Research

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Treatment of sepsis by plasma endotoxin removal: hemoperfusion using a polymyxin-B immobilized column

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Summary A Phase I clinical trial was carried out to evaluate the effects of endotoxin elimination by direct hemoperfusion using a cartridge containing chemically immobilized polymyxin B fibers (PMX-F). Thirty-seven endotoxemic patients with multiple organ failure (MOF) were treated. Direct hemoperfusion for 2 h using a PMX-F column was performed (PMX). PMX could remove circulating endotoxin from severely endotoxemic patients. Plasma endotoxin level was significantly decreased between its inlet and outlet level of PMX-F column ($P < 0.01$). The mean plasma endotoxin concentration of all PMX ($n = 51$) was 83.7 ± 26.7 pg/ml before PMX, and significantly decreased to 56.4 ± 27.9 after ($P < 0.01$). Plasma endotoxin was significantly decreased in survivors, but that of non-survivors did not change with PMX ($P < 0.05$). Body temperature, blood pressure, cardiac index, systemic vascular resistance and the oxygen consumption index improved significantly after PMX. Inotropic and vasopressive drugs were discontinued or reduced with PMX. PMX treatment showed a correlation between reduction of plasma endotoxin level and the improvement of septic syndrome, especially cardiovascular impairments. Also, this new therapy seemed to influence the outcome of severe sepsis or septic MOF patients with endotoxemia.

INTRODUCTION

Between 100 000–300 000 patients die of sepsis in the US annually[1,2] and the overall mortality in Japan has been estimated to be 30 000–50 000. The mortality rate of patients with sepsis and MOF has been reported to be over 70%.[3] Endotoxins, produced by Gram-negative bacteria, act on macrophages and other cells, producing a variety of mediators, including interleukin-1 (IL-1) and tumor necrosis factor-alpha (TNFα), which are associated with the clinical symptoms and signs of sepsis.[4] In Gram-positive infection, especially staphylococcal infection, the toxicity of toxic shock syndrome toxin-1 was reported to be increased 50 000-fold in the presence of a small amount of endotoxin.[5]

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There have been many recent reports on the development of monoclonal antibodies and antagonists against various pro-inflammatory cytokines for the treatment of sepsis. An anti-TNFα antibody[6] and an IL-1 receptor antagonist[7] failed to show efficacies in clinical studies. An anti-IL-6 antibody[8] has been also reported to be useful, but has not yet been clinically applied. These biological response modifiers, however, act on secondary substances released by host cells after stimulation by endotoxins.

The removal or detoxification of endotoxin is a relatively recent therapeutic approach. Anti-lipopolysaccharide (LPS) monoclonal antibodies have been developed against endotoxin, however, to date, clinical studies have shown no obvious clinical benefits.[9,10] Polymyxin B has long been known to specifically bind to endotoxins.[11] However, polymyxin B has renal and neural toxicity, which precludes intravenous administration. In 1982, Duff[12] and Niwa[13] reported a polymyxin-B immobilization technique to remove endotoxin, but they could not proceed to an in vivo study.

In 1983, we developed a method to adsorb endotoxins in the blood, without releasing the polymyxin-B itself, by chemically immobilizing polymyxin-B (PMX-F) onto insoluble fibrous carriers. In vitro endotoxin adsorption experiments showed that 0.52 mg endotoxin/g PMX-F was adsorbed. The treatment of sepsis in both endotoxin and *Escherichia coli* injected dogs followed by arterio-venous direct hemoperfusion (DHP) using a column that was filled with these PMX-F significantly increased the survival rate whereas no beneficial effects were observed with control columns not containing polymyxin B (polystyrene fiber resin alone).[15] PMX-F has also been used together with antibiotics and non-specific immune stimulation in a rodent sepsis model with an additional survival benefit, as compared to using any of the agents individually.[16]

A DHP method using activated charcoal for the removal of endotoxins from the circulation has also been reported.[17] Although this method requires a large amount of activated charcoal and is difficult to apply clinically for use in the adsorption of endotoxins, we compared the effects of PMX-F, anion exchange resin, and activated charcoal on endotoxin adsorption and found PMX-F to be the most effective.[18] The purpose of the present study was to define the safety and capacity of this method of endotoxin adsorption in a clinical trial, by way of the change of parameters with DHP using a PMX-F containing column for treatment of humans with sepsis (Toraymyxin®).

MATERIALS AND METHODS

Design

This study is a prospective, multicenter, non-randomized Phase I clinical trial of the use of DHP with Toraymyxin® in patients with severe sepsis or sepsis with MOF.

The clinical study was carried out in the intensive care units at eight university hospitals during a 2 year period beginning in February, 1989. The protocol and data were managed by two coordinators. Conventional treatments including, resuscitation, antibiotics, hemodynamic monitoring, and organ support in the intensive care unit were continued and remained unchanged. Endotoxin adsorption with DHP using Toraymyxin® is named PMX.

Patients

PMX was performed in patients with culture confirmed infection or those positive for plasma endotoxin and who manifested the sepsis syndrome as defined by Bone.[19] Usually, antibiotics had been used before the initiation of PMX, and patients who were already receiving vasoactive agents to maintain blood pressure or mechanically ventilated were included. During the evaluation of the influences of PMX, conventional treatments were continued as appropriate. MOF was diagnosed according to the MOF diagnostic criteria proposed by Goris et al.[20] The severity of infection was judged according to the septic severity scores (SSS).[21] In evaluation of the level of consciousness, 3 points were given to patients under sedation. Patients younger than 18 years or older than 85 years were excluded. Proper informed consent (oral or written) was obtained from each patient or family.

PMX (endotoxin adsorption with DHP)

PMX-F was produced by immobilizing polymyxin-B on 0.5% weight ratio polystyrene fiber by covalent bonding;[14,15] examination confirmed a firm binding of polymyxin-B without its being released.[15] The polymyxin column for DHP was filled with 53 g PMX-F containing 0.5 vol% of polymyxin-B and saline solution. Columns were stored at room temperature and sterilized using an autoclave supplied by Toray Industries, Inc. (Tokyo, Japan). DHP was performed using an ordinary hemodialysis circuit. Prior to DHP, the column was washed by perfusion with 4 l of physiological saline. Treatment in patients was initiated when sepsis syndrome and organ failure did not respond to conventional measures. For venous access, a double lumen catheter was inserted into the femoral vein, and blood was drawn from the vein and returned to it. The blood flow volume was about 80–100 ml/min in most patients. Each individual session of DHP lasted for 2 h. Heparin, low molecular weight heparin, or nafamostat mesilate (NM)[22] (Torii Pharma Co. Ltd., Tokyo, Japan) were used as anticoagulants. DHP sessions were repeated as deemed necessary by the individual's clinical response.

NM[22] (6-amidino 2-naphthyl 4-guanidino benzoate dimethane sulfonate; molecular weight 539.58) markedly inhibits trypsin, thrombin, plasmin, kallikrein and the

classical complement pathway. NM is a serine protease inhibitor that exerts its anticoagulatory effects primarily by inhibiting thrombin. The half-life of NM is 8 min and anticoagulatory effects were observed only in the extracorporeal circuit. The activated partial thromboplastin time (A-PTT) or prothrombin time (PT) were only[23] negligibly prolonged. In Japan, NM is approved as an anticoagulant for extracorporeal circulation in patients with a tendency to bleed.

Patient evaluation

The survival rate was calculated based on outcome 2 weeks after the termination of the last DHP session. To assess the effects of the PMX, various parameters were monitored before, immediately after, and on the day after PMX. Cardiac index (CI), systemic vascular resistance (SVR) and oxygen consumption index (VO_2I) were measured using pulmonary artery catheters. Body temperature (BT), blood pressure (BP) and heart rate (HR) were also recorded. Platelet counts were serially determined to monitor the safety of the procedure. Plasma endotoxin concentrations were measured before, immediately after, and on the day after PMX, as well as at the inlet and outlet of the Toraymyxin 30 min after DHP was begun.

Endotoxin assay

A new modification of the perchloric acid (PCA) method[24] was used to measure endotoxin. Previously, the plasma protein in a sample was removed by sedimentation using conventional PCA.[25] The protein sediment was redissolved in the new PCA method, since there was a large amount of endotoxin in the protein sediment of patients with endotoxemia. Plasma was first mixed with caustic soda, heated at 37°C for 5 min, mixed with PCA at half the amount used in the conventional PCA method, and then heated for 10 min. In the new PCA method, the resulting sediment was dissolved in caustic soda. The treated sample was mixed with Tris buffer to adjust its pH (finally diluted to 1/10). The sample (100 µl) was then mixed with 100 µl of Endospecy® and heated at 37°C for 30 min. After diazocoupling, absorbance at 545 nm was measured. The upper limit of normal was 10 pg/ml and the sensitivity was 0.003 EU/ml (1 pg/ml = 0.0029 EU).

Endospecy® (Seikagaku Kogyo Co. Ltd, Tokyo, Japan) is a factor G-depleted lysate that does not react with (1-3)-β-glucan and does not promote reactions in the factor G systems, another pathway in the reaction process of endotoxins and *Limulus* lysate. Therefore, this kit does not produce positive reactions in the plasma of patients with fungal sepsis, hemodialysis patients after using a cellulose membrane, or in patients receiving anticancer drugs derived from plant polysaccharide.[25]

Table 1 Characteristics of patients

Characteristic	PMX (n = 37)
Age (18–83 years)	59.2 ± 2.9
Male:female	23:10
Underlying diseases	
Neoplasm	22
Collagen disease	3
Diabetes mellitus	2
Liver cirrhosis	2
Cardiovascular disease	11
Respiratory disease	4
CNS disease	4
Recent surgery	19
Recent trauma	9

n = number of patients.

Statistical methods

All values obtained before, immediately after, and on the day after PMX were expressed as mean ± SE.

Statistical analysis was performed on all of the parameters that were obtained. The values obtained before and after treatment or on the following day were analyzed using Wilcoxon's nonparametric t test in which there is correspondence to pre-treatment values. All P values of less than 0.05 were considered to be significant.

RESULTS

DHP was performed 63 times in 42 patients (33 males and 9 females) between the ages of 18 and 84 years (mean 61.4 years). It was done once in 29 patients, twice in 9, 3 times in 3, and 7 times in one patient. Heparin was used 12 times as the anticoagulant, low-molecular weight heparin 3 times, and NM 48 times. Thirty-seven out of the 42 patients had documented endotoxemia prior to PMX, and these patients underwent 51 DHP sessions. As shown in Table 1, underlying diseases were both varied and multiple, with malignant neoplasms

Table 2 Characteristics of infection

Source	PMX (n = 37)
Abdominal cavity	18 (7)
Respiratory system	10
Biliary tract	4
Cardiovascular system	3
Central nervous system	3
Others	5
Gram-negative infection	22
Gram-positive infection	14
Fungal infection	7
Not detectable	9

n = number of patients.

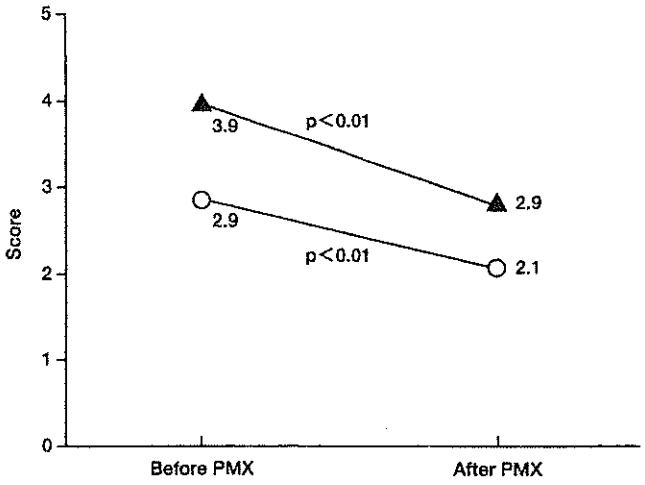

Fig. 1 Improvement of cardiovascular point of SSS with PMX. Open circles, all cases ($n = 37$); closed triangles, in shock ($n = 28$). Both groups show a significant decrease ($P < 0.01$).

being frequently observed. The most commonly isolated micro-organisms were Gram-negative bacteria. Table 2 shows that the most frequent site of infection was in the abdomen, followed closely by pneumonia.

MOF was diagnosed according to Goris's score in 36 of the 37 PMX treated patients: 28 were in shock, 26 received either inotropics, vasopressors, or both to maintain blood pressure and cardiac output. Twenty-eight patients required mechanical ventilation. Coagulation disorders and acute renal dysfunction was seen in 15 and 19 patients, respectively, and hepatic failure in 25 patients. The mean SSS of 46.4 ± 2.9 in the PMX treated patients. The number of failed organs was 3.8 ± 0.4 (Table 3).

Table 3 Severity of sepsis at entry in the patients with endotoxemia

Variable	PMX ($n = 37$)
Septic severity score (SSS)	46.4 ± 2.9
Number of failed organs	3.8 ± 0.4
Shock and/or use of vasopressor	28
Endotracheal intubation and/or respiratory failure	28
Acute renal failure	19
Hepatic failure	25
Coagulation disorder	15

n = number of patients.

Survival rate

Of the 37 endotoxemic patients receiving PMX, 20 (54%) survived more than 14 days after treatment. In patients with SSS under 40, the survival rate was 86% (12/14), but in patients with over 40 SSS the survival rate was 35% (8/23).

Endotoxin concentration

The plasma endotoxin concentration was 10–30 pg/ml in 18 patients (49%) and their survival rate was 67% (Fig. 1); Gram-negative infection was observed in 9 of these 18, and Gram-positive infection in 6. The plasma endotoxin concentration was 30 pg/ml or more before PMX in 19 patients (51%) and their survival rate was 43% (< 30–99 pg/ml) and 25% (100–1000 pg/ml), respectively (Fig. 1) In these, Gram-negative infection was observed in 13 and Gram-positive infection in 8 patients. In the 7 patients showing a pretreatment plasma endotoxin concentration of 10 pg/ml or more, the changes in parameters were evaluated in their 51 DHP sessions. The mean value of plasma endotoxin immediately before PMX was 83.7 ±

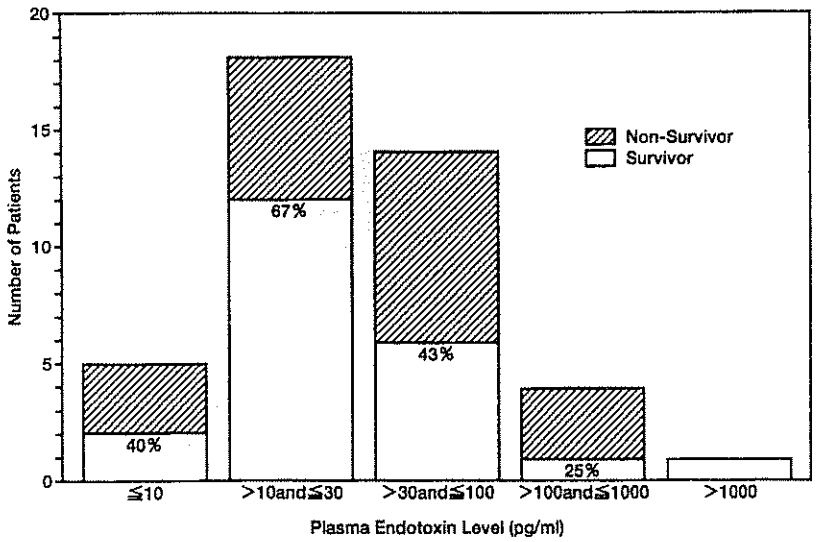

Fig. 2 Plasma endotoxin concentration before PMX and survival. Survival rate was calculated on the 14th day after PMX.

26.7 pg/ml, but significantly decreased to 56.4 ± 27.9 pg/ml ($n = 51$) immediately after PMX ($P < 0.01$). In the 25 samples which could be obtained from patients the following day, these values further decreased from43.4 ± 10.4 before PMX to 28.5 ± 4.1 pg/ml ($P < 0.01$) (Fig. 2) In order to compare the concentration at the entrance of the column to that at the exit, samples were obtained 30 min after the initiation of DHP, In the 37 times that PMX was evaluated, the endotoxin concentration at the inlet of column was 27.0 ± 2.2 pg/ml, and that at the outlet was 22.0 ± 2.7 pg/ml ($P < 0.01$) the decrease was significant (Fig. 2). There was no significant difference in endotoxin concentration before PMX (90 ± 50 versus 119 ± 55 pg/ml) of the patients who survived or died. After PMX, however, those who survived ($n = 20$) demonstrated significantly less endotoxins than those who died ($n = 17$; 18 ± 3 versus 121± 84 pg/ml; $P < 0.05$; Fig. 3).

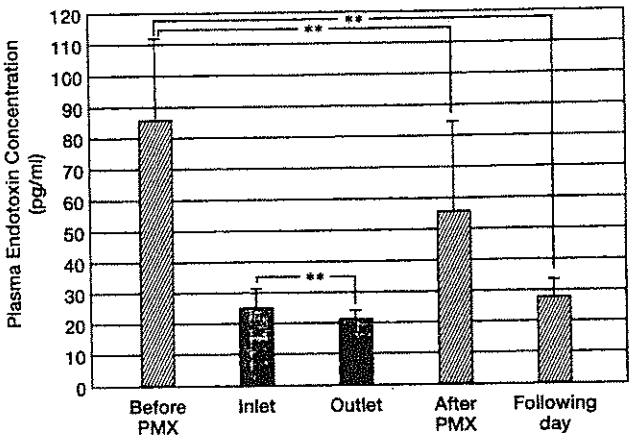

Fig. 3 Changes in blood endotoxin concentrations during PMX. The cross-hatched columns are systemic blood samples with an endotoxin concentration of more than 10 pg/ml. The mean value ± SE was 83.7 ± 26.7 pg/ml before PMX ($n = 51$). This level significantly decreased to 56.4 ± 27.9 pg/ml immediately after PMX (**$P < 0.01$), and to 28.5 ± 4.1 pg/ml ($n = 25$) on the next day (**$P < 0.01$). The dotted columns are the blood endotoxin concentrations at the inlet and outlet of the Toraymyxin® 30 min after the initiation of DHP ($n = 37$). The concentration was 27.0 ± 2.2 pg/ml at the inlet and 22.0 ± 2.7 pg/ml at the outlet ($P < 0.01$).

Hemodynamic parameters

The following parameters were evaluated before and after 51 times of PMX. As shown in Table 4, in 20 times of PMX with a pretreatment body temperature of 38°C or more, the mean value was 39.0 ± 0.2°C before PMX, but significantly decreased to 38.4 ± 0.2°C after PMX ($P < 0.01$) and further decreased to 38.0 ± 0.2°C the following day ($P < 0.01$). In 5 times of PMX with septic shock showing a systolic blood pressure of less than 90 mmHg, the mean value significantly increased from 78.2 ± 2.3 to 93.2 ± 4.0 mmHg after PMX ($P < 0.01$) and to 92.4 ± 5.3 mmHg the next day ($P < 0.01$). The cardiovascular point of SSS was significantly decreased in both the all and subgroup with shock with PMX (2.9 to 2.1 and 3.9 to 2.9, P < 0.01: Fig. 4). In 28 times of PMX in which blood pressure was maintained with an inotropic or vasopressor, the mean value was 127.8 ± 5.4 mmHg before PMX, but significantly increased to 135.8 ± 5.1 mmHg after PMX ($P < 0.05$), and was 133 ± 7.0 mmHg, maintaining the improvement the following day ($P < 0.05$). The vasoactive agents were administered in 22 patients, 9 cases were

Table 4 Results of parameters

Parameters	Number of DHP	Before PMX	After PMX	Following day
Temperature (°C)				
> 36.5	3	35.7 ± 0.1	35.7 ± 0.2	37.1 ± 0.1
36.5–38.0	24	37.2 ± 0.1	37.2 ± 0.1	37.1 ± 0.2
≥ 38.0	20	39.0 ± 0.2	38.4 ± 0.2**	38.0 ± 0.2**
Blood pressure (mmHg)				
> 90	5	78.2 ± 2.3	93.2 ± 4.0**	92.4 ± 5.3**
≤ 90	41	137.7 ± 5.4	143.6 ± 5.3*	138.8 ± 5.8
[≤ 90 drug (+)	28]	127.8 ± 5.4	135.8 ± 5.1*	133.0 ± 7.0*
CI (l/min/m²)				
2.5–4.5	10	3.7 ± 0.1	4.3 ± 0.4	4.4 ± 0.3**
≤ 4.5	18	6.0 ± 0.2	6.3 ± 0.3	5.6 ± 0.3**
SVR (dynes.s.cm^{-5})				
< 1500	3	1906 ± 173	1966 ± 103	1401 ± 147
900–1500	6	1038 ± 47	991 ± 91	1085 ± 70
≥ 900	17	638 ± 37	717 ± 54*	773.49**
VO_2I (ml/min/m²)				
< 140	10	248.6 ± 64.7	247.0 ± 73.7	251.7 ± 70.2
≥ 140	11	100.7 ± 9.4	150.8 ± 22.5**	135.1 ± 17.7

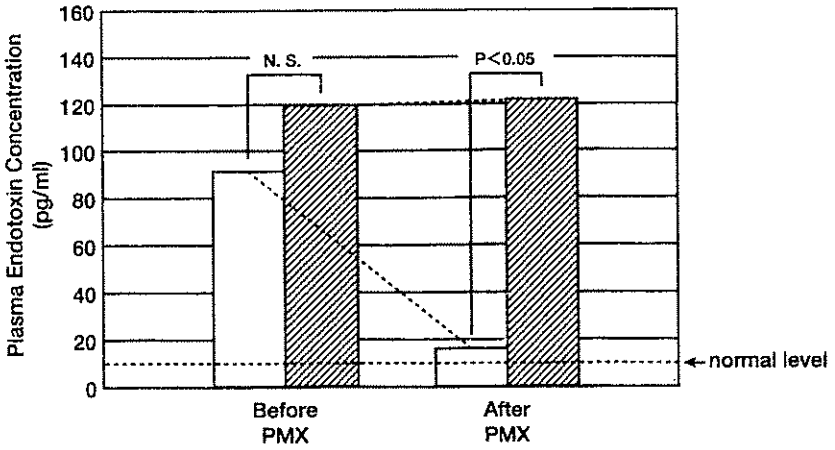

Fig. 4 Plasma endotoxin reduction after PMX. The white and cross-hatched columns are the endotoxin concentration of survivor group and of the group who died during the PMX treatment, respectively. Endotoxin concentrations of the groups were not significantly different before treatment (90 ± 50 versus 119 ± 55 pg/ml), but were after treatment (18 ± 3 versus 121 ± 84 pg/ml; $P < 0.05$).

discontinued during PMX and the dosage was reduced in 8 patients.

In 10 PMX in which patients showed a normal CI of 2.5–4.5 l/min/m^2, the mean value was 3.7 ± 0. 1 l/min/m^2 before PMX which increased to 4.3 ± 0.4 l/min/m^2 after PMX and significantly increased to 4.4 ± 0.3 l/min/m^2 the following day ($P < 0.05$). In 18 times of PMX in which patients had a CI of 4.5 or more, the mean CI was 6.0 ± 0.2 l/min/m^2 before PMX, slightly increased to 6.3 ± 0.3 after PMX, and decreased to 5.6 ± 0.3 l/min/m^2 the following day ($P < 0.01$). In 17 times of PMX in which patients showed an SVR of 900 dyne.s.cm^{-5} or less, the mean value was 638 ± 37 dyne.s.cm^{-5} before PMX which significantly increased to 717 ± 54 dyne.s.cm^{-5} after PMX ($P < 0.05$), and further increased to 773 ± 49 dyne.s.cm^{-5} the next day ($P < 0.01$). The mean pretreatment value in all PMX was 1014 ± 92.4 dyne.s.cm^{-5}.

Among those evaluated for VO_2I, 11 times of PMX had a VO_2I less than 140 ml/min/m^2. The mean value was 100.7 ± 9.4 ml/min/m^2 before PMX, but markedly increased to 150.8 ± 22.5 ml/min/m^2 after PMX ($P <$ 0.01), though it declined to 135.1 ± 17.7 ml/min/m^2 the following day (Table 4).

In 37 times of PMX, a platelet count of at least 50 000/ml prior to PMX, the mean value was 170 100 ± 32 000. This decreased to 101 000 ± 24 000 after PMX and rebounded to 121 000 ± 24 000 the following day. In 12 times of PMX, with a platelet count less than 50 000/ml, the mean value was 30 000 ± 7000/ml before PMX and slightly decreased to 25 000 ± 7000/ml after PMX, but increased to 37 000 ± 10 000/ml the next day. No untoward bleeding was observed in any patients during treatment.

DISCUSSION

In this Phase I clinical study of PMX treatment for sepsis, strict controls were not used because the majority of subjects in the study were in a very severe or life-threatening state. Furthermore, these very ill patients, have usually failed to survive even though they received conventional therapies, including various other forms of hemoperfusion such as hemofiltration, hemodialysis and plasma exchange.

The survival rate after PMX was 54% (20/37), which was impressive in a severely ill patient population such as this. This is much higher than that which has previously been reported in septic MOF,[3] with similar degrees of severity. The mean SSS score of the 37 endotoxemic patients was 46.4 ± 2.9. Stevens reported a mortality rate of 82% in patients who had an SSS score above 40.[21] Furthermore, the PMX decreased mortality even in severe cases in which an SSS of over 40 was determined. Except for endotoxin negative patients, outcome appeared to be improved when plasma endotoxin concentrations became lower. The data would suggest that endotoxin adsorption may be less beneficial in those without documented endotoxemia, such as those who had only the systemic inflammatory response syndrome (SIRS)[26] without documented concomitant infection.

A study on endotoxin infusion in humans by Suffredini[27] and a review by Parrillo[28] have shown that endotoxin caused marked changes in blood pressure, CI and SVR. In our study after PMX, the use of a vasoactive agents was discontinued in 9 patients with shock and the dosage was reduced in another 8 out of 22. The CI of

patients in a hyperdynamic state also significantly decreased. The mean SVR in all patients was nearly normal (1007 dyne.s.cm^{-5}), but was the result of an equal number of patients in hyperdynamic and hypodynamic states. Hyperdynamic patients, however, showed significant improvement after PMX. In patients who had a body temperature of 38°C or more, the mean temperature before treatment was nearly 39°C, but was significantly lower the following day. All symptoms which improved after PMX were similar to those observed after endotoxin infusion in humans or typical abnormal parameters during septic shock.[27,28]

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. The endotoxin concentrations determined by this method significantly decreased after treatment, and further decreased the next day; the elimination rate was 66%. In addition, the endotoxin concentrations at the inlet and outlet of the PMX-F column evaluated 30 min after the initiation of DHP also significantly decreased. These data according to the capacity of endotoxin removal of PMX will be supported by in vivo experiments using canine endotoxin shock model, which was previously reported else where. These changes in the column are not associated with the clinical course, but indicate that endotoxins were removed by Toraymyxin®.

The amount of removed endotoxins and the apparent degree of improvement in symptoms suggest that symptoms may be improved by the removal of even a small amount of endotoxin. Tracy et al[29] proposed that TNFα, a proinflammatory cytokine, is a primary mediator of septic shock. Other studies have shown both a marked potentiation of the effects of TNF by a small amount of endotoxin and the lethal effects of the simultaneous presence of TNF and endotoxin in mice.[30] Small amounts of endotoxin, therefore, may play a key role in the presence of other proinflammatory cytokines and mediators in the development of the symptoms of sepsis. A similar finding has been observed with endotoxins and TSST-1. The toxicity of TSST-1 in staphylococcal infection increases by 50 000 times in the presence of endotoxin.[5] In this study, PMX was performed in 8 patients with methicillin-resistant *Staphylococcus aureus* infection, and significant improvement was found. This improvement may be due to the removal of a small amount of endotoxin in Gram-positive or mixed infection and, therefore, such a reduction may be effective in patients with toxic symptoms caused by endotoxins or bacteria.

In recent years, the mechanisms of septic shock have been clarified, and the involvement of many cytokines has been suggested. Treatment methods to inhibit each cytokine have subsequently been reported. Anti-LPS antibody treatments have shown significant improvement of the survival rate of Gram-negative sepsis patients at 28 days after treatment, but endotoxemia was not defined in their studies.[9] Subsequent studies have shown a lack of benefits, including treatment directed against TNF[6] and IL-1.[7]

This multicenter Phase I study demonstrates a decrease in the plasma endotoxin concentration as the result of treatment for endotoxemia, and correlates this with a reduction in morbidity and improvement of several parameters in septic patients. It was shown that this treatment could be performed safely and its function was true. Further studies are needed to firmly establish efficacy in outcome, on the duration and frequency of endotoxin adsorption for patients in whom the initial reduction of plasma endotoxin with PMX is suspected to be insufficient. Randomized prospective clinical trials are warranted based on the encouraging results of endotoxin removal by DHP presented here.

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APPENDIX

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