Therapeutic Apheresis and Dialysis
10(1):12–18. Blackwell Publishing Asia Pty Ltd

Endotoxin Adsorption Therapy for Septic Shock Using Polymyxin B-Immobilized Fibers (PMX): Evaluation by High-sensitivity Endotoxin Assay and Measurement of the Cytokine Production Capacity

Masahiro Kojika,[1] Nobuhiro Sato,[1] Yasunori Yaegashi,[1] Yasusi Suzuki,[1] Kenji Suzuki,[2] Hajime Nakae,[3] and Sigeatu Endo[1]

[1]Department of Critical Care Medicine and [2]Department of Anesthesiology, Iwate Medical University, School of Medicine, Morioka, and [3]Department of Integrated Medicine, Division of Emergency and Critical Care Medicine, Akita University, School of Medicine, Akita, Japan

Abstract: Because of its low sensitivity, the conventional measurement method for endotoxin (ET) is not the most appropriate for monitoring the effect of ET adsorption therapy. Thus, the efficacy of ET adsorption therapy was investigated using a newly developed high-sensitivity ET assay method. The changes in the cytokine production capacity of whole blood were also examined. We treated 24 peritonitis patients who had developed postoperative septic shock with ET adsorption therapy using a column of polymyxin B-immobilized fibers (PMX) and their serum ET levels were measured using the high-sensitivity ET assay based on the kinetic turbidimetric Limulus assay. In addition, the changes in the tumor necrosis factor-(TNF-α) production capacity of whole blood following lipopolysaccharide (LPS) stimulation and clinical outcome in the study patients were also examined. The 28-day mortality rate was 12%. PMX-direct hemoperfusion (PMX-DHP) was associated with elevation of the mean arterial pressure and urine output, reduction in the mean dose requirement of vasopressor agents, and recovery from the shock state in all the patients. The PaO_2/FIO_2 ratio also showed significant improvement. Using the high-sensitivity ET assay, ET was detected in the blood of 20 out of the 24 patients (80%) before the PMX-DHP, and a significant reduction in the ET level was noted after the PMX-DHP. The TNF-α production capacity of whole blood, which was found to be lower in the septic shock patients than in healthy subjects, was significantly increased after PMX-DHP. Elimination of ET by PMX-DHP in septic shock patients was confirmed by the high-sensitivity ET assay. PMX-DHP is thus considered to be a useful adjuvant therapeutic technique in the treatment of septic shock. Also, PMX-DHP might alleviate the immunosuppression associated with severe sepsis.
Key Words: Endotoxin, PMX, Sepsis, Shock, TNF-α.

Despite advances in critical care medicine, the mortality associated with severe sepsis worldwide is still reported to be in the range of 30–50%. In the USA, of the 750 000 cases of sepsis reported annually, 225 000 prove fatal (1–4). Various treatments for sepsis are constantly being investigated and developed, however, the mortality associated with this condition continues to remain high. Endotoxin (ET)-induced responses of the body have been implicated as playing a major role in the development of multiple organ failure as a complication of sepsis. ET induces excessive generation of cytokines via activation of macrophages, and these mediators, in turn, are considered to activate neutrophils and cause tissue damage. It has been suggested that elimination from the body of ET and of the inflammatory cytokines present in excess might inhibit the progression to multiple organ failure in cases of sepsis (5–9).

ET adsorption therapy was developed as one of the techniques to counter the effects of severe sepsis; it consists of blood purification using a blood purification column filled with a fibrous material on to which polymyxin B (PLB) is immobilized (10). While the potential clinical usefulness of this column has

Received October 2005.
Address correspondence and reprint requests to Dr Masahiro Kojika, Department of Critical Care Medicine, Iwate Medical University, School of Medicine, 19-1 Uchimaru, Morioka 020-0807, Japan. Email: Kojimasa@iwate-med.ac.jp

been suggested, the mechanisms underlying the effectiveness of ET adsorption therapy still remain to be established, particularly because the existing measurement method for ET has a low sensitivity and also a low specificity.

We developed a high-sensitivity method for ET measurement based on the kinetic turbidimetric Limulus assay, and investigated the effectiveness of ET adsorption therapy (11). The effectiveness of ET adsorption therapy against another aspect of severe sepsis, immunosuppression, was also investigated in the present study. The possibility of elimination of ET-inducing changes in the immune responses was assessed by evaluation of the changes of the tumor necrosis factor-α (TNF-α) production capacity of whole blood.

MATERIALS AND METHODS

The protocol of this prospective cohort study was approved by the Ethics Committee of Iwate Medical University School of Medicine (H15-35).

Patients

The present prospective study was carried out on sereal patients with peritonitis who developed septic shock despite appropriate emergency surgical treatment by excision and drainage of the infective foci. The exclusion criteria were: (i) pregnancy; (ii) perioperative administration of steroids; (iii) history of chemotherapy or irradiation; (iv) serum total bilirubin > 10.0 mg/dL; and (v) hepaplastine test result <40%. The patients were enrolled in this study within 1 h of the development of septic shock. Written informed consent was obtained from either the patients themselves or their immediate family members prior to the start of the study.

The scoring systems used for determining the severity of sepsis were the SOFA scoring system and the APACHE II scoring system. Septic shock was defined in accordance with the definition established at the ACCP/SCCM Consensus Conference; i.e. low blood pressure despite appropriate fluid therapy (systolic pressure under 90 mm Hg or blood pressure at least 40 mmHg lower than the usual systolic pressure of the patient) in the presence of sepsis.

Methods

The blood access route was venous-venous blood of the femoral vein or the subclavian vein. The ET adsorption column used was PMX-20R (Toray Industries, Tokyo, Japan), and the blood flow volume was 80–120 mL/min. PMX-direct hemoperfusion (PMX-DHP) carried out for 120 min was considered as one session of therapy. Therapy was discontinued when adverse events appeared or it was judged that PMX-DHP would be difficult to continue. When a patient failed to recover from the shock state after one course of therapy, another course was repeated after 24 h.

Blood sampling

Blood samples were collected before the start of PMX-DHP and immediately after PMX-DHP.

Endotoxin measurement

Heparinized whole blood samples were centrifuged at 3000 r.p.m. for 10 min, and the separated plasma was used for the assay. The high-sensitivity assay was carried out by kinetic turbidimetric Limulus assay using a MT-251 Toxinometer (Wako, Osaka, Japan), which theoretically can measure up to 0.01 pg/mL. In a previous study, the median ET level in sepsis patients measured by this assay method was 2.29 pg/mL. When the cut-off ET level for the diagnosis of sepsis was set at 1.1 pg/mL, the sensitivity and specificity of the detection were 81.3% and 86.1%, respectively (Figs 1,2). Changes in the ET level following PMX-DHP were also evaluated by the present high-sensitivity measurement method.

Tumor necrosis factor-α production capacity

Whole blood samples were mixed with 1 μg of lipopolysaccharide (LPS) per mL of blood to induce cytokine production and the mixture was incubated at 37°C in 5% CO_2 for 24 h. The separated plasma

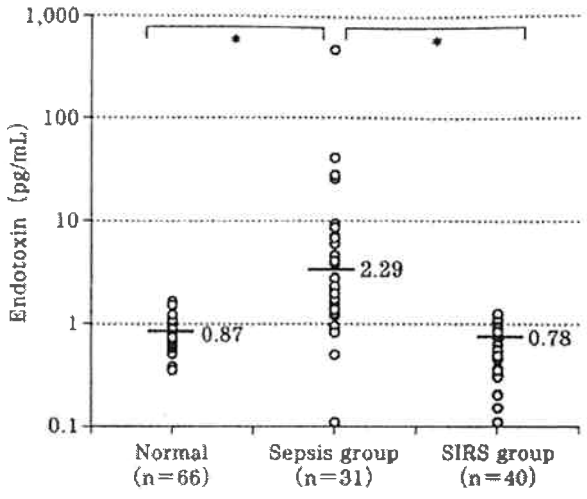

FIG. 1. High-sensitivity measurement of blood endotoxin (ET) levels in various diseases. Cited from Ref (14).

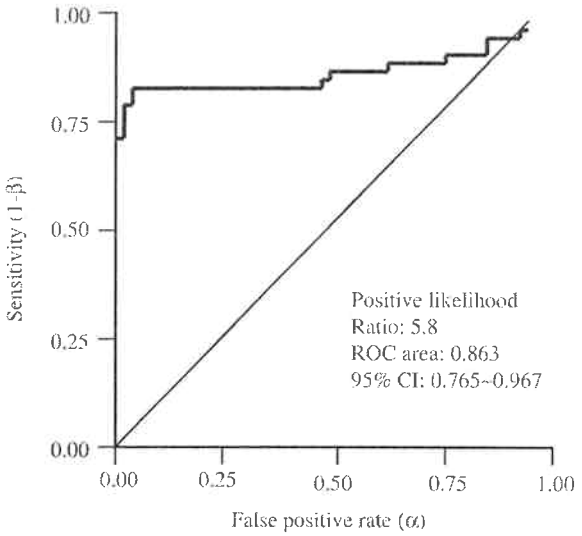

FIG. 2. Receiver-operator curves (ROC) from the high-sensitivity endotoxin (ET) measurement assay for the diagnosis of sepsis. Cited from Ref (14).

specimens were stored at −80°C until the analysis. The TNF-α level in the supernatant was measured by enzyme-linked immunosorbent assay. Similarly, whole blood samples collected from 10 healthy subjects were also assayed as controls.

Clinical outcome

The clinical outcome was evaluated from the mean arterial pressure, urine output, mean dose requirement of vasopressor agents (DOB or DOA), and the PaO_2/FIO_2 ratio as an indicator of the pulmonary-oxygenating capacity.

Statistical analysis

Stat View 5.0 (SAS Institute Inc., Cary, NC, USA) software for exclusive use with a Macintosh computer (Apple Computer Inc., USA) was used for the statistical analyzes. Values were expressed as mean ± SD. The differences were analyzed by Wilcoxon's test, and differences at $P \leq 0.05$ were considered to be significant.

RESULTS

Background factors

Table 1 shows the background characteristics of the patients. There were 24 patients in all (15 male, nine female), with a mean age of 70.3 ± 17 years. The most frequently encountered cause of the sepsis was colonic perforation. Bacterial culture of the ascitic fluid collected during the operation showed only Gram-negative bacilli in 10 patients, both Gram-negative and Gram-positive bacteria in four patients, and both Gram-negative bacteria and fungi in two patients. Thus, Gram-negative bacteria were implicated in the development of sepsis in 16 of the 24 patients. The mean SOFA and APACHE II scores were 6.5 ± 3.2 and 14.2 ± 4.0, respectively.

Clinical outcome

All the 24 patients enrolled in the study recovered from shock after the PMX-DHP. Of the patients, 18 received a single session of PMX-DHP, while six patients received two sessions. Three of the 24 patients died within 28 days of the development of shock, and all three died of multiple organ failure occurring within a week of the development of shock (Table 1).

Mean arterial pressure, urine output and mean dose requirement of vasopressor agents

The mean arterial pressure was 87.1 ± 17.9 mm Hg before PMX-DHP and 110.9 ± 29.8 mm Hg at the end of PMX-DHP, indicating a significant ($P = 0.0009$) increase of the blood pressure following PMX-DHP. The mean dose requirement of vasopressor drugs (average of the sum of the dopamine and dobutamine doses) before the PMX-DHP was 5.1 ± 2.6 μg/kg/min, and the corresponding requirement at the end of PMX-DHP was 4.4 ± 2.3 μg/kg/min, indicating a significant ($P = 0.018$) decrease in the dose requirement following PMX-DHP (Fig. 3).

Pulmonary-oxygenating capacity

The pulmonary-oxygenating capacity was determined by measurement of the PaO_2/FIO_2, and the changes in this parameter after PMX-DHP was evaluated. The PaO_2/FIO_2 was 251 ± 99.9 mm Hg before the PMX-DHP and 281 ± 69.8 mm Hg after PMX-DHP, indicating a significant ($P = 0.027$) improvement of the pulmonary-oxygenating capacity after PMX-DHP (Fig. 4).

Plasma endotoxin level

The peripheral blood ET levels in the septic shock patients before and after PMX-DHP were 8.84 ± 15.11 pg/mL and 2.11 ± 4.62 pg/mL, respectively, suggesting a significant ($P = 0.0061$) decrease of the peripheral blood ET level after PMX-DHP (Fig. 5).

Tumor necrosis factor-α production capacity of whole blood

With regards to the changes in the TNF-α production capacity of LPS-stimulated whole blood, the TNF-α levels before and after PMX-DHP were

TABLE 1. *Clinical course of patients*

Patient no.	Gender	Age (years)	Cause of sepsis	APACHE II	SOFA score	No. PMX-DHP	Bacteria (ascites)	Outcome
1	male	21	Liver abscess	14	9	2	Bacteroides caccae	Survived
2	female	29	Liver abscess	2	2	2	Candida albicans	Survived
3	female	51	Perforation of the colon	14	7	1	Enterococcus faecalis, E.coli	Survived
4	male	55	Ischemic colitis	10	12	2	Morganella morganii, Enterococcus avium/Bacteroides caccae	Survived
5	female	64	Acute appendicitis	8	4	1	Pseudomonas aeruginosa	Survived
6	male	79	Ischemic colitis	14	5	1	–	Survived
7	male	75	Perforation of the colon	13	4	2	Bacteroides caccae	Survived
8	male	72	Ileus	18	6	1	–	Survived
9	male	78	Liver abscess	14	4	1	Enterococcus avium	Died
10	male	81	Ischemic colitis	12	5	1	–	Survived
11	female	79	Perforation of the colon	17	2	1	Klebsiella pneumoniae ssp. Pneumoniae	Survived
12	male	87	Ischemic colitis	14	2	1	–	Survived
13	male	81	Perforation of the gastric ulcer	18	7	2	Enterobacter aerogenes, Candida glabrata	Survived
14	female	71	Perforation of the uterus	13	6	1	–	Survived
15	male	77	Ischemic colitis	18	5	2	–	Died
16	female	74	Perforation of the small intestine	13	6	1	Klebsiella pneumoniae ssp. Pneumoniae	Survived
17	male	82	Cholecystitis	12	5	1	–	Survived
18	male	67	Perforation of the colon	15	5	1	Enterococcus faecalis	Survived
19	female	78	Perforation of the colon	21	5	1	Enterococcus faecalis, E.coli	Died
20	male	75	Ischemic colitis	15	7	1	–	Survived
21	female	80	Retroperitorieal abscess	14	5	1	Klebsiella pneumoniae ssp. Pneumoniae, Pseudomonas aeruginosa	Survived
22	male	75	Perforation of the gastric cancer	15	4	1	Candida albicans, Candida glabrata	Survived
23	female	83	Perforation of the colon	21	7	1	E.coli	Survived
24	female	74	Ischemic colitis	19	6	1	–	Survived

PMX-DHP, polymyxin B-immobilized fibers direct hemoperfusion.

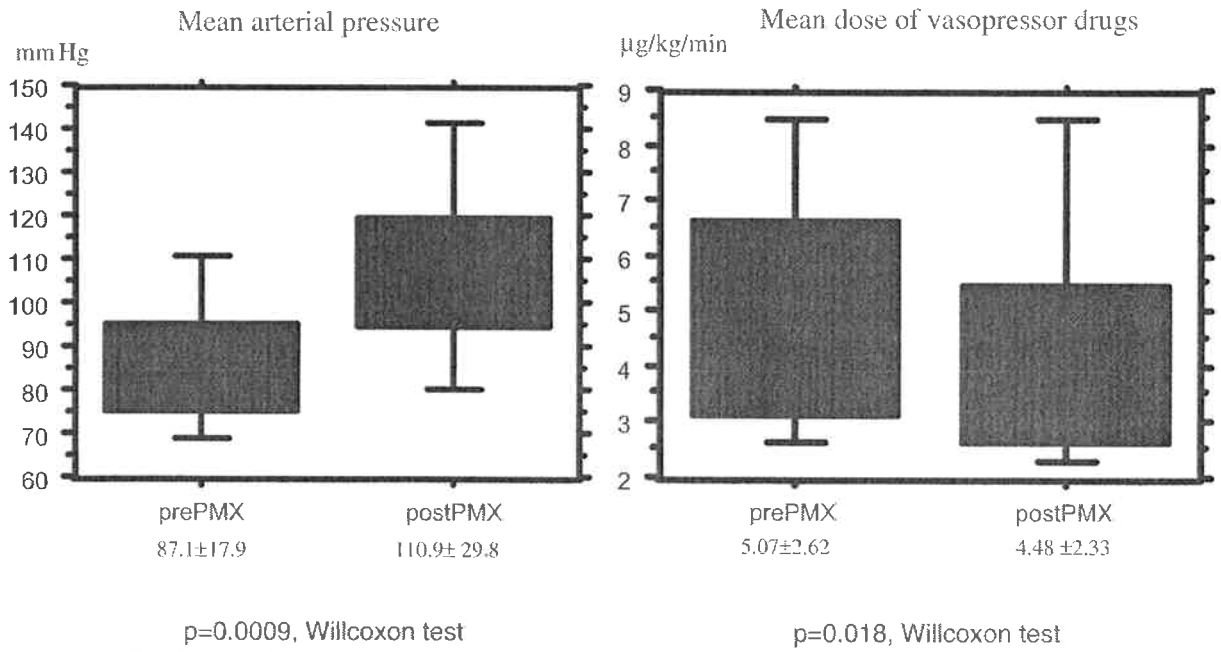

FIG. 3. Changes in the mean arterial pressure and mean dose requirement of vasopressor drugs after PMX-DHP.

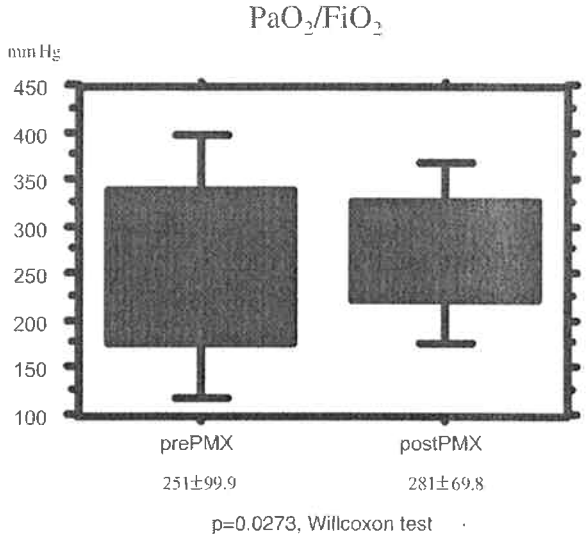

FIG. 4. Changes in the PaO_2/FiO_2 values after polymyxin B-immobilized fibers direct hemoperfusion (PMX-DHP).

580 ± 363 pg/mL and 1018 ± 1008 pg/mL, respectively, indicating a significant ($P = 0.017$) improvement of the TNF-α-generating capacity following PMX-DHP (Fig. 6). The mean TNF-α production capacity of whole blood in healthy volunteers was 3386.6 ± 1263.2 pg/mL, suggesting that the TNF-α production capacity of whole blood was significantly suppressed in septic shock patients.

DISCUSSION

ET-induced excessive release and hyperactivity of intrinsic inflammatory mediators have been implicated in the development of multiple organ failure as a complication in sepsis. Several therapeutic methods have been developed to counter the strong toxicity of ET and of inflammatory mediators, including anti-ET antibody therapy (12–14) and high-dose steroid therapy (15–21) however, none of the methods has until now been established to be clinically useful. In recent years, an increasing number of reports have appeared, suggesting that acute blood purification therapy designed to eliminate the inflammatory mediators present in excess, might be effective in retarding the progression to multiple organ failure in cases of sepsis (6–11). In Japan, ET adsorption therapy, in which a column of PLB-coated fibers (PMX-20R) prepared based on the binding property of PLB to lipid A is used, is clinically applied to treat septic shock (10).

It is important to monitor serum ET levels to evaluate the effectiveness of PMX-DHP, however, the conventional measurement method for ET has a rather low sensitivity. In the present study, we used the high-sensitivity ET measurement method developed by us. The sensitivity of this measurement method was found to increase with increasing observation time. Measurement using this high-sensitivity assay showed a blood ET level of 0.87 in the healthy group ($n = 71$), 2.29 in the sepsis group ($n = 55$; 10 patients with acute appendicitis, 10 with perforated duodenal ulcer, 12 with colonic perforation, four with pyelonephritis, nine with cholangitis, seven with thrombosis of the superior mesenteric artery, one with perforated gastric ulcer, four with small intestinal perforation, and nine with other conditions), and

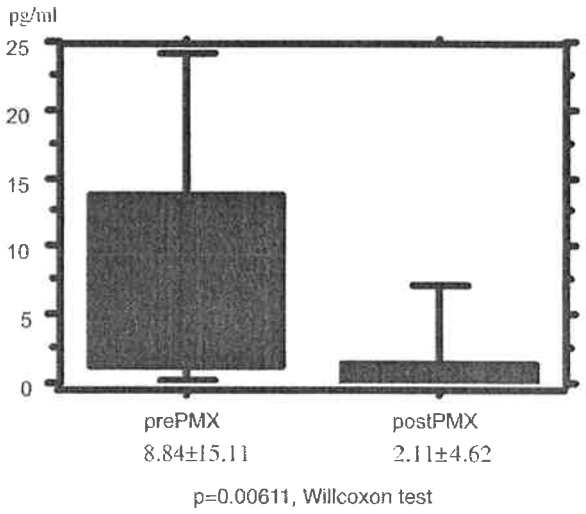

FIG. 5. Changes in the blood ET levels after polymyxin B-immobilized fibers direct hemoperfusion (PMX-DHP).

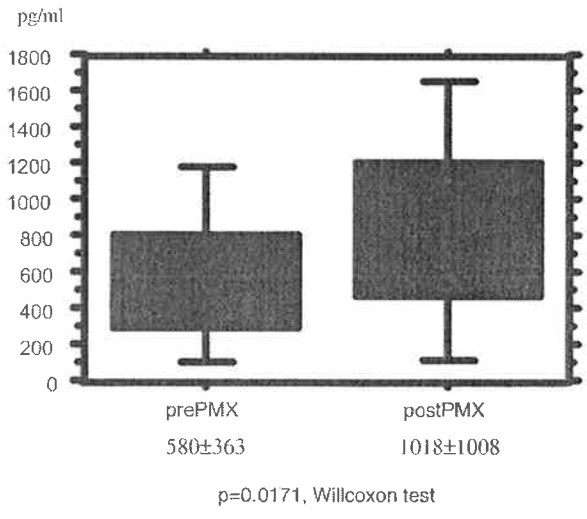

FIG. 6. Changes in the tumor necrosis factor-α (TNF-α)-generating capacity of whole blood after polymyxin B-immobilized fibers direct hemoperfusion (PMX-DHP).

0.78 in the systemic inflammatory response syndrome (SIRS) group ($n = 40$; eight with myocardial infarction, three with carbon monoxide poisoning, three with head injury, three with hepatopathy, two with fat embolism, two with pelvic fracture, and 17 with other conditions). The high-sensitivity method is sensitive to even differences in values as small as 1 pg/dL. Using this high-sensitivity method, the ET levels before and after PMX-DHP were determined to be 8.8 ± 15.1 pg/mL and 2.1 ± 4.6 pg/mL, respectively. Thus, significant elimination of ET by PMX-DHP was confirmed.

Measurement of the cytokine-production capacity of whole blood following stimulation with LPS has increasingly been recognized as a useful method for evaluating a patient's immunological competence. Some reports have shown that the cytokine-production capacity of whole blood is strongly suppressed in patients with sepsis, surgical stress, and injury. The influence of elimination of ET and recovery from the shock state following PMX-DHP on the immunosuppression in sepsis was also assessed in the present study; the TNF-α production capacity was shown to improve after PMX-DHP. Hotta et al. reported an increase in the count of HLA-DR+ monocytes following PMX-DHP (22). These results suggest that PMX might indeed alleviate the immunosuppression caused by sepsis. However, no significant correlation was found between the rate of decrease of ET levels and the improvement in the TNF-α production capacity of whole blood following LPS stimulation in this study (data not shown). One of the reasons for this result could be that the number of patients included in this study was small. Furthermore, although the TNF-α production capacity of whole blood increased following PMX-DHP, the TNF-α levels still did not reach the levels encountered in healthy subjects. In this context, the mechanisms underlying the development of immunosuppression in cases of sepsis have not yet been clarified. Further studies are thus needed to investigate the immunosuppression associated with sepsis. Mediators, receptors and signal transduction pathways are all under investigation. PMX-DHP was found to result in improvement of the cytokine production, suggesting that some mediators might be involved in the immunosuppression associated with sepsis.

In the present study, ET-negative patients and patients with Gram-positive bacteremia also recovered from septic shock following PMX-DHP. Thus, factors other than the elimination of ET might be involved in the effectiveness of PMX-DHP in cases of septic shock. In this context, Ezoe et al. showed that anandamide is also eliminated by PMX-DHP (23). Ebihara et al. showed that the increased plasma ET-1 levels and monocyte ET-1 mRNA levels in patients with sepsis decreased significantly after PMX-DHP (24). These observations suggest that the elimination of diverse mediators by PMX-DHP might be involved in the effectiveness of this treatment modality for septic shock. PMX-DHP resulted in improvement of the sepsis-induced deterioration of pulmonary-oxygenating capacity. Permeability edema, the ventilation-perfusion ratio, etc. also seemed to improve after PMX-DHP. These observations clearly suggest that PMX-DHP also eliminates humoral mediators other than ET.

PMX-DHP is thus considered to be a useful adjuvant therapeutic technique in the treatment of sepsis, after appropriate excision and drainage of the infective focus causing the sepsis. PMX-DHP has also been shown to have some beneficial effects in interdisciplinary oncotherapy. Further studies are needed in the future to investigate the mechanisms underlying the effectiveness of PMX-DHP in the clinical setting.

REFERENCES

1. Angus DC, Birmingham MC, Balk RA. E5 murine monoclonal antiendotoxin antibody in gram-negative sepsis: a randomized controlled trial. E5 Study Investigators. *JAMA* 2000;283:1723–30.
2. Rangel-Frausto MS, Pittet D, Costigan M, Hwang T, Davis CS, Wenzel RP. The natural history of the systemic inflammatory response syndrome (SIRS): a prospective study. *JAMA* 1995;273:117–23.
3. Annane D, Sebille V, Troche G, Raphael JC, Gajdos P, Bellissant E. A 3-level prognostic classification in septic shock based on cortisol levels and cortisol response to corticotropin. *JAMA* 2000;283:1038–45.
4. Centers for Disease Control (CDC). Increase in National Hospital Discharge Survey rates for septicemia - United States, 1979–1987. *JAMA* 1990;263:937–8.
5. Gotloib L, Barzilay E, Shustak A, Wais Z, Jaichenko J, Lev A.. Hemofiltration in septic ARDS. The artificial kidney as an artificial endocrine lung. *Resuscitation* 1986;13:123–32.
6. Blinzler L, Hausser J, Bodeker H, Zaune U, Martin E, Gebhardt C. Conservative treatment of severe necrotizing pancreatitis using early continuous venovenous hemofiltration. *Contrib Nephrol* 1991;93:234–6.
7. Lee PA, Matson JR, Pryor RW, Hinshaw LB. Continuous arteriovenous hemofiltration therapy for Staphylococcus aureus-induced septicemia in immature swine. *Crit Care Med* 1993;21:914–24.
8. 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.
9. Ronco C, Tetta C, Lupi A et al. Removal of platelet-activating factor in experimental continuous arteriovenous hemofiltration. *Crit Care Med* 1995;23:99–107.
10. Shoji H, Minaga M, Sasaki Y et al. Design and development of endotoxin detoxifying column (PMX) and its clinical application. *Jpn J Artif Organs* 1993;22:204–11. (Japanese with English abstract.)

11. Yaegashi Y, Shirakawa K, Sato N et al. Evaluation of a newly identified soluble CD14 subtype as a marker for sepsis. *J Infect Chemother* 2005;11:234–8.
12. Zeni F, Freeman B, Natanson C. Anti-inflammatory therapies to treat sepsis and septic shock: a reassessment. *Crit Care Med* 1997;25:1095–100.
13. Natanson HW, Hoffman WD, Suffredini AF, Eichacker PQ, Danner RL. Selected treatment strategies for septic shock based on proposed mechanisms of pathogenesis. *Ann Int Med* 1994;120:771–83.
14. Baumgartner JD, Heumann D, Gerain J, Weinbreck P, Grau GE, Glauser MP. Association between protective efficacy of anti-lipopolysaccharide (LPS) antibodies and suppression of LPS-induced tumor necrosis factor alpha and interleukin 6. Comparison of O side chain-specific antibodies with core LPS antibodies. *J Exp Med* 1990;171:889–96.
15. Bennett IL, Finland M, Hamborger M. The effectiveness of hydrocortisone in the management of severe infection. *JAMA* 1963;183:462–5.
16. Luce JM, Montgomery AB, Marks JD. Ineffectiveness of high-dose methylprednisolone in preventing parenchymal lung injury and improving mortality in patients with septic shock. *Am Rev Respir Dis* 1988;138:62–8.
17. The Veterans Administration Systemic Sepsis Cooperative Study Group. Effect of high-dose glucocorticoid therapy on mortality in patients with clinical signs of systemic sepsis. *N Eng J Med* 1987;317:659–65.
18. Bone RC, Fisher CJ Jr, Clemmer TP, Slotman GJ, Metz CA, Balk RA. A controlled trial of high dose methylprednisolone in the treatment of severe sepsis and septic shock. *N Engl J Med* 1987;317:653–8.
19. Sprung C, Caralis P, Marcial E et al. The effects of high dose corticosteroids in patients with septic shock. A prospective, controlled study. *N Engl J Med* 1984;311:1137–43.
20. Schmer W. Steroid in the treatment of clinical shock. *Ann Surg* 1976;184:133–9.
21. Klastersky J, Cappel R, Debusscher L. Effectiveness of betamethasone in management of severe infections. A double-blind study. *N Engl J Med* 1971;284:1248–50.
22. Ebihara I, Nakamura T, Shimoda N, Shoji H, Koide H. Effect of hemoperfusion with polymyxin B-immobilized fiber on plasma endothelin-1 and endothelin-1 mRNA in monocytes from patients with sepsis. *Am J Kidney Dis* 1998;32:953–61.
23. Ezoe E, Imaizumi H, Maruyama I. Analysis of Effect with PMX-DHP Regard as Blood Pressure. *Jpn J Crit Care Endotoxemia* 2001;5:150–7. (Japanese with English abstract.)
24. Hotta O. Effects of Endotoxin Elimination Therapy on Immune Cells. *ICU & CCU* 2000;24:875–80. (Japanese with English abstract.)