Artificial Organs
22(12):1038–1044, Blackwell Science, Inc.

Therapeutic Apheresis for Septic Patients with Organ Dysfunction: Hemoperfusion using a Polymyxin B Immobilized Column

Tohru Tani, Kazuyoshi Hanasawa, Yoshihiro Endo, Toyokazu Yoshioka, Masashi Kodama, *†Masamitsu Kaneko, *†Yoshimitsu Uchiyama, *‡Tadao Akizawa, *§Kodo Takahasi, and *§§Katsuo Sugai

*First Department of Surgery, Shiga University of Medical Science, Shiga; *PMX Clinical Study Group, and †Department of Traumatology and Critical Care Medicine, Sapporo University School of Medicine, Sapporo; ‡Showa University Fugigaoka Hospital, Yokohama; §Department of Emergency and Critical Care Medicine, Nippon Medical School, Tama Nagayama Hospital, Tama; and §§Department of Emergency and Critical Care Medicine, Chiba University School of Medicine, Inohana, Japan*

Abstract: A prospective clinical study was performed to evaluate a new method of treatment of endotoxin shock, a column containing polystyrene fibers with covalently bound immobile polymyxin B. Direct hemoperfusion using the column removes circulating endotoxin by adsorption. All of the patients studied, 37 in the treatment group and 33 in the control group, had endotoxemia and failure of 1 or more organs. The perfusion was performed 1–7 times per patient, 2 h/session. The survival rate was significantly higher in the treatment group (54%) than in the controls (36.4%). The mean plasma endotoxin concentration was significantly lowered by the treatment from 83.7 pg/ml before perfusion to 56.4 pg/ml immediately after and 28.5 pg/ml the day after the treatment, and the posttreatment level was much lower in those who survived (mean, 18.8 pg/ml) compared to those who died (mean, 88 pg/ml). Various parameters of cardiac function also improved after the treatment. **Key Words:** Endotoxin—Sepsis—Multiple organ failure—Polymyxin—Hemoperfusion—Systemic inflammatory response syndrome.

The main objective of therapeutic apheresis is the removal of toxic substances although the method can also be applied to immunomodulation as we have previously reported (1). The development of technology has made possible the application of apheresis to increasingly varied target substances and diseases.

The possibility of fatality in septic patients rises when shock develops, and the mortality rate of patients with septic multiple organ failure (MOF) has been reported to be over 70% (2). Endotoxin produced by gram-negative bacteria acts on macrophages and leukocytes, producing various mediators and cytokines, and induces septic symptoms. Even a small amount of endotoxin plays a major role in the development of toxic symptoms both in gram-negative and gram-positive infections. Therapeutically, the removal of endotoxins themselves or detoxification of endotoxin would be ideal. An anti-lipopolysaccharide (LPS) monoclonal antibody was developed against endotoxin, and experimental (3) and clinical studies (4) have been performed, but clinical trials have failed (5).

Polymyxin B has long been known to specifically bind with endotoxin (6), but it cannot be used in patients directly because of its renal and neural toxicity. Niwa et al. (7) reported (8) in 1982 a new technique of removing endotoxin from a solution using polymyxin B immobilized on sepharose. However, so far this method of removing endotoxin from blood has not been clinically applied to the treatment of sepsis.

In 1983, we developed a method of adsorbing endotoxin in the blood by polymyxin B immobilized on polystyrene fibers (8). The polymyxin B immobilized

Received October 1997; revised April 1998.
Address correspondence and reprint requests to Dr. Tohru Tani, First Department of Surgery, Shiga University of Medical Science, Seta, Otsu-shi, Shiga 520-21, Japan.

fiber (PMX-F) adsorbed endotoxin at the ratio of 0.52 mg/g fiber without releasing polymyxin B. The efficacy of the device already had been proven in in vitro studies and in vivo with large animal models both by administering purified (9) endotoxin and live bacteria (10). In this paper, the result of the clinical trial using the endotoxin adsorbing Toraymyxin PMX-F column with direct hemoperfusion (DHP) is reported. The efficacy of the Toraymyxin column in the removal of circulating endotoxin and improvement of symptoms of sepsis and survival rates is described. Some basic problems involved in clinical studies of mechanical devices for treatment also are discussed.

PATIENTS AND METHODS

Design

This prospective clinical study was carried out at 8 university hospitals during a 2 year period beginning February 1989. The protocol and data were managed by 2 coordinators. Each patient's condition before treatment (DHP using a PMX-F column; hereafter referred to as PMX treatment) was compared to his condition after treatment. Conventional treatments, all of which are routinely applied to MOF patients in intensive case units, were continued. The PMX treatment was initiated when a patient did not respond to conventional treatments for sepsis. The prognosis was compared to that of a control group of endotoxemic patients treated in the same institutions during the period of the study. The control group received the same conventional treatments as the PMX group.

Subjects

PMX treatment was performed in patients with endotoxemia and sepsis with 1 or more organs in failure. Conventional treatment had been initiated before PMX treatment, and the patients were receiving vasoactive agents to maintain blood pressure or were under mechanical ventilation and/or receiving a sedative. Patients in the control group were chosen by the same criteria as those for the PMX treated group with the minimum requirement that they had endotoxemia and at least 1 failed organ. Patients under 18 or over 85 years of age were excluded from the study. Proper informed consent (oral or written) was obtained from each patient or patient's family.

Severity of the disease

The functions of the lungs, kidneys, liver, and the coagulation system were evaluated, and MOF was diagnosed according to the MOF diagnostic criteria proposed by Goris et al. (11). Briefly explained, these criteria consist of pulmonary, hepatic, renal, cardiac, gastrointestinal tract, central nervous system, and coagulation disorders. The worst score is 7 points. The severity of infection was evaluated using the septic severity score (SSS) (12), and 3 points were given to patients under sedation in the evaluation of the consciousness level.

PMX-F and PMX treatment

The PMX-F was produced by immobilizing polymyxin B on polystyrene fiber at the ratio of 0.5% g of polymyxin to 1 g fiber by covalent bonding (10). Biological detecting tests confirmed a firm binding of polymyxin B to the fiber without its release (8). The holding blood volume of this column is 200 ml. The column for DHP was filled with 53 g PMX-F and physiological saline. The columns, supplied by Toray Industries, Inc. (Tokyo, Japan) were stored at room temperature and sterilized using an autoclave. DHP was performed using conventional equipment for hemoperfusion and a circuit for hemodialysis. The column was washed by perfusion with 4 L of physiological saline. A double lumen catheter was inserted into the femoral vein, and blood was drawn from the vein and returned to the vein (V-V method). The perfusion was carried out at a rate of 80–100 ml/min for 2 h (13). DHP was performed twice at most for each patient. Heparin, low molecular weight heparin, or nafamostat mesilate (NM), a serine protease inhibitor obtained from Torii Pharmaceuticals, Co., Ltd. (Tokyo, Japan) was used as the anticoagulant (14).

Evaluation of patients

The survival rate was calculated 2 weeks after the termination of DHP or, in the control group, 2 weeks after the detection of endotoxemia. To evaluate the effects of the PMX treatment, various parameters were monitored before, immediately after (2 h after the start of DHP), and on the day after DHP (24 h after the treatment). The cardiac index (CI), systemic vascular resistance (SVR), and oxygen consumption index (Vo_2I) were measured using a Swan Ganz catheter. The body temperature (BT), blood pressure (BP), and heart rate (HR) were also recorded. The platelet count was determined to monitor the safety of the procedure. The endotoxin concentration was measured. The concentrations of endotoxin in the blood before the entry into and after the exit from the PMX-F column were also measured 30 or 60 min after the initiation of treatment. The arterial ketone body ratio (AKBR) (15), as the marker of metabolic change, was determined before PMX and on the following day.

Measurement of endotoxin

A new perchloric acid (PCA) method was used (16). Plasma was mixed with sodium hydroxide, heated at 37°C for 5 min, mixed with PCA at half the amount used in the conventional PCA method (17), and heated again for 10 min. The resulting sediment was dissolved in a × 9 volume of molarity sodium hydroxide. The sample (100 μl) was mixed with 100 μl of Endospecy (Seikagaku Kogyo Co., Ltd., Tokyo, Japan) solution and heated at 37°C for 30 min. After diazo coupling, absorbance at 545 nm was measured. The upper limit of the normal value is 10 pg/ml, and the minimum detectable level is 0.003 EU/ml (1 pg/ml = 0.0029 EU). Endospecy is a factor G-depleted lysate that does not react with (1-3)-β-glucan and does not promote reactions in the factor G system. Therefore, this agent does not produce false positive reactions in the plasma of patients with fungal sepsis, hemodialysis patients after using a cellulose membrane, or patients receiving anticancer drugs derived from plant polysaccharides (17).

Statistical methods

The prognosis, severity of sepsis, and degree of MOF were compared between the PMX treated group and the control group. Statistical analysis was done with the Mantel-Haenszel test. In the PMX group, all values obtained before, immediately after, and on the day after treatment were expressed as the mean ± standard error and were analyzed by the nonparametric test of Wilcoxon's *t* test; p values less than 0.05 were considered to indicate significance.

RESULTS

PMX treatment was performed in 37 patients (33 males and 10 females) aged between 18 and 83 years (mean age, 57.9 ± 3 years). Heparin was used 12 times as the anticoagulant, low molecular weight heparin 3 times, and NM 49 times. In the control group, 33 patients were included. As shown in Table 1, the underlying diseases varied, and some patients had more than 1 disease, but malignant neoplasms were most frequently observed in both groups. Table 2 shows that the most frequent site of infection in both groups was the abdomen, followed by the respiratory system. The most frequently detected microorganisms were gram-negative bacteria. The characteristics of the backgrounds and infections in both the control and PMX treated groups were similar. MOF was diagnosed according to Goris's criteria in 36 of the 37 PMX treated patients and in 31 of 33 in the control group. The distribution of organ failure was also similar in both groups. The SSS of 46.2 ± 3.2 in the PMX treated patients was significantly higher than the 39.1 ± 2.7 in the control group ($p < 0.05$). The number of failed organs was also significantly higher in the PMX group than the control, 3.8 ± 0.3 vs. 3.2 ± 0.2 ($p < 0.05$) (Table 3).

TABLE 1. *Characteristics of patients*[a]

Characteristics	Groups	
	Control (n = 33)	PMX (n = 37)
Age (years) (18–83)	57.9 ± 3	59.2 ± 2.9
Male:Female	29:8	23:10
Underlying Diseases		
Neoplasm	11	22
Collagen disease	0	3
Diabetes mellitus	1	2
Liver cirrhosis	0	2
Cardiovascular disease	3	11
Respiratory disease	0	4
CNS disease	4	4
Recent surgery	18	19
Recent trauma	9	9

[a] Underlying diseases show the same distribution in both groups.

EFFECTIVENESS OF PMX

Survival rate

There were significant differences in the survival rates, not only between the control and the PMX treated groups, but also between more severely affected patients in the 2 groups. The survival rate in the treated group (54%) was significantly higher than that in the control group (36.4%, $p < 0.05$) even though the patients in the PMX treated group were in much worse condition initially with respect to the number of failed organs and SSS. There was no difference between the patients with 4 or more organ failure and 40 or more SSS in age distribution between the control and treated groups. In the MOF patients with 4 or more failed organs, the survival

TABLE 2. *Characteristics of infection*[a]

	Groups	
	Control (n = 33)	PMX (n = 37)
	Number of patients	
Source		
Abdominal cavity (trauma)	18	18(7)
Respiratory system	9	10
Biliary tract	1	4
Cardiovascular system	1	3
Central nervous system	1	3
Others	3	5
Microorganism		
Gram (−) bacteria	27	26
Gram (+) bacteria	4	8
Fungus	1	0
Not detectable	1	9

[a] Source of infection and microorganisms show the same.

TABLE 3. *Severity of sepsis at admission*[a]

Variable	Groups	
	Control (n = 33)	PMX (n = 37)
Septic severity score (SSS)	39.1 ± 2.7	46.2 ± 3.2[b]
Number of failed organs	3.2 ± 0.2	3.8 ± 0.3[b]
Shock and/or use of vasopressor	25	27
Endotracheal intubation and/or respiratory failure	24	28
Acute renal failure	9	19
Hepatic failure	18	25
Coagulation disorder	12	15

[a] Failing organs show the same distribution in both groups.
[b] $p < 0.05$ between control and PMX groups. Illness severity was greater in the PMX group than in the control.

rate of the PMX treated group was 33% (7/21) and that of the control group was 8% (1/13) ($p < 0.01$).

In patients with SSS of under 40, the survival rate was 86% (12/14) in the PMX treated group and 83% (9/12) in the controls, but in patients with SSS of over 40, the survival rate of the PMX treated group (35%, 8/23) was higher than that of the control group (14%, 3/21) (Table 4).

Endotoxin concentration

The plasma endotoxin concentration of all sessions in PMX treated groups was 83.7 ± 26.7 pg/ml pretreatment. The endotoxin concentration in the survivors was 90.0 ± 50.0 pg/ml in the PMX treated group pretreatment; in nonsurvivors, it was 121.4 ± 110.8 pg/ml in the PMX treated group pretreatment. There was no significant difference in the pretreatment endotoxin concentration of the patients in the PMX treated group who survived and those who died. The endotoxin reduction by PMX treatment was evaluated as the difference in plasma endotoxin concentrations before and after DHP.

The circulatory endotoxin concentration was reduced significantly 2 h after all DHP sessions. In the PMX treated group, the mean ± SE immediately before DHP, 83.7 ± 26.7 pg/ml, was significantly decreased to 56.4 ± 27.9 pg/ml (n = 51) immediately after PMX treatment ($p < 0.05$). In the 25 samples obtained from evaluated sessions the following day, the endotoxin concentration was 28.5 ± 4.1 pg/ml, which was significantly lower ($p < 0.05$). There was no significant difference in endotoxin concentrations prior to PMX treatment between survivors and nonsurvivors. After DHP, the endotoxin concentration in the survivors (n = 20) was significantly less than in those who died (n = 17) (18 ± 3 vs. 121 ± 84 pg/ml, $p < 0.05$) (Table 5).

TABLE 4. *Survival rate*[a]

Conditions	Groups	
	Control (n = 33)	PMX (n = 37)
	Number of survival patients	
Total	12/33 (36.4%)	20/37 (54%)[b]
>40 SSS	3/31 (14%)	8/23 (35%)
<40 SSS	9/12 (75%)	12/14 (86%)
>4 failed organs	1/13 (8%)	7/21 (33%)[c]

[a] Pretreatment conditions of patients in PMX treated group are significantly more severe than those of the control group in terms of the total and number of failed organs.
[b] $p < 0.05$ between control and PMX groups.
[c] $p < 0.01$ between control and PMX groups.

Endotoxin adsorption

Endotoxin adsorption by the PMX-F column was evaluated by comparing the endotoxin concentrations at the entrance to and exit from the column in the circuit. There were significant differences between the 2 concentrations at both 30 and 60 min after the start of DHP. Table 5 shows the results at 60 min. In the 14 samples evaluated, the decrease of endotoxin concentration between the entrance and that of the exit was significant.

Improvement of cardiovascular parameters

Systolic arterial blood pressure

In the 7 sessions evaluated with patients in septic shock with systolic blood pressure of less than 90 mm Hg, the pressure significantly increased from 78.2 ± 2.3 to 93.2 ± 4.0 mm Hg after DHP ($p < 0.01$) and to 92.4 ± 5.3 mm Hg on the following day ($p < 0.01$). In the 28 sessions evaluated with patients whose blood pressure was maintained above 90 mm Hg with an inotropic agent or a vasopressor, usually dopamine, dobutamine, or noradrenalin, the mean blood pressure was 127.8 mm Hg immediately before the treatment but significantly increased to 135.8 mm Hg immediately after the treatment ($p < 0.05$) and was 133 mm Hg on the following day ($p < 0.05$). The vaso-

TABLE 5. *Endotoxin concentrations*

	PMX treated pg/ml (mean ± SE)	
	Prior to PMX	After PMX
Plasma endotoxin (session)	83.7 ± 26.7	56.4 ± 27.9[a]
Survivors	90.0 ± 50.0	18.0 ± 3.0
Nonsurvivors	119.0 ± 55.0	121.0 ± 84.0
Change by PMX column	(Entrance)	(Exit)
60 min after treatment	128.8 ± 141.0	114.9 ± 127.8[a]

[a] $p < 0.05$ between level prior to PMX and level after PMX or entrance and exit levels of PMX column. PMX treatment significantly reduces plasma endotoxin ($p < 0.05$). Endotoxin concentrations before the entrance and after the exit of the PMX-F column show a significant difference ($p < 0.05$). There is no significant difference between the control and PMX treated groups in the pretreatment levels.

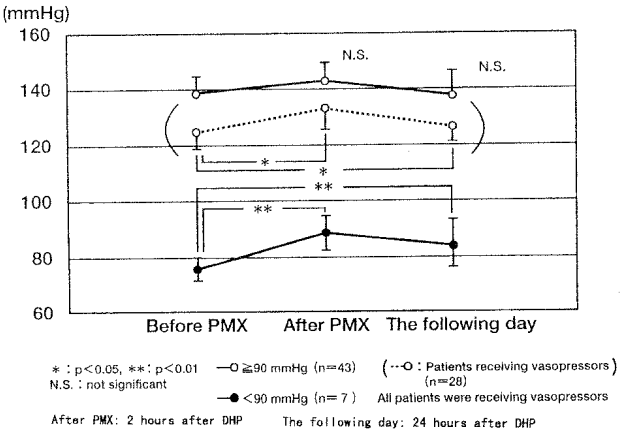

FIG. 1. Systolic blood pressure before and after PMX treatment is shown.

active agents could be discontinued after the treatment in 6 patients, and their dosages could be reduced in 9 (Fig. 1).

Cardiac index

The CI results were divided into 3 groups, hyperdynamic, normodynamic, and hypodynamic, according to the pre-DHP CI of each patient. In the 10 sessions evaluated with patients showing a normal CI of 2.5–4.5 L/min/m^2, the CI was 3.7 ± 0.1 L/min/m^2 before treatment and increased to 4.3 ± 0.4 L/min/m^2 immediately after treatment and to 4.4 ± 0.3 L/min/m^2 on the following day ($p < 0.05$). In the 18 sessions evaluated with patients in a hyperdynamic state showing a CI of 4.5 L/min/m^2 or more, the CI was 6.0 ± 0.2 L/min/m^2 prior to the PMX treatment and slightly increased to 6.3 ± 0.3 L/min/m^2 immediately after the treatment, but significantly decreased to 5.6 ± 0.3 L/min/m^2 on the following day ($p < 0.01$) (Fig. 2).

Systemic vascular resistance

The SVR results were also divided into 3 groups in the same manner as the CI results (Fig. 3). In the 18 evaluated sessions in which patients had initial SVR

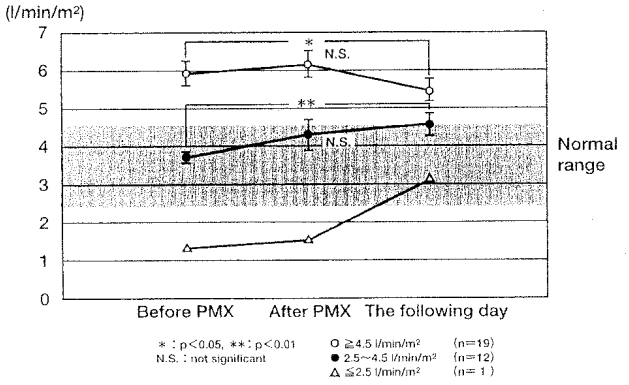

FIG. 2. CI before and after PMX treatment is shown.

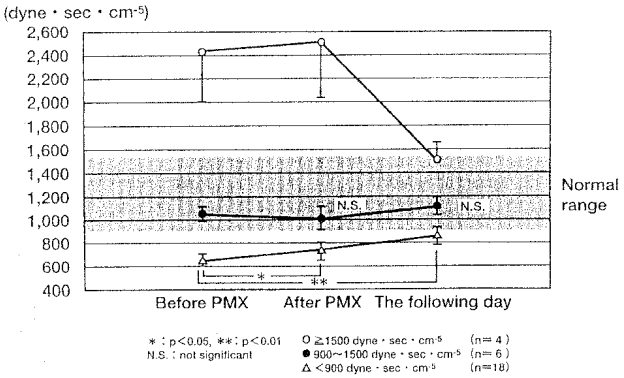

FIG. 3. SVR before and after PMX treatment is shown.

(dyne·s·cm^{-5}) of 900 or less, the mean value was 638 ± 37 dyne·s·cm^{-5} immediately before treatment but significantly increased to 717 ± 54 dyne·s·cm^{-5} after PMX treatment ($p < 0.05$) and further increased to 773 ± 49 dyne·s·cm^{-5} on the following day ($p < 0.01$). The mean pretreatment value in all patients was 1,014 ± 92.4 dyne·s· cm^{-5}.

Oxygen consumption index

The results of $\dot{V}O_2I$ were similarly divided into 2 groups, one with $\dot{V}O_2I$ <140 ml/min/m^2 and the other ≥140 ml/min/m^2. In the 11 evaluated sessions with patients with a $\dot{V}O_2I$ level of less than 140 (ml/min/m^2), the mean value was 100.7 ± 9.4 ml/min/m^2 prior to PMX treatment but markedly increased to 150.8 ± 22.5 ml/min/m^2 immediately after treatment ($p < 0.01$) although it declined somewhat to 135.1 ± 17.7 ml/min/m^2 on the following day (Fig. 4).

Metabolic change (arterial ketone body ratio)

These were divided into normal (≥0.7) and abnormally low (<0.7). The normal group values were not changed significantly by PMX treatment, but the abnormally low group showed a significant increase after treatment ($p < 0.05$) (Fig. 5).

No bleeding nor aggravation of bleeding tendency

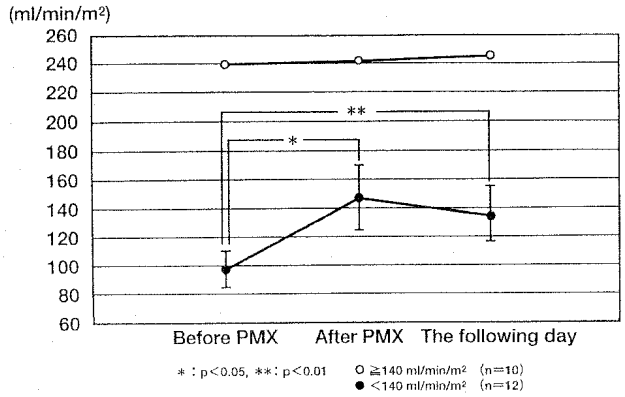

FIG. 4. $\dot{V}O_2$ I before and after PMX treatment is shown.

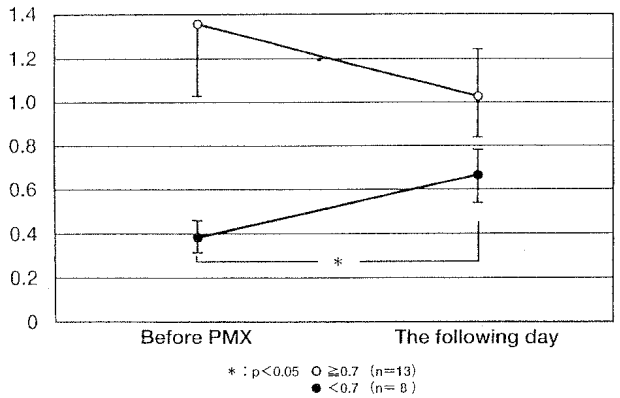

FIG. 5. AKBR before and after PMX treatment is shown.

was observed in any of the patients during the treatment with DHP even with those whose platelet counts were as low as $2 \times 10^4/mm^3$.

DISCUSSION

This clinical study was not double blind. PMX treatment was applied to those patients who did not respond to conventional treatment and had no other treatment available for cure. The control group consisted of patients with endotoxemia or sepsis with organ failure who were treated by conventional methods in the same institutions during the same period as the PMX treated group. The patients in the PMX treated group were in much graver condition than those in the control group. Furthermore, the use of a sham device necessary for a truly double blind study is hazardous as we have reported (18) and would have been ethically unacceptable.

Even though the PMX group had patients who were more severely ill than those in the control group in terms of the extent of MOF and SSS, the survival rate in the PMX group was significantly higher than in the control group, 54% versus 38% ($p < 0.05$). PMX treatment was effective even in severe cases with SSS of over 40.

The method used to measure endotoxins in this study was a new PCA method in which 1,3-β-glucan is eliminated and the endotoxins that precipitate with protein are recovered (16). The plasma endotoxin levels in the DHP circuit significantly decreased after passage through the PMX-F column at 30 and 60 min after the initiation of PMX treatment, indicating that the column successfully adsorbed circulatory endotoxin. Furthermore, the plasma endotoxin level of the patients was significantly decreased after PMX treatment. The decrease in endotoxin concentration after PMX treatment was significantly greater in those who survived than in those who did not. Thus, PMX treatment was effective both in achieving a higher survival rate compared to conventional treatment in patients with severe endotoxemia and in reducing plasma endotoxin.

PMX treatment markedly improved the cardiovascular function in sepsis. A study using endotoxin infusion in humans by Suffredini et al. (19) and a review by Parrillo et al. (20) have shown that endotoxin causes marked changes in cardiovascular parameters such as BP, CI, and SVR. These parameters were abnormal in our cases as well, but PMX treatment improved them so much that after the treatment, the use of vasoactive agents was discontinued in 6 patients and the dosage was reduced in another 9. Even when the patients were in shock, PMX treatment significantly elevated the arterial pressure to over 100 mm Hg in the survivors although not in the nonsurvivors. Even in normotensive patients in whom the pressure was maintained by vasosuppressors, the blood pressure was improved significantly. The CI of patients in a hyperdynamic state also significantly decreased. Hyperdynamic patients with SVR of 900 $dyne \cdot s \cdot cm^{-5}$ or less also showed significant improvement immediately after DHP.

The $\dot{V}O_2I$ value also showed a significant increase after PMX treatment in the patients who had abnormally low values (<140). The AKBR, which is an indicator of the cell metabolism, is reported in MOF and septic shock (15). However, in our study, DHP with the PMX-F column improved the AKBR in those with abnormally low values. All the symptoms improved by PMX treatment were similar to those observed after endotoxin infusion in humans (21) and clinically in septic shock. This can be explained by the decrease in the endotoxin concentration in the blood by PMX treatment.

This paper reports a reduction in the plasma endotoxin concentration by treatment for endotoxemia and demonstrates that a reduction of plasma endotoxin can lead to the improvement of symptoms.

The correlation between the amount of endotoxins removed and the degree of improvement in the cardiovascular parameters suggests that endotoxin removal with PMX could ameliorate septic conditions by the removal of even small amounts of endotoxin. Rothstain et al. (22) showed in mice both a marked potentiation of the effect of tumor necrosis factor (TNF) by a small amount of endotoxin and the lethal effects of the simultaneous presence of sublethal doses of TNF and endotoxin. It would seem, then, that even a small amount of endotoxin can play a key role in the presence of cytokines and mediators in the development of symptoms of endotoxemia. Endotoxin can be active at a level of several

pg/ml in human plasma or whole blood in the presence of CD14 or LPS binding protein (23). This paper demonstrated that a very small amount of endotoxin at the level of pg/ml can still be toxic for the host.

Therefore, a reduction in endotoxin concentration may be effective in patients with toxic symptoms caused by endotoxins or bacteria. In recent years, anti-LPS antibody treatments developed by Centocor, Inc. (6) and by Xoma, Inc. (6) have shown significant improvement of the survival rate of gram-negative sepsis patients at 28 days after treatment. However, endotoxemia was not confirmed in the patients in their studies, and none of the parameters used, such as BP, HR, etc., showed any significant improvement during a 72 h period after the administration of the antibody (24).

In contrast, we show in this study that PMX treatment was effective even in patients who did not respond to conventional treatment methods, and the beneficial effects were evident within 24 h. The effectiveness of PMX treatment shown in this study may also serve to explain why PMX treatment was effective on the patients with severe sepsis.

One potentially serious side effect of PMX treatment is a decrease in the platelet count. However, none of the patients showed aggravation of coagulopathy or a bleeding tendency after DHP. Further studies are needed to determine the optimal duration and frequency of DHP.

Acknowledgments: This study was supported by a Grant-in-Aid for Development of Scientific Research (6287005) from the Japanese Ministry of Education and by Toray Industries, Inc. We also appreciate Kazuhiro Moriyama and Akira Yokoyama for their role in the adjustment of data.

REFERENCES

1. Tani T, Oka T, Hanasawa K. The treatment of cancer by direct hemoperfusion with an endotoxin immobilized fiber. In: Oda T, ed. *Therapeutic plasmapheresis (IV).* New York: Schattauer 1985:177–81.
2. Ruokonen E, Takala J, Kari A, Alhava E. Septic shock and multiple organ failure. *Crit Care Med* 1991;19:1146–51.
3. Wolff SM. Monoclonal antibodies and the treatment of gram-negative bacteremia and shock. *N Engl J Med* 1991;324:486–8.
4. Ziegler EJ, Fisher CJ, Sprung CL, Straube RC, Sadoff JC, Foulke GE, Wortel CH, Mitchell PF, Dellinger RP, Tand NNH, Allen IE, Berger HJ, Knatterud GL, LoBuglio AF, Smith CR, and the HA-1A Sepsis Study Group. Treatment of gram-negative bacteremia and septic shock with HA-1A human monoclonal antibody against endotoxin. *N Engl J Med* 1991;324:429–36.
5. Quezado ZMN, Banks SM, Natanson C. New strategies for combatting sepsis: The magic bullets missed the mark . . . but the search continues. *Tiblech Febrary* 1995;13:56–63.
6. Newton BA. A fluorescent derivative of polymyxin: Its preparation and use in studying the site of the antibiotic. *J Gen Microbiol* 1955;12:226–36.
7. Niwa M, Umeda M, Ohashi K. A novel endotoxin binding substance, polymyxin sepharose. *Jpn J Med Sci Biol* 1982;35: 114–5.
8. Endo Y, Hanasawa K, Tani T, Kodama M. The antimicrobial and the endotoxin detoxifying of activities polymyxin-B immobilized fiber. In: Ishigami J, ed. *Recent advances in chemotherapy.* Tokyo: University of Tokyo Press 1985:257–8.
9. Kodama M, Hanasawa K, Tani T. New therapeutic method against septic shock. Removal of endotoxin using extracorporeal circulation. In: Friedman H, Klein TW, Nakano M, Nowotny A, eds. *Endotoxin.* New York and London: Plenum Press. 1989:953–65.
10. Hanasawa K, Tani T, Kodama M. New approach to endotoxin and septic shock by means of polymyxin B immobilized fiber. *Surg Gynecol Obstet* 1989;168:323–31.
11. Goris JA, Boekhorst TPA, Nuytinck JKS, et al. Multiple-organ failure. *Arch Surg* 1985;120:1109–15.
12. Stevens LE. Gauging the severity of surgical sepsis. *Arch Surg* 1983;118:1190–2.
13. Aoki H, Kodama M, Tani T, Hanasawa K. Treatment of sepsis by extracorporeal elimination of endotoxin using polymyxin B-immobilized fiber. *Am J Surg* 1994;167:412–7.
14. Endo Y, Tani T, Oka T, Kodama M. Application of protease inhibitor to hemoperfusion and plasma exchange as a regional anticoagulant. *Trans Am Soc Artif Intern Organs* 1985;31: 429–32.
15. Shimahara Y, Kohno Y, Ukikusa M, et al. Significance of mitochondrial enhancement in endotoxin shock. *Eur Surg Res* 1980;12:127–8.
16. Inada K, Endo S, Takahashi K, Suzuki M, Narita T, Yoshida T, Suda H, Komuro T, Yoshida M. Establishment of a new perchloric acid treatment method to allow determination of the total endotoxin content in human plasma by the limulus test and clinical application. *Microbiol Immunol* 1991;35: 303–14.
17. Obayashi T, Tamura H, Tanaka S, Ohki M, Takahashi S, Kawai T. A new chromogenic endotoxin-specific assay using recombined limulus coagulation enzymes and its clinical applications. *Clin Chem Acta* 1985;149:55–65.
18. Hanasawa K, Tani T, Oka T, Yoshioka T, Endo Y, Matsuda K, Kodama M. Comparison among three adsorbents in detoxifying endotoxin. *Jpn J Artif Organs* 1986;15:1414–8.
19. Suffredini AF, Fromm RE, Parker MM, Brenner M, Kovacs JA, Wesley RA, Parrillo JE. The cardiovascular response of normal humans to the administration of endotoxin. *N Engl J Med* 1989;321:280–7.
20. Parrillo JE, Parker MM, Natanson C, Suffredini AF, Danner RL, Cunnion RE, Ognibene FB. Septic shock in humans. *Ann Intern Med* 1990;113:227–42.
21. Kuhns DB, Alvord WG, Gollin JI. Increased circulating cytokines, cytokine antagonists, and E-selectin after intravenous administration of endotoxin in humans. *J Infect Dis* 1995;171: 145–52.
22. Rothstein JL, Schreiber H. Synergy between tumor necrosis factor and bacterial products causes hemorrhagic necrosis and lethal shock in normal mice. *Proc Natl Acad Sci USA* 1988; 85:607–11.
23. Dedrick RL, Conlon PJ. Prolonged expression of lipopolysaccharide (LPS)-induced inflammatory genes in whole blood requires continual exposure to LPS. *Infect Immun* 1995;63: 1362–8.
24. Kett DH, Quartin AA, Sprung CL, Fisher CJ Jr, Pena MA, Heard SO, Zimmerman JL, Albertson TE, Panacek EA, Eidelman LA, Schein RMH. An evaluation of the hemodynamic effects of HA-1A human monoclonal antibody. *Crit Care Med* 1994;22:1227–34.