baseline.

Timely changed concentrations of different solutes during the course of PDF

As PDF was going on, all serum levels of the tested cytokines decreased significantly. This was also the case for serum creatinine. After 60 and 120 min PDF, all concentrations of these mentioned solutes decreased by at least 50%, whereas serum creatinine decreased by 90%. The serum level of albumin, however, increased (Table 2, Fig. 2).

Sieving coefficient

The sieving coefficient decreased significantly after one hour of PDF, and then remained stable during

TABLE 1. *Characteristics of* in vitro *model of sepsis*

	Baseline	After exposure to LPS	*P*-value
TNF-α (pg/mL)	21.5 ± 0.7	84.9 ± 0.9	0.012
HMGB1 (pg/mL)	3.4 ± 0.1	15.6 ± 0.4	0.008
IL-1β (pg/mL)	9.8 ± 0.5	77.7 ± 0.4	0.006
IL-1ra (pg/mL)	23.0 ± 1.4	1430.6 ± 24.6	0.007
IL-2 (pg/mL)	16.9 ± 0.1	129.5 ± 1.3	0.005
IL-2r (pg/mL)	19.0 ± 0.1	283.6 ± 6.2	0.010
IL-6 (pg/mL)	16.2 ± 0.4	80.6 ± 1.4	0.008
IL-8 (pg/mL)	26.0 ± 1.4	778.7 ± 13.1	0.007
IL-10 (pg/mL)	16.1 ± 0.9	44.2 ± 1.5	0.039
SCr (μmol/L)	451.0 ± 22.630	455.5 ± 20.510	0.726
Alb (g/L)	20.321 ± 0.563	19.874 ± 0.438	0.572

TNF-α, tumor necrosis factor-α (monomer 17 kD, trimer 54 kD); HMGB1, high-mobility group box 1 protein (30 kD); IL-1β, interleukin-1β (17 kD); IL-1ra, interleukin-1 receptor antagonist (17-22 kD); IL-2, interleukin-2 (15 kD); IL-2r, interleukin-2 receptor (75 kD); IL-6, interleukin-6 (26 kD); IL-8, interleukin-8 (8 kD); IL-10, interleukin-10 (39 kD); SCr, serum creatinine (113 D); Alb, albumin (67 kD), respectively. LPS, lipopolysaccharide.

the next 60 min. The reduction rate of sieving coefficient after one hour of PDF ranged from 10% to 60% (Fig. 3).

Clearances in PDF

Although all total clearance rates decreased after a 2 h PDF treatment, they did not reach a statistically significant difference. This was also the case for most clearance rates by convection and diffusion (Table 3).

TABLE 2. *The decline rate of different solutes during the course of plasmadiafiltration (PDF)*

	After 60 min PDF(%)*	After 120 min PDF(%)*
TNF-α	61.87 ± 16.79	73.73 ± 11.80
HMGB1	50.00 ± 0.00	73.57 ± 1.37
IL-1β	50.00 ± 0.00	86.42 ± 1.08
IL-ra	75.12 ± 0.96	88.14 ± 0.16
IL-2	55.62 ± 3.39	77.61 ± 0.11
IL-2r	74.99 ± 2.44	80.20 ± 0.14
IL-6	54.64 ± 6.96	91.20 ± 0.10
IL-8	54.24 ± 1.86	77.44 ± 0.32
IL-10	51.95 ± 2.68	62.03 ± 0.05
SCr	91.54 ± 5.40	99.34 ± 0.03
Alb	–7.99 ± –2.47	–19.26 ± –6.84

*The rate was calculated as compared to the baseline. Alb, albumin; IL, interleukin; HMGB1, high-mobility group box 1 protein; SCr, serum creatinine; TNF, tumor necrosis factor.

DISCUSSION

Sepsis refers to a systemic inflammatory response syndrome resulting from a microbial infection. Despite recent advances in antibiotic therapy and intensive care, sepsis is still the most common cause of death in intensive care units. The prevailing theories of sepsis is an uncontrolled inflammatory response, as manifested by excessive release of inflammatory mediators such as TNF, IL-1β, IL-2, IL-6 (15–20) and HMGB1. Not only that, but increased levels of anti-inflammatory cytokines, such

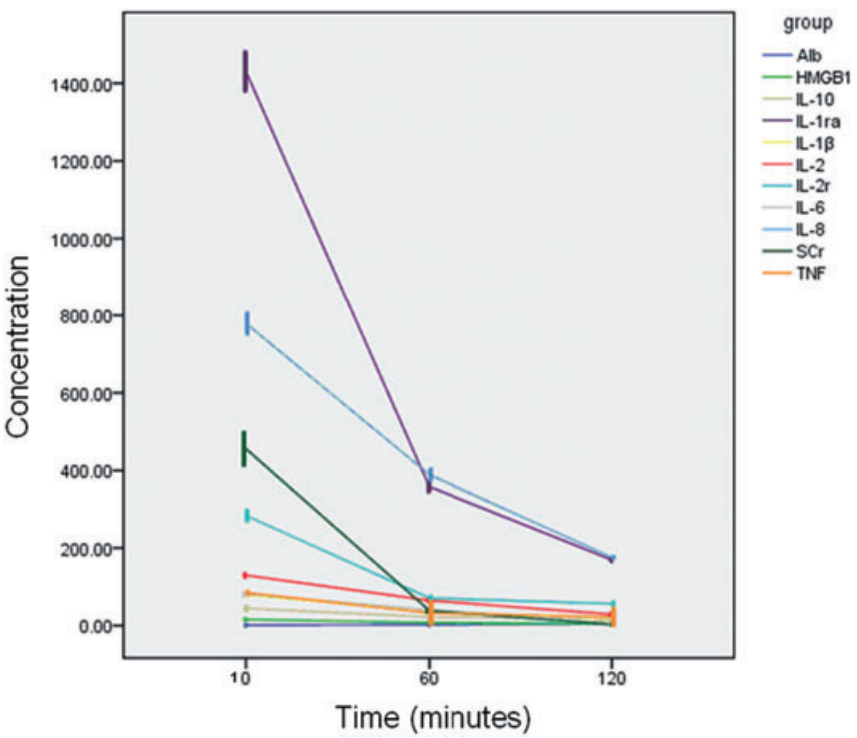

FIG. 2. Time changed concentrations of different solutes. Their concentrations at 10, 60, 120 min after PDF were as follows, respectively: TNF-α (84.93 ± 0.96, 32.46 ± 14.63, 22.37 ± 10.28 pg/mL), HMGB1 (15.62 ± 0.36, 7.81 ± 0.18, 4.13 ± 0.21 pg/mL), IL-1β(77.70 ± 0.42, 38.85 ± 0.21, 10.55 ± 0.78 pg/mL), IL-1ra(1430.60 ± 24.61, 357.65 ± 6.15, 169.60 ± 0.57 pg/mL), IL-2 (129.50 ± 1.28,64.75 ± 0.64, 29.10 ± 0.42 pg/mL), IL-2r(283.60 ± 6.22, 70.90 ± 1.56, 56.15 ± 1.63 pg/mL), IL-6(80.58 ± 1.45, 40.29 ± 0.72, 7.09 ± 0.21 pg/mL), IL-8(778.74 ± 13.10, 389.37 ± 6.55, 175.70 ± 0.44 pg/mL), IL-10(44.24 ± 1.47, 22.11 ± 0.75, 16.80 ± 0.54 pg/mL), SCr(455.50 ± 20.51, 38.50 ± 0.71, 3.00 ± 0 μmol/L), albumin(20.15 ± 1.20, 21.75 ± 0.49, 24.00 ± 0.28 g/L).

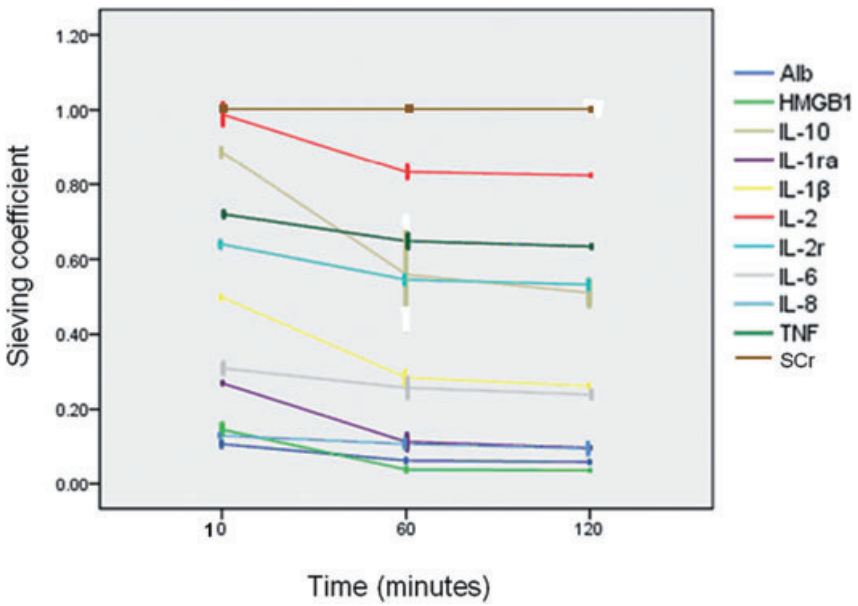

FIG. 3. Time changed sieving coefficient.

as IL-8 (21) and IL-10 (22) are found in the circulation of septic patients. The early kinetics of systemic TNF accumulation in sepsis makes it a difficult therapeutic target in clinical settings, prompting a search for other, late pro-inflammatory mediators, such as HMGB1, that may offer a wider therapeutic window. Accumulating evidence indicates that HMGB1 can stimulate migration of cells, facilitate recognition of bacterial products, activate innate immune cells, and inhibit phagocytotic elimination of apoptotic neutrophils (23). These mediators have therefore received attention as potential targets in the treatment of sepsis (24–26). Blockade of only one mediator, however, may be insufficient to down-regulate the inflammatory response, since clinical trials with monoclonal antibodies have failed to show a survival benefit (27). Blood purification, which can remove excess proinflammatory and anti-inflammatory mediators by sieving and or by adsorption, is being applied to treat severe sepsis and is beginning to show its clinical efficacy (28–32).

Although blood purification strategies for unselective cytokine removal were suggested, it has never been demonstrated in experimental models or in the clinical setting that plasma cytokine reduction per se leads to survival benefit. One explanation would be that the cut-off point of the membrane was not large enough to allow the entire pathogenic solutes pass across the membrane by means of convection. On the other hand, the balance between cytokine clearances and albumin loss during the application of high-cut-off membranes should be taken into consideration. Plasmadiafiltration with the EVACURE-2A had been successfully treated on severe liver dysfunction.

TABLE 3. *Time changed clearance rates*

	Total clearance rate (mL/min)			Clearance by convection and diffusion (mL/min)		
	10 min	120 min	*P* value	10 min	120 min	*P*-value
TNF-α	65.54 ± 5.81	55.60 ± 6.53	0.779	10.365 ± 1.53	37.31 ± 5.18	0.779
HMGB1	23.46 ± 1.10	11.46 ± 2.45	0.425	10.37 ± 2.95	7.69 ± 1.24	0.425
IL-1β	21.56 ± 4.40	18.45 ± 2.38	0.968	19.65 ± 1.25	17.66 ± 2.50	0.968
IL-1ra	14.77 ± 1.46	8.87 ± 1.26	0.798	13.43 ± 2.75	7.58 ± 1.46	0.798
IL-2	75.47 ± 6.81	66.54 ± 8.20	0.780	22.60 ± 2.53	21.00 ± 3.01	0.780
IL-2r	42.71 ± 2.94	29.61 ± 3.86	0.487	40.46 ± 6.72	26.65 ± 6.92	0.487
IL-6	79.55 ± 12.25	59.53 ± 5.28	0.155	50.64 ± 3.01	28.73 ± 4.33	0.155
IL-8	18.31 ± 1.48	5.70 ± 2.84	0.215	10.72 ± 2.46	4.49 ± 1.61	0.215
IL-10	66.77 ± 8.55	56.75 ± 8.65	0.073	60.51 ± 6.97	37.20 ± 4.05	0.073
Scr	127.50 ± 6.36	117.50 ± 10.61	0.186	124.50 ± 6.36	117.50 ± 12.02	0.330
Alb	2.02 ± 1.39	0.28 ± 0.15	0.045	1.88 ± 0.59	0.26 ± 0.11	0.343

Alb, albumin; IL, interleukin; HMGB1, high-mobility group box 1 protein; SCr, serum creatinine; TNF, tumor necrosis factor.

So, in this study, we chose a selective plasma separator EVACURE-2A as a hemofilter to test whether it could ensure high clearances for the different cytokines, with an acceptable loss of albumin.

Our data showed that the concentration of proinflammatory and anti-inflammatory mediators reduced about 60%–70% after one hour of PDF. There were two phenomenon observed: firstly, the ratio between the clearance of diafiltration and the total clearance was around 60%–70%; secondly, the sieving coefficient decreased significantly after one hour's PDF and remained stable thereafter. The first point implicates convection and diffusion as being predominant in deleting cytokines compared to absorption. The second phenomena may be explained by the sieving coefficient lowering as the filter absorbed more and more substance that would reduce the pore size and block some pores. As adsorption was saturated, usually in no more than 60 min, the sieving coefficient stopped decreasing and then diafiltration became the dominant way of clearance.

Convective removal of a solute depends not only on pore size, but also on molecular weight (MW) and structure of the solute. Tumor necrosis factor-α is present in the circulation as a biologically active trimer with a MW of 54 kD, together with the nonactive monomer of 17 kD. Although MW of both the trimer and monomer are lower than the cut-off point of the EVAL membrane, and their average sieving coefficient reached 0.642 ± 0.014, similar to that in the European study with polysulphone membrane of 100 kD cut-off point (14), is it surprising that so large a trimeric TNF-α had so high a clearance? We supposed that ELISA may have measured the split products of trimeric TNF-α. Similar to 75 kD IL-2r, which is a heterotrimeric protein that consists of three subunits, alpha, beta and gamma chain, the sieving coefficient was unexpectedly high probably because of the single chain largely detected in the ultrafiltrate by the immuno-assay. Only this form could pass the average pore size of the EVAL membrane. The MW values of HMGB1 (30 kD), IL-1β (17 kD), IL-1ra (17 to 22 kD), IL-2 (15 kD), IL-6 (26 kD), IL-8 (8 kD), IL-10 (39 kD) were allowed across the EVACURE hemofilter, but, not all of the solute with a lower MW had a higher sieving coefficient due to the different physical and chemical characteristics. This may be the case for IL-8, possibly in relation to its high cationicity and its consequent binding to heparin (33). In in vitro model of sepsis, PDF was associated with about 70% reduction of the concentration of cytokines. The results indicate that optimal cytokine removal with PDF with EVAL membrane could be achieved with a combination of ultrafiltration and dialysate rate.

Albumin loss should be monitored when performing PDF with a filter of 60 kD cut-off point. Our data showed that the PDF sieving coefficient of albumin was 0.1 at first and then decreased to 0.05 after one hour's therapy. The clearance rate of albumin was 2.02 ± 1.39, 0.28 ± 0.15 mL/min at 10, 120 min, respectively. The total albumin loss was 0.5 g after one hour's therapy (dialysate rate 2000 mL/h, net ultrafiltration rate 300 mL/h). Replacing with plasma diluted with two times volume of normal saline would compensate for the loss of plasma. If the flow rate of the replacing fluid were set at 400 mL/h, 2 liters of plasma would be sufficient for a 24 hour therapy session, and PDF can be accepted as a continuous therapy.

CONCLUSION

Performed with a high cut-off point the EVAL membrane provides a new approach to removing sepsis-associated mediators more effectively with a relatively low loss of albumin. Whether PDF has a role in improving the survival of septic patients with multi-organ failure needs further studies.

Acknowledgments: The project was supported by "The major research and development project of innovative drugs, Ministry of Science and Technology" (2008ZX09312-010).Sincerely thank Prof Yutaka Eguchi (Critical and Intensive Care Medicine, Shiga University of Medical Science, Seta Tsukinowa, Ostsu, Shiga, Japan) for his continuous guidance during the whole study, and also deepest thanks to Claudio Ronco (Department of Nephrology, Dialysis and Transplantation, St. Bortolo Hospital, Vicenza, Italy) for his careful review and a lot of helpful suggestions.

REFERENCES

1. Cohen J. The immunopathogenesis of sepsis. *Nature* 2002;420:885–91.
2. Annane D, Bellissant E, Cavaillon JM. Septic shock. *Lancet* 2005;365:63–78.
3. Tracey KJ. Physiology and immunology of the cholinergic antiinflammatory pathway. *J Clin Invest* 2007;117:289–96.
4. Ziegler EJ, Fisher CJ Jr, Sprung CL et al. Treatment of gram-negative bacteremia and septic shock with HA-1A human monoclonal antibody against endotoxin. A randomized, double-blind, placebo-controlled trial. The HA-1A Sepsis Study Group. *N Engl J Med* 1991;324:429–36.
5. Abraham E, Wunderink R, Silverman H et al. Efficacy and safety of monoclonal antibody to human tumor necrosis factor alpha in patients with sepsis syndrome. A randomized, controlled, double-blind, multicenter clinical trial. *JAMA* 1995;273:934–41.
6. Honore PM, Jamez J, Wauthier M et al. Prospective evaluation of short-term, high-volume isovolemic hemofiltration on the hemodynamic course and outcome in patients with intractable circulatory failure resulting from septic shock. *Crit Care Med* 2000;28:3581–7.

7. Cole L, Bellomo R, Journois D et al. High-volume haemofiltration in human septic shock. *Intensive Care Med* 2001;27: 978–86.
8. Cornejo R, Downey P, Castro R et al. High-volume hemofiltration as salvage therapy in severe hyperdynamic septic shock. *Intensive Care Med* 2006;32:713–22.
9. Uchino S, Bellomo R, Goldsmith D et al. Cytokine removal with a large pore cellulose triacetate filter: an ex vivo study. *Int J Artif Organs* 2002;25:27–32.
10. Morgera S, Klonower D, Rocktäschel J et al. TNF-αelimination with high cut-off haemofilters: a feasible clinical modality for septic patients. *Nephrol Dial Transplant* 2003;18: 1361–9.
11. Morgera S, Slowinski T, Melzer C et al. Renal replacing therapy with high cut-off hemofilters: impact of convection and diffusion on cytokine clearances and protein status. *Am J Kidney Dis* 2004;43:444–53.
12. Nakae H, Igarashi T, Tajimi K et al. A case report of hepatorenal syndrome treated with plasma diafiltration(selective plasma filtration with dialysis). *Ther Apher Dial* 2007;11:391–5.
13. Nakae H, Igarashi T, Tajimi K et al. A case report of pediatric fulminant hepatitis treated with plasma diafiltration. *Ther Apher Dial* 2008;12:329–32.
14. Mariano F, Fonsato V, Lanfranco G et al. Tailoring high-cut-off membranes and feasible application in sepsis-associated acute renal failure: in vitro studies. *Nephrol Dial Transplant* 2005;20:1116–26.
15. Kriegler M, Perez C, DeFay K et al. A novel form of TNF/cachectin is a cell surface cytotoxic transmembrane protein: ramifications for the complex physiology of TNF. *Cell* 1988;53: 45–53.
16. Dinarello CA. The interleukin-1 family: 10 years of discovery. *Faseb J* 1994;8:1314–25.
17. Arend WP, Malyak M, Guthridge CJ et al. Interleukin-1 receptor antagonist: role in biology. *Annu Rev Immunol* 1998; 16:27–55.
18. Waldmann TA. The biology of interleukin-2 and interleukin-15: implications for cancer therapy and vaccine design. *Nature Rev Immun* 2006;6:595–601.
19. Smith KA. The interleukin 2 receptor. *Annu Rev Cell Biol* 1989;5:397–425.
20. van der Poll T, Keogh CV, Guirao X et al. Interleukin-6 gene-deficient mice show impaired defense against pneumococcal pneumonia. *J Infect Dis* 1997;176:439–44.
21. Baggiolini M, Clark-Lewis I. Interleukin-8, a chemotactic and inflammatory cytokine. *FEBS Lett* 1992;307:97–101.
22. Mocellin S, Panelli MC, Wang E et al. The dual role of IL-10. *Trends* 2004;24:36–43.
23. Wang HC, Zhu S, Zhou RR et al. Therapeutic potential of HMGB1-targeting agents in sepsis. *Expert Rev Mol Med* 2008; 10:e32–56.
24. Oberholzer C, Oberholzer A, Clare-Salzler M et al. Apoptosis in sepsis:a new target for therapeutic exploration. *FASEB J* 2001;15:879–92.
25. Oberholzer A, Oberholzer C, Moldawer LL. Understangding the role of innate and acquired immunity. *Shock* 2001;16:83–96.
26. Hotchkiss RS, Karl JE. The pathophysiology and treatment of sepsis. *N Engl J Med* 2003;348:138–50.
27. Marshall JC. Clinical trials of mediator-directed therapy in sepsis:what have we learned? *Intensive Care Med* 2000; 26(Suppl 1):S75–83.
28. Ronco C, Tetta C, Mariano F et al. Interpreting the mechanisms of continuous renal replacing therapy in sepsis: the peak concentration hypothesis. *Artif Organs* 2003;27:792–801.
29. Ronco C, Bellomo R, Homel P et al. Effects of different doses in continuous veno-venous haemofiltration on outcomes of acute renal failure: a prospetcive randomised trial. *Lancet* 2000;356:26–30.
30. Ronco C, Bonello M, Bordoni V et al. Extracorporeal therapies in non-renal disease: treatment of sepsis and the peak concentration hypothesis. *Blood Purif* 2004;22:164–74.
31. Piccinni P, Dan M, Barbacini S et al. Early isovolaemic haemofiltration in oliguric patients with septic shock. *Intensive Care Med* 2006;32:80–6.
32. Lonnemann G, Behme TC, Lenzner B et al. Permeability of dialyzer membranes to TNFα-inducing substances derived from water bacteria. *Kidney Int* 1992;42:61–8.
33. Kuschert GS, Coulin F, Power CA et al. Glycosaminoglycans interact selectively with chemokines and modulate receptor binding and cellular responses. *Biochemistry* 1999;38: 12959–68.