World J. Surg. 25, 660–668, 2001
DOI: 10.1007/s002680020028

WORLD
Journal of
SURGERY

Correlation between Plasma Endotoxin, Plasma Cytokines, and Plasminogen Activator Inhibitor-1 Activities in Septic Patients

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Published Online: April 12, 2001

Abstract. The objective of this study was to evaluate the relation between the clinical and plasma parameters and the changes in plasma endotoxin activity with 2 hours of endotoxin-adsorbing therapy using polymyxin B (PMX). A total of 88 consecutive patients were admitted for PMX treatment of severe sepsis or septic organ failure. Standard supportive care was continued without alteration during PMX treatment. Endotoxin, tumor necrosis factor-α (TNFα), interleukin-6 (IL-6), IL-10, and plasminogen activator inhibitor-1 (PAI-1) activities and clinical parameters were measured before, immediately after, and the day after PMX treatment. The mean APACHE II and III scores were 24.2 ± 1.0 and 85.8 ± 3.0, respectively. The 2-week survival rate was 51.1%. In survivors, TNFα, IL-6, IL-10, and PAI-1 activities were significantly decreased during the 2-hour PMX treatment, the following day, or both times. There was no significant change in the parameters, except for TNFα, after PMX in nonsurvivors. In the subgroup whose plasma endotoxin decreased more than 30%, IL-6, TNFα, and PAI-1 significantly decreased after 2 hours of PMX or the following day (or both), but all four parameters in nonsurvivors showed no significant change. Hence PMX adsorbed plasma endotoxins and contributed to reductions in plasma proinflammatory cytokine levels and to improved clinical parameters during the 2-hour treatment. Changes in these parameters correlated with changes in plasma endotoxin activity in survivors whose plasma endotoxin levels were adequately reduced.

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Severe sepsis is thought to be the main cause of multiple organ failure (MOF). The outcome of MOF is serious, and its associated mortality is high even today [1]. Many new agents, such as anti-endotoxin monoclonal antibodies (HA-IA [2], E5 [3]) and cytokine antagonists [an anti-tumor necrosis factor (TNF) monoclonal antibody and an interleukin-1 (IL-1) receptor antagonist], have undergone clinical trials, but poor results have been reported [4]. The studies relating to anti-endotoxin antibodies did not measure plasma endotoxin activities. The issue of the importance of endotoxins in severe sepsis or septic MOF has been reopened by this series of clinical trials. Animal studies of endotoxemia or endotoxic shock have frequently been reported, but clinical reports especially regarding the relation between plasma endotoxin activities and clinical symptoms or parameters are rare [5].

Danner et al. emphasized the significance of plasma endotoxins in the outcome of sepsis in patients but did not mention any correlation with clinical parameters [6]. Wortel et al. reported that plasma TNF activity was significantly decreased after administration of HA-1A. This might be the only mention of a relation between changes in levels of a suspected endotoxin and a mediator in a clinical study without the actual plasma endotoxin values [7].

We invented an endotoxin-adsorbing device in 1982 [8], and

successful results in mongrel dogs were reported in 1989 [9]. A preliminary clinical trial was reported in 1994 [10]. Subsequent phase II clinical results showed that plasma endotoxin activity was significantly decreased by direct hemoperfusion with polymyxin B (Toraymyxin) (Toray Medical, Tokyo, Japan) (PMX) given over 2 hours and that clinical parameters could be returned to normal levels [11]. Subsequent clinical trials have not yet established the relation between changes in plasma endotoxin activities, clinical parameters, and mediators.

This clinical study aimed to define the mechanisms underlying the reducing plasma endotoxin levels with PMX. The relation between changes in plasma endotoxin levels and those in clinical parameters, plasma cytokine levels, and endothelial cell-related mediators were also investigated.

Methods

Study Design

This prospective consecutive study was conducted at 15 university or community hospitals in Japan from the beginning of February to December 1995. All parameters before treatment were compared with those after treatment and the following day. Standard treatments were given to MOF patients in intensive care units.

Subjects

The PMX regimen was given to patients who were confirmed to have an infection or their plasma endotoxin assay was positive, consisting of the systemic inflammatory response syndrome (SIDS) and at least one organ failure. Usually antimicrobial agents selected by the primary physician had been used before initiation of PMX treatment. Patients who were already receiving vasoactive agents to maintain blood pressure or who were under mechanical ventilation or receiving a sedative were maintained on these treatments during PMX. Standard treatments were continued, decreasing them when appropriate during the study. Patients less than 18 or more than 85 years of age were excluded. Proper informed consent (oral or written) was obtained from each patient or family.

PMX and Treatments

The polymyxin B immobilized fiber was produced by immobilizing polymyxin B on (0.5% weight ratio) polystyrene fiber using covalent bonding without its release. The column for direct hemoperfusion (DHP) contained 53 g of polymyxin B immobilized fiber, supplied by Toray Industries (Tokyo, Japan) [11]. DHP was performed using an ordinary machine for hemoperfusion and a circuit for hemodialysis. Before DHP the column was washed by perfusion with 4 liters of physiologic saline. For blood access, a double-lumen catheter was inserted into the femoral vein, and blood was withdrawn from the vein and returned to the vein (venovenous method). The blood flow volume was about 80 to 100 ml/min in most patients. DHP was performed two times at most. Each DHP continued for 2 hours. Heparin or nafamostat mesylate (NM) (Torii, Tokyo, Japan) was applied as an anticoagulant. NM is 6-amidino-2-naphthyl-4-guanidinobenzoate, or dimethanesulfonate (molecular weight 539.58). It markedly inhibits trypsin, thrombin, plasmin, kallikrein, and the classic complement pathway, and it is a serine protease inhibitor that exerts its anticoagulation effects primarily by inhibiting thrombin [12]. The half-life of NM is 8 minutes, and anticoagulation effects are observed only in the extracorporeal circuit. The activated partial thromboplastin time and prothrombin time are only negligibly prolonged.

Clinical and Laboratory Evaluation

The impairment of kidneys, liver, respiratory system, central nervous system, cardiovascular system, coagulation, and gastrointestinal systems were estimated by the Goris definition [13]. The severity of infection was evaluated according to the Sepsis Severity Score (SSS) [14]. The severity of the critical illness was evaluated by the number of failed organs, the Acute Physiology and Chronic Health Evaluation (APACHE) II [15] and III [16], and the Goris' score, and if the patient was in shock. For estimating the level of consciousness, 3 points in the Goris' score were assigned to patients under sedation. The survival rate, improvement in the Goris' score, and number of failed organs were calculated 2 weeks after PMX. Various parameters were monitored before, immediately after, and on the day after DHP. Body temperature (BT), blood pressure (BP), heart rate (HR), and the PaO_2/FiO_2 ratio (P/F) were also recorded. The endotoxin, TNFα, IL-6, IL-10, and plasminogen activator inhibitor-1 (PAI-1) activities were monitored in the same manner. Samples for plasma mediators were centrifuged at 3000 rpm for 10 min and stored in a frozen tube at -20°C until measurement.

Measurement of Endotoxin

With the conventional perchloric acid (PCA) method [17] (Endospecy; Seikagaku Kogyo, Tokyo, Japan), the plasma protein in a sample is removed by sedimentation. With the new PCA method [18] the protein sediment is redissolved, as there is a large amount of endotoxin in the plasma protein sedimented. Initially, plasma was mixed with caustic soda, heated at 37°C for 5 minutes, mixed with PCA at half the amount used for the conventional PCA method, and heated for 10 minutes. The treated sample was mixed with Tris buffer to adjust its pH (finally diluted 1:10). It was then subjected to the following endotoxin measurement. The sample (100 ml) was mixed with 100 ml of aliquot Endospecy and heated at 37°C for 30 minutes. After diazo coupling, absorbance was measured at 545 nm. The lower limit of normal is 9.8 pg/ml, and the sensitivity is 0.003 EU/ml (1 pg/ml = 0.0029 EU).

Endospecy is a factor G-depleted lysate that does not react with β-1,3-glucan and does not promote reactions in the factor G system, another pathway in the reaction process of endotoxins and limulus lysate. Therefore this agent does not produce 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 [18].

Cytokine Measurement

Cytokines were measured by an enzyme-linked immunosorbent assay (ELISA) described elsewhere. All assays were performed in duplicate. The kit for TNFα was from Amersham (Buckinghamshire, UK). This ELISA was sensitive to 5.0 pg/ml. The kit for IL-6 was from Fujirebio (Tokyo, Japan). This ELISA was sensitive to 4.0 pg/ml. IL-10 was measured by ELISA (Medgenix, Fleurus,

Table 1. Patient characteristics prior to PMX.

Characteristic	Data
No. of patients	88
Male	71.6%
Age (years), mean ± SE	61.9 ± 1.6
Plasma endotoxin detected	79.5%
Culture-positive	85.5%
Multiple organ failure	94.3%
In shock	89.8%
No. of failed organs (mean ± SE)	4.6 ± 0.2
Septic severity score (mean ± SE)	54.3 ± 1.7
APACHE II score (mean ± SE)	24.2 ± 1.0
APACHE III score (mean ± SE)	85.8 ± 3.0

PMX: endotoxin-absorbing therapy with polymyxin B; APACHE: Acute Physiology and Chronic Health Evaluation.

Belgium). The sensitivity of this assay was 10 pg/ml. PAI-1 was measured by an LPIA assay (Mitsubishi Chemical, Tokyo, Japan), which quantitatively measured whole antigen of PAI-1 in plasma. Briefly, the latex aggregation reaction was measured as the amount of turbidity at wavelength 950 nm. Anti-PAI-1 polyclonal antibody from the rabbit was obtained by sensitization with the PAI-1 antigen derived from the WI38VA13RA cell line. The low normal range of this assay was 2 ng/ml.

Statistical Methods

The effect of PMX on the severity of sepsis and MOF were compared prior to and 2 weeks after PMX. Statistical analysis was done with the Mantel-Haenszel test. Measured values before, immediately after, and on the day after treatment were expressed as the mean ± standard error (SEM). The values obtained before and after treatment or the following day were analyzed by the nonparametric test of Wilcoxon's *t*-test, in which there is correspondence to pretreatment values. All p values < 0.05 were considered significant.

Results

Patient Demographics

A total of 96 patients were enrolled in this study. Eight were excluded because of insufficient data. Of the 88 patients evaluated for PMX efficacy, 77 (80%) had plasma endotoxin values other than zero. Patient demographics are presented in Table 1. Most of the patients (94.3%) exhibited MOF according to the Goris definition, and 89.8% were in shock. The mean SSS was 54.3 ± 1.7, indicating severe sepsis.

The mean number of failed organs was 4.6 ± 0.2. Mean APACHE II and III scores were 24.2 ± 1.0 and 85.8 ± 3.0, respectively (Table 1). All data obtained prior to the initial PMX treatment showed the patients' illness to be severe. Among the culture-positive patients, gram-negative bacteria were isolated from 54 patients, gram-positive bacteria from 36, and fungus from 26.

Outcomes and Endpoints

Survival rates 2 and 4 weeks after PMX were 51.1% and 39.8%, respectively (Table 2). The Goris score significantly improved from 6.1 ± 0.3 to 3.0 ± 0.4 ($p = 0.0001$) 2 weeks after PMX. The mean number of failed organs also improved, from 4.6 ± 0.2 to 2.4 ± 0.3 ($p = 0.0001$). These significant improvements in the survivors were also apparent 2 weeks after PMX.

Table 2. Outcome: survival and changes in major clinical parameters.

Outcome of 88 patients	Data	p
Survival rate 2 weeks after	51.1%	
Survival rate 4 weeks after	39.8%	
Goris score (mean ± SE)		
Prior to PMX treatment	6.1 ± 0.3	0.0001
Two weeks after[a] (mean ± SE)	3.0 ± 0.4	
Mean no. of failed organs		
Prior to PMX treatment	4.6 ± 0.2	0.0001
Two weeks after[b] (mean ± SE)	2.4 ± 0.3	

Statistical analysis was conducted using Wilcoxon's signed rank test.
[a]Evaluable cases 2 weeks after PMX ($n = 43$).
[b]Cases evaluable for failed organs 2 weeks after PMX ($n = 44$).

Table 3. Disease severity and outcome.

Variable	Survivors ($n = 45$)	Nonsurvivors ($n = 43$)	p
SSS	48.2 ± 2.3	60.7 ± 2.0	0.0001
In shock	39 (86.7%)	40 (93.0%)	NS
Goris score	4.9 ± 0.3	7.2 ± 0.3	0.0001
No. of failed organs	3.9 ± 0.2	5.3 ± 0.2	0.0001
APACHE II score	22.1 ± 1.2	26.4 ± 1.5	0.0436
APACHE III score	80.2 ± 3.7	92.1 ± 4.6	0.0835(NS)

Results are given as the mean ± SE.
Survival was determined 2 weeks after completing PMX treatment. Statistical analysis was conducted using the Mann-Whitney U-test. The Sepsis Severity Score (SSS), Goris score, number of failed organs, and APACHE II score showed a significant difference between survivors and nonsurvivors.

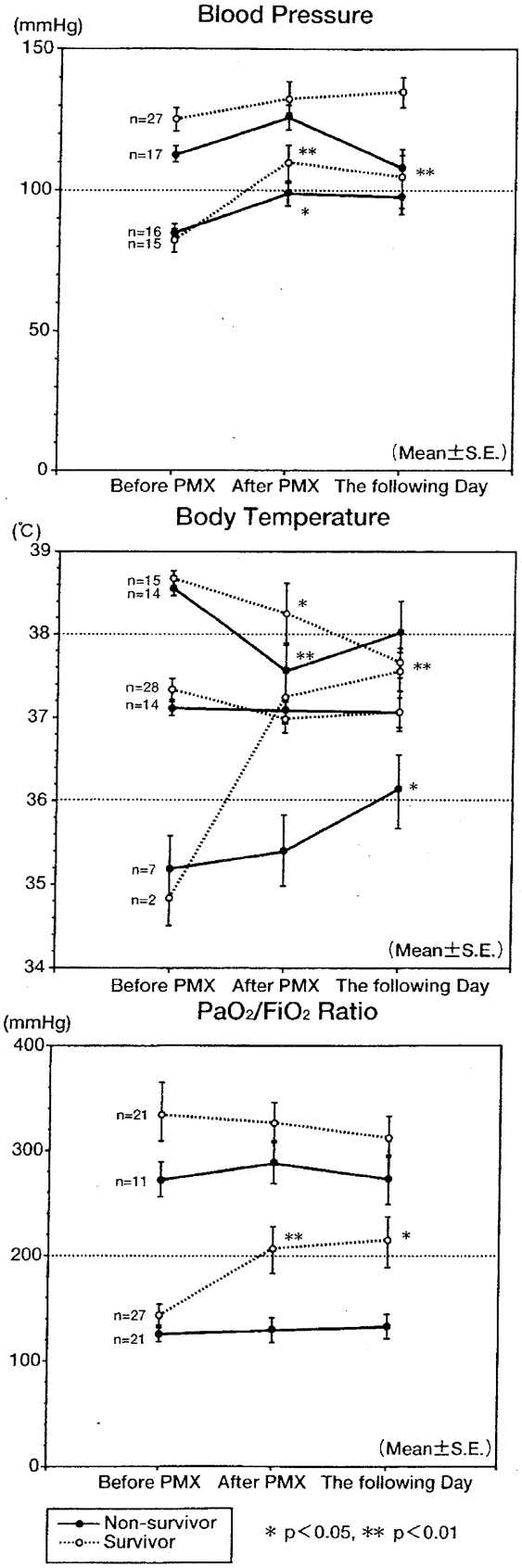
Clinical and Laboratory Factors

Comparisons of the data for survivors and nonsurvivors at 2 weeks are shown in Table 3. Scores for severity, such as the SSS, Goris score, number of failed organs, and APACHE II score, indicated a significantly less severe condition in survivors; there was no significant difference in APACHE III scores or the number of patients in shock.

Prior to the first PMX treatment, the mean BT, frequency of respiration, Pa/FiO_2 ratio, and BP were 37.4°C, 21.3/min, 215.9 mmHg, and 105.1 mmHg, respectively. The average endotoxin concentration was 48.2 ± 15.5 pg/ml, and the platelet count was 11.1×10^4/ml. Mean TNFα, IL-6, IL-10, and PAI-1 activities were 59.8 ± 10.1 pg/ml, 12,717 ± 3,179 units, 204.5 ± 40.6 pg/ml, and 422.2 ± 59.9 ng/ml, respectively.

Clinical Parameters and Outcome

Values for each clinical factor were divided into two or three groups—abnormally high, normal, abnormally low—and were then compared for survivors and nonsurvivors. BT values were divided into ≥ 38°C, ≥ 36° but < 38°C, and < 36°C. The group with a BT of 36° to 38°C did not show a significant temperature change after PMX, whereas the groups with abnormally high or



low BTs exhibited significant changes, to around 37.6°C. BP was divided into two groups, ≥ 90 mmHg and < 90 mmHg. In the < 90 mmHg group, the BP in survivors rose significantly to > 100 mmHg after PMX and remained there the following day. On the other hand, BP in nonsurvivors was not elevated to over 100 mmHg by PMX. In the ≥ 90 mmHg group, BP showed the tendency to rise after PMX in both groups. The PaO_2/FiO_2 ratio was divided into ≥ 200 mmHg and < 200 mmHg. In the < 200 mmHg group, changes in the PaO_2/FiO_2 ratio after PMX showed a trend similar to that of the BP values. Survivors showed a significant improvement in PaO_2/FiO_2 values, with increases to > 200 mmHg after PMX (Fig. 1).

Relation with Plasma Endotoxin Concentrations

Plasma mediator values for all three time points (before, after, the following day) were serially compared with data for survivors and nonsurvivors. All four mediators (TNFα, IL-6, IL-10, PAI-1) were significantly reduced in survivors after PMX or the following day. IL-6 activities significantly decreased during the 2-hour treatment or the day after direct hemoperfusion (Fig. 2). In the nonsurvivor group, three of the four parameters (except TNFα) showed no significant change after PMX, whereas the IL-10 and PAI-1 values in nonsurvivors became significantly higher than in survivors after PMX and the following day.

To analyze any correlation between changes in plasma endotoxin activity and changes in mediator activities around the time of PMX treatment, patients with plasma endotoxin activity measurements were divided into two groups. In the first, endotoxin activity levels decreased by more than 30% from baseline during the 2-hour PMX treatment (D group). In the second group, endotoxin activity levels increased by more than 30% from baseline despite PMX (I group).

In the D group, IL-6 levels decreased rapidly and significantly after PMX in survivors. Differences in IL-6, IL-10, and PAI-1 activities between survivors and nonsurvivors became significant after PMX or the following day. None of the four parameters exhibited significant changes after PMX in nonsurvivors (Figs. 3–6). These results were similar to the changes in mediator levels in survivors and nonsurvivors as a group (Fig. 2). In the I group, none of the four parameters showed a significant decrease in nonsurvivors. Survivors in the I group had significantly lower IL-10 and PAI-1 values prior to PMX compared with those in nonsurvivors (Figs. 3–6).

Fig. 1. Changes in parameters after polymyxin B (PMX) treatment. Blood pressure (BP) was divided into two groups: ≥ 90 mmHg and < 90 mmHg. BP in the group with shock rose significantly to > 100 mmHg and was maintained on the following day. Body temperature (BT) was divided into three groups: ≥ 38°C, > 36° but < 38°C, and < 36°C. Both groups with abnormal values showed a significant change to the normal range. No significant change occurred in the group with normal BT. The PaO_2/FiO_2 ratio was divided into two groups: ≥ 200 mmHg and < 200 mmHg. Survivors in the low PaO_2/FiO_2 group showed significant elevations to > 200 mmHg after PMX. Statistical analysis was conducted using Wilcoxon's signed rank test.

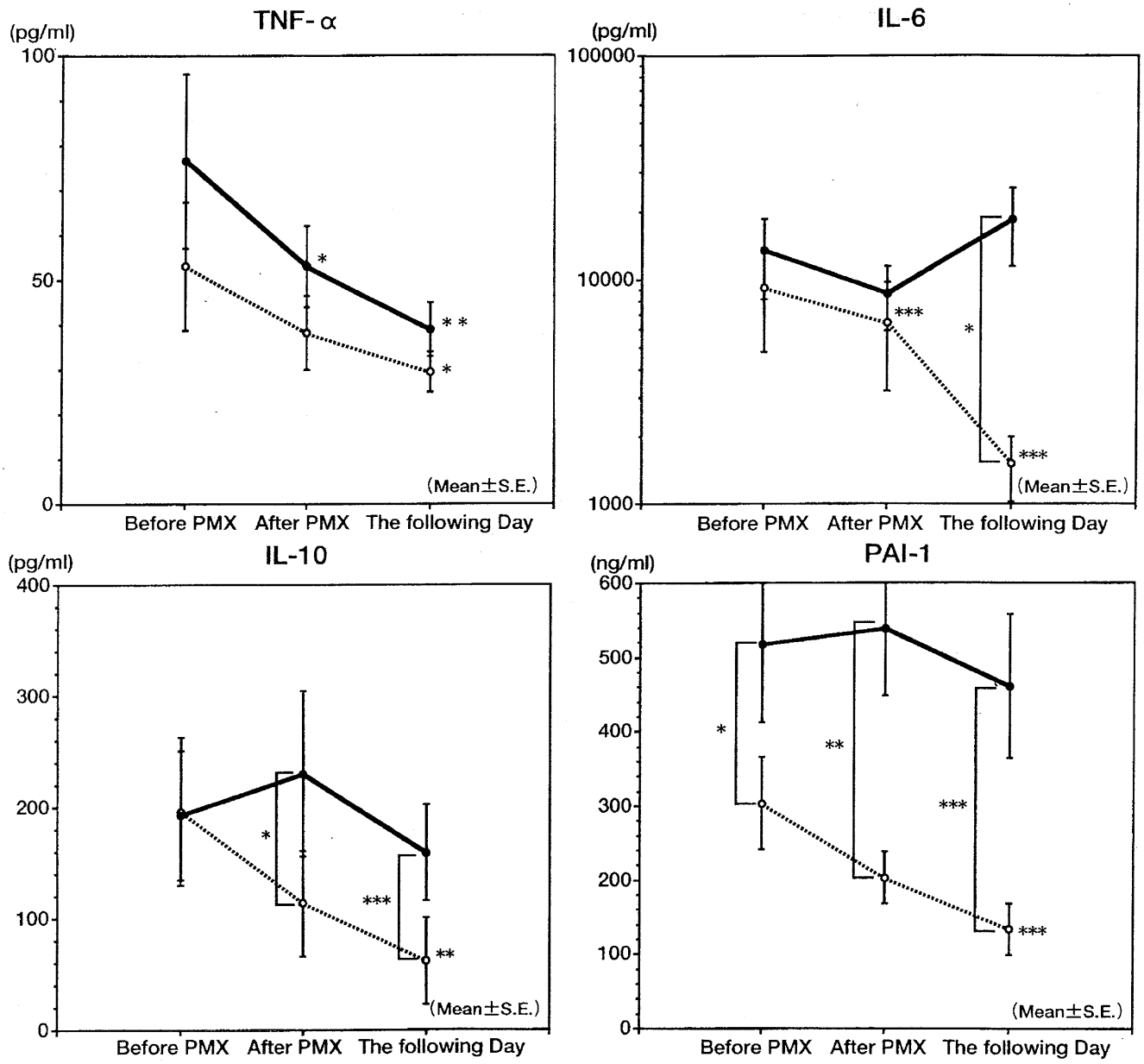


Fig. 2. Mediator changes after PMX treatment. Survivors demonstrated rapid and significant reductions after PMX. Plasminogen activator inhibitor-1 (PAI-1) **(bottom right)** and interleukin-10 (IL-10) **(bottom left)** levels in nonsurvivors increased during PMX, and IL-6 **(top right)** levels increased after PMX. Wilcoxon's signed rank test was used for statistical analysis. Data for nonsurvivors are shown by a solid line and for survivors by a dotted line. $*p < 0.05$; $**p < 0.01$; $***p < 0.001$.

Discussion

We hypothesized that plasma endotoxin adsorption with polymyxin B would have a beneficial effect on the outcome and clinical symptoms of severe sepsis [9–11]. The mechanism by which plasma endotoxin removal improves organ failure or dysfunction, and the relation between removal of plasma endotoxins and changes in clinical symptoms or mediator activities, especially for cytokines, have not been defined until now. Many studies regarding plasma endotoxins during sepsis have been reported, but only a few have mentioned relations between plasma endotoxins and mediators [7] or outcome [5]. There are no reported studies of the relation between changes in plasma endotoxin activity and such clinical parameters.

Our previous studies were the first to show that reduction of plasma endotoxins in patients with severe sepsis was effective, using actual endotoxin values [10, 11]. However, the relation between endotoxin reduction and its influence on septic parameters has been not analyzed. In this study, an improvement in both the Goris score and the number of failed organs was apparent after 2 weeks in all patients and in those who survived more than

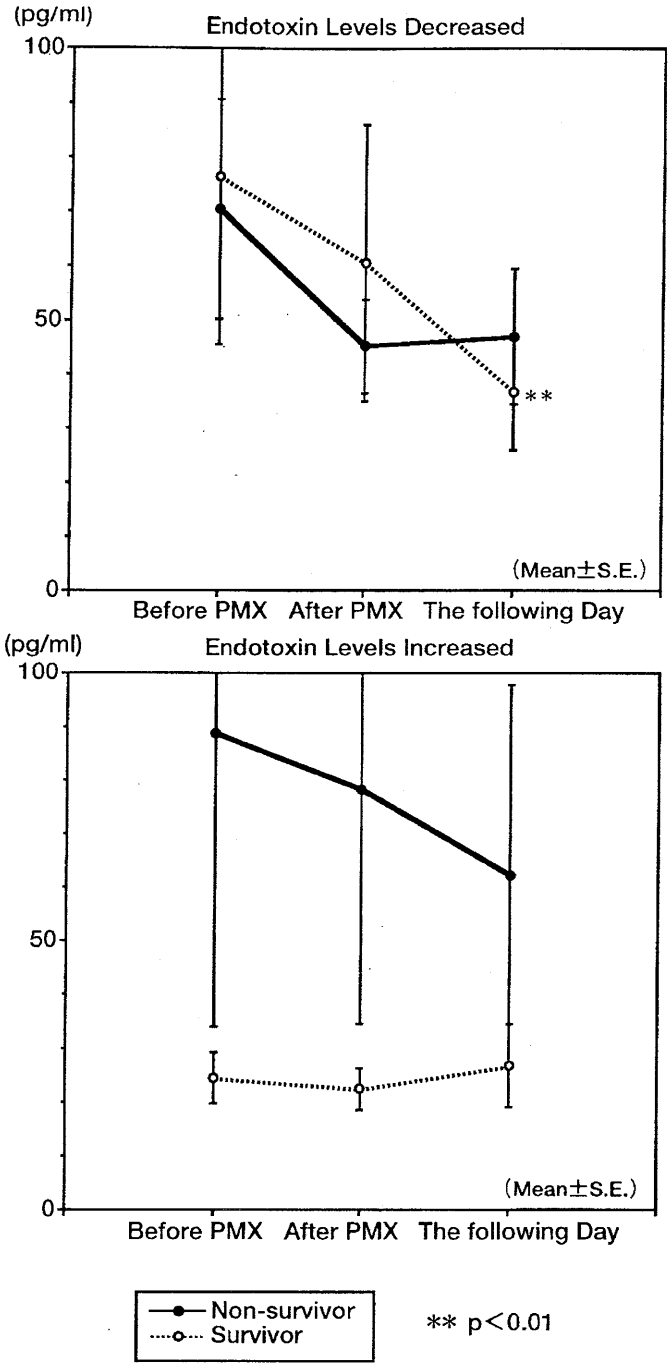


Fig. 3. Changes in tumor necrosis factor-α (TNFα) after PMX treatment. **Top.** Data for the group whose endotoxin activity level decreased by more than 30% after PMX. **Bottom.** Data for the group whose endotoxin activity level increased by more than 30% during PMX. Statistical analysis was performed for a comparison in a group using Wilcoxon's signed rank test and the Mann-Whitney U-test for a comparison between groups. $^{*}p < 0.05$; $^{**}p < 0.01$.

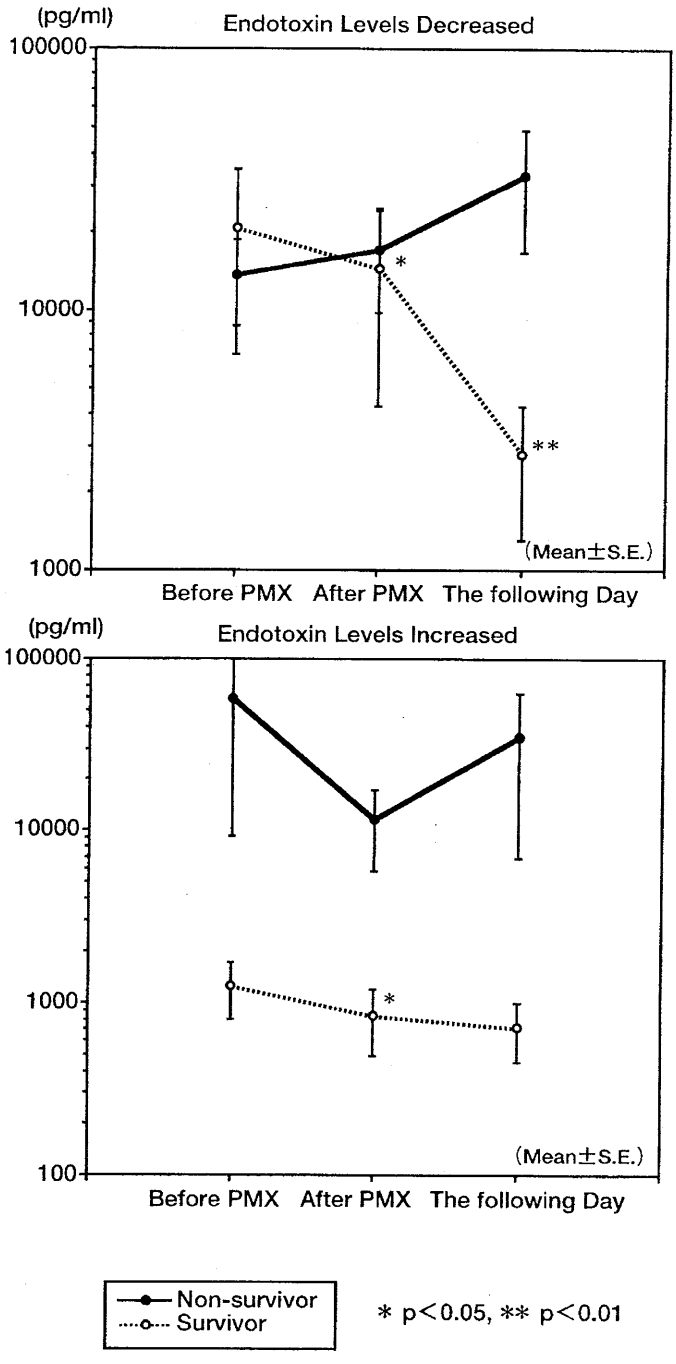


Fig. 4. Changes in IL-6 after PMX treatment. The groups were divided by the same conditions as in Figure 3. The change in the decreased endotoxin level group (**top**) was similar to the results in Figure 2. The change in the increased endotoxin level group (**bottom**) was similar to the results of PAI-1 in Figure 2.

2 weeks. These good outcomes despite the severe conditions mentioned above strongly indicate the efficacy of PMX. In addition, other clinical parameters, including BT, BP, and the PaO_2/FiO_2 ratio, were normalized by PMX. Abnormally high values were decreased, and abnormally low values increased, as previously reported [11].

The mechanism underlying the efficacy of endotoxin adsorption in patients with severe sepsis has been unclear. Many experimental studies have shown that several mediators, including TNFα,

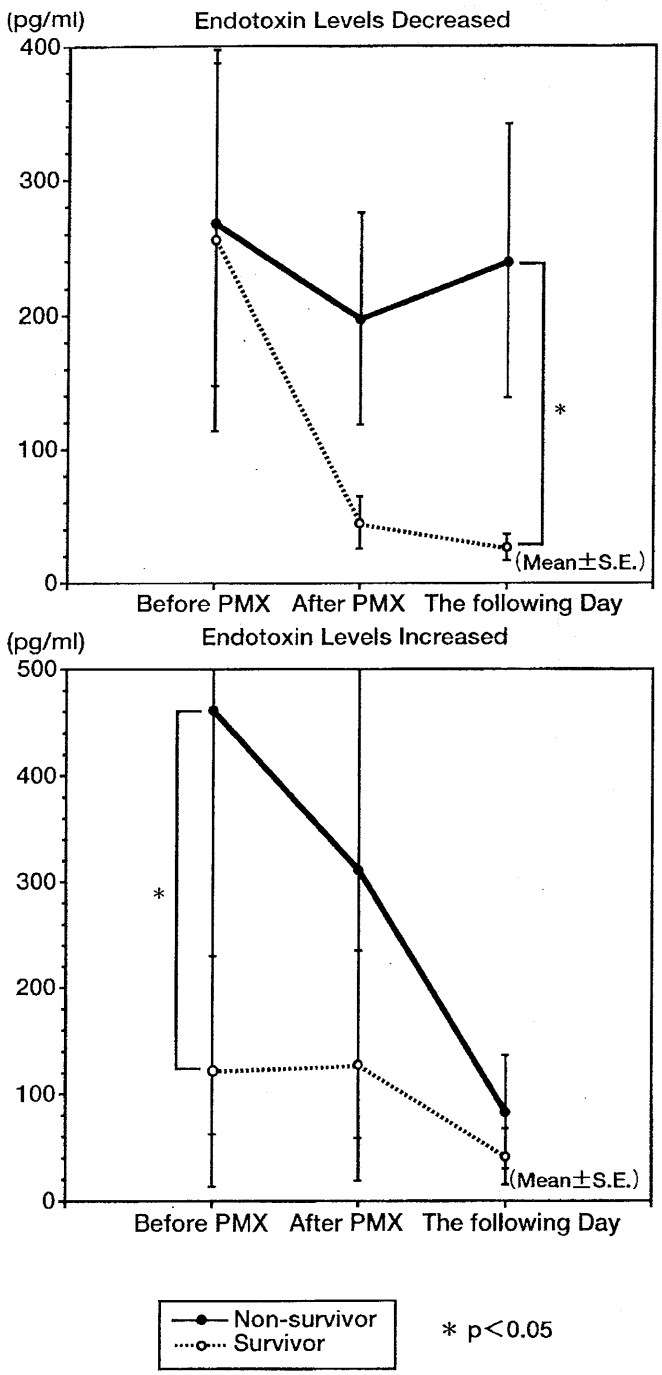


Fig. 5. Changes in IL-10 after PMX treatment. The groups were divided by the same conditions as in Figure 3. The changes of both groups demonstrated the same tendency shown in Figure 6.

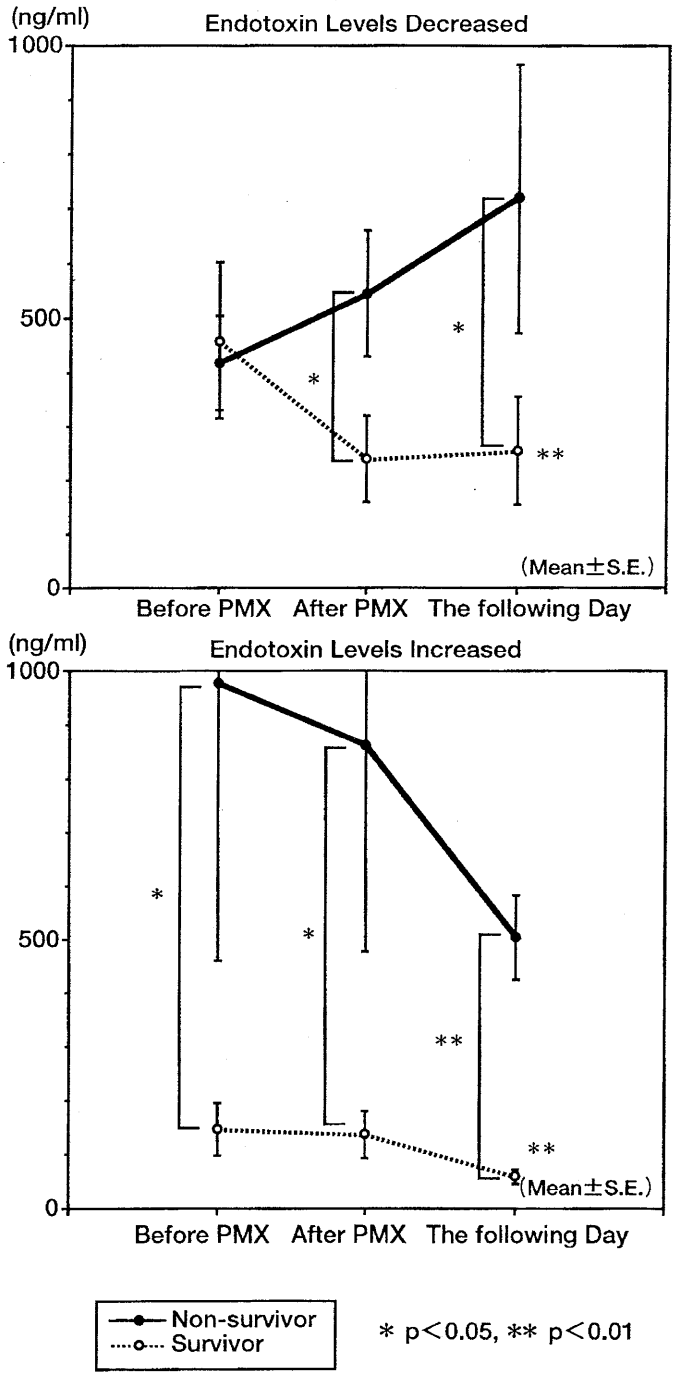


Fig. 6. Changes in PAI-1 after PMX treatment. The groups were divided by the same conditions as in Figure 3.

IL-1, IL-6, and PAI-1, have pivotal roles in sepsis or its progression to MOF. We hypothesized that the removal of plasma endotoxin contributed to a decrease in cytokines and endothelial cell-related mediators. Polymyxin B suppressed TNFα levels in a canine sepsis model [19] but did not adsorb TNFα, IL-6, or IL-8 in vitro (unpublished data). In our study, TNFα and IL-6 decreased rapidly during a 2-hour direct hemoperfusion. The difference between survivors and nonsurvivors was demonstrated to be significant for IL-10 and PAI-1 activities after PMX treatment.

Further evidence for a relation between changes in plasma endotoxins and cytokines were analyzed in this study. Patients were divided into two groups (D and I groups) depending on the direction of the plasma endotoxin changes. IL-6 levels in survivors with adequately reduced endotoxin levels were seen to be signif-

icantly decreased with PMX. Nonsurvivors did not have a significant decrease in any of the four factors during PMX treatment or on the following day. IL-6, IL-10, and PAI-1 levels were significantly higher in nonsurvivors than in survivors after PMX treatment and the following day. For the survivors in D group, TNFα, IL-6, IL-10, and PAI-1 activities might depend on plasma endotoxin activity and be reduced in those in whom plasma endotoxins were adequately removed.

Survivors in the I-group exhibited significantly lower levels of PAI-1 and IL-10 prior to receiving PMX treatment. These results suggest that the causes of these increases in critically ill patients with severe sepsis, in whom cytokine levels were low prior to PMX treatment, did not depend primarily on plasma endotoxin activity. On the other hand, reduced plasma endotoxin levels in patients whose initial TNFα, IL-6, or PAI-1 levels were relatively high but whose levels significantly decreased on the following day would be predictive of cure with PMX. In the I group the activities of these parameters did not correlate with changes in endotoxin activity because PMX treatment might have been inadequate.

Rapid reductions in cytokines following endotoxin neutralization have been reported in vitro. Huang et al. demonstrated the same phenomenon with E-selectin secretion from the endothelium [20]. A reduction in plasma endotoxins by endotoxin adsorption was thought to contribute to the cessation of cytokine gene expression and the excretion of cytokines. The influence of this rapid reduction in plasma endotoxins might be expressed not only in monocytes but also in the endothelium. This mechanism brought about a rapid decrease in plasma levels of cytokines and mediators derived from the endothelium.

Plasma endotoxin adsorption suppressed both TNFα and IL-6 gene expression and neoprotein production; at the same time, antiinflammatory cytokine gene expression (that of IL-10) was suppressed owing to a decrease in inflammatory cytokines. Because endothelial cells would also receive less stimulation by the endotoxin, they would produce less PAI-1. These changes in the cascade due to endotoxin removal would result in alleviation of septic symptoms.

Conclusions

Plasma endotoxin reduction due to 2 hours of PMX treatment correlated with changes in the parameters of patients whose endotoxins were adequately removed. We therefore suggest that plasma endotoxins are the major cause of severe sepsis, and that reduction of their levels contributes to a decrease in mediator levels; it alleviates the clinical symptoms and abnormalities in BP, BT, and the PaO_2/FiO_2 ratio associated with sepsis. In future, new strategies for the use of PMX should be devised to treat current nonsurvivors, whose plasma cytokine levels were not significantly reduced after PMX or whose plasma endotoxin levels increase.

Résumé

But: Evaluer le rapport entre les paramètres cliniques et plasmatiques et les modifications d'activité d'endotoxines plasmatiques, d'une part, et l'utilisation de polymyxin B (PMX, Toray Medical Co., Ltd., Tokyo, Japon), un absorbant d'endotoxine, pendant 2 heures, d'autre part. Patients et méthodes: Au total, 88 patients consécutifs, admis pour traitement d'un sepsis sévère ou une défaillance viscérale par PMX. Pendant le traitement, la réanimation était sans particularité. On a mesuré, avant, immédiatement et 24 h après l'administration de PMX, les facteurs endotoxine et tumor necrosis factor (TNF)-alpha, l'interleukine (IL)-6, IL-10, les activités du plasminogen activator inhibitor-1 (PAI-1), et certains autres paramètres cliniques. Résultats: Les scores moyens APACHE II et III ont été, respectivement, de 24,2 ± 1,0 et de 85,8 ± 3,0. La survie à deux semaines a été de 51,1%. Les activités du TNF-alpha, d'IL-6, d'IL-10 et de PAI-1 ont été significativement réduites chez les survivants pendant le traitement par PMX et/ou le jour après. Chez les non survivants, aucune modification significative n'a été notée pour ces paramètres sauf en ce qui concerne le TNF-alpha. Dans le sous-groupe de patients où l'endotoxine plasmatique a diminué de plus de 30%, on a remarqué une diminution significative de l'IL-6, du TNF-alpha et du PAI-1 après 2 h et/ou le jour d'après, mais aucun de ces quatre paramètres n'a changé chez les non-survivants. Conclusion: Le PMX, pendant les deux heures du traitement, contribue à absorber des endotoxines du plasma et réduit le taux de cytokines inflammatoires, améliorant les paramètres cliniques.

Resumen

Objetivo: Evaluar la relación existente entre los parámetros clínicos y plasmáticos y los cambios en la actividad de la endotoxina plasmática tras utilizar durante dos horas una terapia absorbente de endotoxinas: Toraymaxin^R (PMX, Toray Medical Co., Ltd. Tokyo, Japón). Métodos: 88 pacientes con sepsis grave o fallo multiorgánico séptico fueron tratados mediante el PMX. Se determinaron antes, al finalizar la terapia y al día siguiente del tratamiento con PMX, la actividad de endotoxina, TNF a, interleucinas IL-6, IL-10, así como la del inhibidor-activador 1 del plasminógeno (PAI-1). Resultados: La puntuación media en las escalas APACHE II y III fueron 24.2 ± 1.0 y 85.8 ± 3.0. La supervivencia a las dos semanas fue del 51.1%. La actividad del TNFα, IL-6, IL-10 y PAI-1 disminuyó significativamente durante las dos horas del tratamiento con PMX y/o al día siguiente en aquellos que sobrevivieron. Los tres parámetros, excepción hecha del TNFα, no mostraron cambios significativos tras el PMX en los pacientes que fallecieron. En el subgrupo de pacientes en los que la endotoxina plasmática disminuyó más del 30%, la IL-6, TNFα y PAI-1 descendieron significativamente tras el tratamiento durante dos horas con PMX y/o en el siguiente día, pero los cuatro parámetros no se modificaron en aquellos pacientes que fallecieron. Conclusión: El PMX es capaz de absorber a las endotoxinas plasmáticas, contribuyendo así a la reducción de los niveles de citocinas pro-inflamatorias en el plasma, lo que supone una mejoría en los parámetros clínicos al menos durante las 2 horas que dura el tratamiento.

Acknowledgments

We thank Mr. and Mrs. Yokoyama and Mrs. Miyata in Toray Industries (Toray Medical Co., Tokyo, Japan) for their advice in the preparation of this manuscript. This study was supported by Toray Industries, Toray Medical Co., Ltd., Tokyo, Japan.

References

1. Zimmerman, J.E., Knaus, W.A., Wagner, D.P., Sun, X., Hakim, R.B., Nystrom, P-O.: A comparison of risks and outcomes for patients with organ system failure: 1982–1990. Crit. Care Med. *24*:1633, 1996
2. Ziegler, E.J., Fisher, C.J., Sprung, C.L., Staraube, R.C., Sadoff, J.C., Foulke, G.E., Wortel, C.H., Fink, M.P., Dellinger, R.P., Teng, N.N.H., Allen, I.E., Berger, H.J., Knatterud, G.L., LoBuglio, A.F., Smith, C.R.: Treatment of gram-negative bacteremia and shock with HA-1A human monoclonal antibody against endotoxin. N. Engl. J. Med. *324*:429, 1991
3. Greenman, R.L., Schein, R.M.H., Martin, M.A., Wenzel, R.P., Macintyre, N.R., Emmanuel, G., Chmel, H., Kohler, R.B., McCarthy, M., Plouffe, J., Russell, J.A.: A controlled clinical trial of E5 murine monoclonal IgM antibody to endotoxin in the treatment of gram-negative sepsis. J.A.M.A. *266*:1097, 1991
4. Quezado, Z.M.N., Banks, S.M., Natanson, C.: New strategies for combatting sepsis: the magic bullets missed the mark ... but the search continues. Trends Biotechnol. *13*:56, 1995
5. Brandtzaeg, P., Kierulf, P., Gaustad, P., Skulberg, A., Bruun, J.N., Halvorsen, S., Sorensen, E.: Plasma endotoxin as a predictor of multiple organ failure and death in systemic meningococcal disease. J. Infect. Dis. *159*:195, 1989
6. Danner, R.L., Elin, R.J., Hosseini, J.M., Wesley, R.A., Reilly, J.M., Parillo, J.E.: Endotoxemia in human septic shock. Chest *99*:169, 1991
7. Wortel, C.H., von der Mohlen, M.A.M., van Deventer, S.J.H., Splung, C.L., Jastremski, M., Lubbers, M.J., Smith, C.R., Allen, I.E., ten Cate, J.W.: Effectiveness of a human monoclonal anti-endotoxin antibody (HA-1A) in gram-negative sepsis: relationship to endotoxemia and cytokine levels. J. Infect. Dis. *166*:1367, 1992
8. Hanasawa, K., Tani, T., Oka, T., Hashimoto, T., Endoh, Y., Kodama, M., Teramoto, K., Nishiumi, S., Murakami, M., Takiuchi, H., Yoshioka, T.: Experimental study of endotoxin adsorption using polymyxin B immobilized fiber. Jpn. J. Artif. Organs *13*:825, 1984
9. Hanasawa, K., Tani, T., Kodama, M.: New approach to endotoxin and septic shock by means of polymyxin B immobilized fiber. Surg. Gynecol. Obstet. *168*:323, 1989
10. Aoki, H., Kodama, M., Tani, T., Hanasawa, K.: Treatment of sepsis by extracorporeal elimination of endotoxin using polymyxin B-immobilized fiber. Am. J. Surg. *167*:412, 1994
11. Kodama, M., Tani, T., Maekawa, K., Hirasawa, H., Ootsuka, T., Takahashi, Y., Kaneko, M.: Endotoxin eliminating therapy in patients with severe sepsis: direct hemoperfusion using polymyxin B immobilized column. Jpn. J. Surg. *96*:277, 1995
12. Endo, Y., Oka, T., Hanasawa, K., Yoshioka, T., Tani, T., Nakane, Y., Kodama, M.: Applications of protease inhibitor to hemoperfusion and plasma exchange as a regional anticoagulant. Trans. Am. Soc. Artif. Intern. Organs *31*:429, 1985
13. Goris, R.J.A., to Boekhorst, T.P.A., Nuytink, T.K.S.: Multiple organ failure: generalized autodestructive inflammation? Arch. Surg. *120*:1109, 1985
14. Stevens, L.E.: Gauging the severity of surgical sepsis. Arch. Surg. *118*:1190, 1983
15. Knaus, W.A., Draper, E.A., Wagner, D.P., Zimmerman, J.E.: APACHE II: a severity of disease classification system. Crit. Care Med. *13*:818, 1985
16. Knaus, W.A., Wagner, D.P., Draper, E.A., Zimmerman, J.E., Berger, M., Bastos, P.G., Sirio, C.A., Murphy, D.J., Lotring, T., Damiano, A., Harrell, F.E.: The APACHE III prognostic system. Chest *100*:1619, 1991
17. Obayashi, T., Tamura, H., Tanaka, S., Ohki, M., Takahashi, S., Arai, M., Masuda, M., Kawai, T.: Removal of limulus test-interfering factors in blood samples with perchloric acid and the improvement of the specificity of the limulus test by fractionating amebocyte lysate. Prog. Clin. Biol. Res. *23*:357, 1987
18. Inaba, 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. *95*:303, 1991
19. Huang, K., Fishwild, D., Wu, H-M., Dedrick, R.L.: Lipopolysaccharide (LPS)-induced E-selectin expression requires continuous presence of LPS and is inhibited by bactericidal/permeability-increasing protein. Inflammation *19*:389, 1995
20. Sato, T., Orlowski, J.P., Zborowski, M.: Experimental study of extracorporeal perfusion for septic shock. A.S.A.I.O. J. *39*:M790, 1993