

Hemoperfusion With an Immobilized Polymyxin B Fiber Column Inhibits Activation of Vascular Endothelial Cells

Hidehiko Kushi, Jun Nakahara, Takahiro Miki, Kazuhiko Okamoto, Takeshi Saito, and Kastuhisa Tanjo

Department of Emergency and Critical Care Medicine, Nihon University School of Medicine, Tokyo, Japan

Abstract: Involvement of the activation of neutrophils and vascular endothelial cells in the pathology of sepsis has recently been reported. We therefore investigated whether direct hemoperfusion (DHP) with a polymyxin B immobilized fiber column (PMX) could reduce the level of plasminogen activator inhibitor-1 (PAI-1), an index of vascular endothelial cell activation. Twelve sepsis patients satisfying the following criteria were enrolled in the study: (i) stable global oxygen metabolism (oxygen delivery index >500 mL/min/m² and oxygen consumption index >120 mL/min/m²); (ii) abnormal tissue oxygen metabolism (PCO₂ gap: gastric mucosal PCO₂ minus arterial PCO₂ difference >8 mm Hg); and (iii) mean blood pressure ≥ 60 mm Hg. Direct hemoperfusion with PMX was performed twice (for

3 h each time) within 24 h. Plasminogen activator inhibitor-1 was measured a total of 5 times: before PMX-DHP, immediately after the first DHP with PMX session (3 h after the start), and 24, 48, and 72 h afterward. The PAI-1 value was 150 ± 30.0 ng/mL before DHP with PMX, 178 ± 60.0 ng/mL immediately after DHP with PMX, 90 ± 22.1 ng/mL at 24 h after, 65 ± 21.0 ng/mL at 48 h after, and 64 ± 18.3 ng/mL 72 h after. The values were significantly lower from 48 h onward compared with baseline. These data suggest that DHP with PMX inhibits vascular endothelial cell activation. **Key Words:** Endothelial cell, Plasminogen activator inhibitor-1, Polymyxin B immobilized fiber column (PMX), Sepsis, Systemic inflammatory response syndrome (SIRS).

A new concept of sepsis was proposed in 1992, when it was defined as systemic inflammatory response syndrome (SIRS) associated with infection (1). Sepsis remains a major cause of multiple organ failure (MOF) with a high mortality rate (2) even 13 years later. Although clinical studies have been performed in Europe and the USA using an anticytokine antibody to suppress the inflammatory response, favorable results have not been obtained (3).

A polymyxin B immobilized fiber column (PMX; Toray Industries Inc., Tokyo, Japan) was developed in Japan in 1994 and it has been used for treatment of endotoxemia. Reduction of the blood endotoxin level by this column has been recognized (4) and

improvement of the circulation (blood pressure and systemic vascular resistance index) has been reported as well (4). Although the detailed mechanism is not known, this column also lowers inflammatory cytokine and plasminogen activator inhibitor-1 (PAI-1) levels immediately after direct hemoperfusion (DHP) with PMX (5). Based on such evidence, PMX has been tried in Japan for peritonitis due to colorectal perforation (6), acute cholecystitis (7), or acute lung injury (8) and its usefulness has been reported.

Toxins such as lipopolysaccharide (LPS) bind to monocytes or endothelial cells, and endothelial cells are activated by these toxins. Activated endothelial cells then release various mediators, including pro-inflammatory cytokines and PAI-1, and induce the production of adhesion molecules (9). Plasminogen activator inhibitor-1 is considered to be an index of endothelial cell activation (9). There are, however, no reports of measurements over time periods as long as 72 h after DHP with PMX. We measured the time course of changes in PAI-1 to investigate whether PMX could prevent the endothelial cell activation.

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Address correspondence and reprint requests to Dr Hidehiko Kushi, Department of Emergency and Critical Care Medicine, Nihon University School of Medicine, 1-8-13 Kanda, Surugadai, Chiyoda-ku, Tokyo 101-0062, Japan. Email: hkushi@med.nihon-u.ac.jp

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PATIENTS AND METHODS

Selection of patients

Patients with a clinical diagnosis of sepsis according to the criteria of the American College of Chest Physicians/Society of Critical Care Medicine (ACCP/ACCM) Consensus Conference were enrolled in the study (1). Before the start of PMX treatment, global oxygen metabolism and tissue oxygen metabolism were measured. A thermodilution catheter (Edwards Lifesciences LLC, Irving, TX, USA) was used to determine the oxygen delivery index (DO_2I), oxygen consumption index (VO_2I), and oxygen extraction ratio (O_2ER) as parameters of global oxygen metabolism. Gastric tonometer (TRIP TGS catheter, Tonometrics, Worcester, MA, USA) was used for measurement of tissue oxygen metabolism and gastric intramucosal PCO_2 was measured using a gas tonometer (Tonocap, Datex Ohmeda, Helsinki, Finland). Then the gastric mucosal-arterial PCO_2 difference (PCO_2 gap) was calculated as the gastric mucosal PCO_2 minus arterial PCO_2 . A PCO_2 gap ≥ 8 mm Hg was used to define abnormal tissue oxygen metabolism (10).

The criteria for inclusion of patients in the study were the following findings within the previous 24 h: (i) signs of systemic inflammatory response syndrome due to infection, including hyperthermia or hypothermia (temperature $>38^\circ C$ or $<36^\circ C$), tachycardia (>90 beats/min), tachypnea (>20 beats/min) or $PaCO_2$ less than 32 mm Hg or mechanical ventilation, and a white blood cell count greater than $12.0 \times 10^4/L$ or less than $4.0 \times 10^4/L$ or 10% or more immature neutrophils; (ii) mean arterial pressure >60 mm Hg (irrespective of the use of catecholamines); (iii) stable global oxygen metabolism ($DO_2I > 500$ mL/min/m² and $VO_2I > 120$ mL/min/m²) with abnormal tissue oxygen metabolism (PCO_2 gap > 8 mm Hg); (iv) controlled infection; and (v) use of an antibiotic to minimize LPS release for by Gram-negative rods. Exclusion criteria were patients under 18 years old and a mean blood pressure of 60 mm Hg or less irrespective of the use of catecholamines. Administration of histamine H2 receptor antagonists or enteral feeding were not performed for 24 h prior to DHP with PMX. Hypovolemia was corrected with appropriate infusion as necessary upon initiation of DHP with PMX.

Direct hemoperfusion

Access to the blood for DHP with PMX adsorbent therapy was achieved via a double-lumen catheter inserted into the femoral vein of each patient. Direct

hemoperfusion was carried out for 3 h at a flow rate of 80–100 mL/min.

Direct hemoperfusion with PMX was performed twice within 24 h. Heparin or nafamostat mesilate (Torii Co. Ltd, Tokyo, Japan) was used as an anticoagulant.

PAI-1 assay

Plasminogen activator inhibitor-1 was measured in duplicate by an enzyme-linked immunosorbent assay (ELISA; Fuji Rebio Inc., Tokyo, Japan). The total PAI-1 level was measured 5 times: before DHP with PMX, immediately after the first session of DHP with PMX, 24 h after completion of the second session of DHP with PMX, 48 h afterward, and 72 h afterward.

Evaluation of patients

A total of 12 subjects who satisfied the above criteria were enrolled in the study. The sepsis-related organ failure assessment (SOFA) score (11) was measured 3 times: before DHP with PMX, 72 h afterward, and on discharge from the intensive care unit (ICU). In this study, DHP with PMX was performed in patients with either Gram-negative or Gram-positive infection who satisfied the definition of sepsis (1).

Statistical analysis

Results are expressed as the mean \pm SE. Differences were analyzed by Wilcoxon's generalized test and statistical significance was established at the $P < 0.05$ level.

RESULTS

Direct hemoperfusion with PMX was performed 24 times in 12 patients (10 males and 2 females) between the ages of 36 and 81 years (mean 61 ± 14.6 years). It was performed twice (for 3 h each time) within 24 h. As shown in Table 1, the underlying diseases were both varied and multiple. All patients were discharged alive. Table 2 shows the

TABLE 1. Demographic characteristics and underlying disease

Patients (N)	12
Age (years)	61 ± 14.6 (36–81)
Gender (M : F)	10 : 2
Underlying disease	
Pneumonia	5
Cerebrovascular disease	3
Diabetes mellitus	2
Recent trauma	1
Chronic liver disease	1

TABLE 2. Distribution of anatomical sources of bacteria

Respiratory system	6
Abdominal cavity	3
Urinary tract	1
Central nervous system	1
Others	1

anatomical sites of infection. Pneumonia was the most frequent primary focus and 17 bacteria were detected in 12 subjects. Table 3 shows the causative organisms. The most commonly isolated microorganisms were Gram-negative bacteria. Antibiotic therapy was judged to be adequate when the patient received antibiotics to which each isolated organism was sensitive.

The SOFA score was 12 ± 1.7 before DHP with PMX, 9 ± 1.4 at 72 h, and 5 ± 0.8 on discharge from ICU, showing a significant decrease from 72 h onward (Fig. 1).

Plasminogen activator inhibitor-1 was 150 ± 30.0 ng/mL prior to PMX with DHP, 178 ± 60.0 ng/mL immediately after the initial PMX with DHP, 90 ± 22.1 ng/mL at 24 h, 65 ± 21.0 ng/mL at 48 h, and 64 ± 18.3 ng/mL at 72 h afterwards, showing a significant decrease from 48 h onward compared with baseline (Fig. 2).

DISCUSSION

Patient selection

Direct hemoperfusion with PMX has conventionally been used in patients with severe sepsis or septic shock and its clinical effect has been reported. When shock persists, organ failure becomes irreversible and the prognosis is dismal. It was reported that an interleukin (IL)-6 level ≥ 1000 pg/mL (12), an acute physiology and chronic health evaluation (APACHE) II score ≥ 25 (5), an APACHE II score ≥ 30 (13), and a cardiac index ≥ 6 L/min/m² (14) are factors indicating

TABLE 3. Causative organisms and number of cases

Gram-negative bacteria	
<i>Escherichia coli</i>	1
<i>Pseudomonas aeruginosa</i>	3
<i>Klebsiella pneumonia</i>	1
<i>Neisseria</i>	2
<i>Vibriocitrobacter</i>	1
Gram-positive bacteria	
<i>Staphylococcus aureus</i>	2
<i>Staphylococcus epidermidis</i>	1
<i>Corynebacterium</i>	1
α - <i>Streptococcus</i>	1
<i>Enterococcus faecalis</i>	1
Fungus	
<i>Candida albicans</i>	3

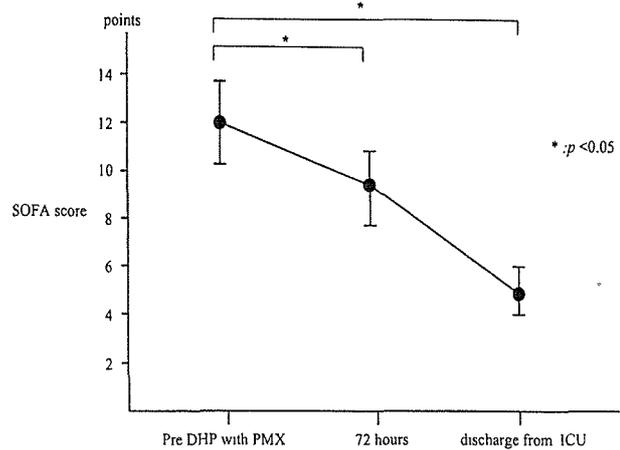


FIG. 1. Changes of the sepsis-related organ failure assessment (SOFA) score are shown. The SOFA score was 12 ± 1.7 before direct hemoperfusion with polymyxin B immobilized fiber column, 9 ± 1.4 at 72 h, and 5 ± 0.8 on discharge from the intensive care unit (ICU), showing a significant decrease from 72 h onward.

a poor prognosis at the start of DHP with PMX. Once multiple organ failure develops, cellular dysfunction progresses at the molecular level and it becomes difficult to assess the effect of DHP with PMX, but the patients in this study were carefully selected to allow assessment. In other words, this study enrolled patients who satisfied the definition of sepsis (1), in whom infection was controlled and organ perfusion was maintained (a mean blood pressure ≥ 60 mm Hg), and whose global oxygen metabolism was stable although tissue oxygen metabolism was disturbed. Disturbed global oxygen metabolism is indicated by a reduced oxygen consumption index although the oxygen delivery index is maintained, and a functional disorder at the cellular level or wide-

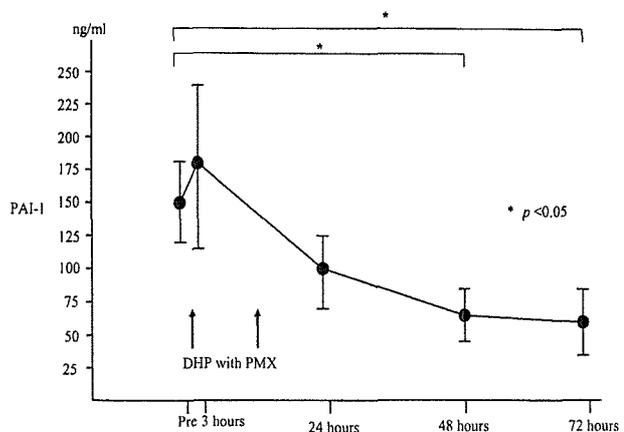


FIG. 2. Changes of the plasminogen activator inhibitor-1 (PAI-1) level are shown. PAI-1 was 150 ± 30.0 ng/mL prior to direct hemoperfusion with polymyxin B immobilized fiber column (DHP with PMX), 178 ± 60.0 ng/mL immediately after the initial PMX with DHP, 90 ± 22.1 ng/mL at 24 h, 65 ± 21.0 ng/mL at 48 h, and 64 ± 18.3 ng/mL at 72 h afterwards, showing a significant decrease from 48 h onward compared with baseline.

spread minor circulatory changes are suggested. Patients with disturbed global oxygen metabolism were excluded. Because the gastrointestinal tract, which has a low resistance to invasion, was used to measure tissue oxygen metabolism, it was likely to be affected by catecholamines, hypovolemia, SIRS (sepsis), histamine H₂ receptor antagonists, or enteral feeding. Histamine H₂ receptor antagonists or enteral feeding were not allowed before DHP with PMX and hypovolemia was corrected, so the increased PCO₂ gap was considered to be attributable to sepsis itself or to catecholamine therapy in some of the patients.

Appropriateness of direct hemoperfusion with polymyxin B immobilized fiber column for non-Gram-negative bacterial infection

The clinical effect of DHP with PMX has often been reported. Shoji et al. (15) reported the adsorption of endotoxin in clinical application and Sato et al. (4) reported improvement of the circulation. Direct hemoperfusion with PMX was recently reported to be effective not only for Gram-negative rods but also for methicillin-resistant *Staphylococcus aureus* (MRSA) (16), and the mechanism was postulated to be inhibition of TNF- α produced by lipoteichoic acid (17). The clinical application of DHP with PMX is thus expanding to patients with sepsis due to Gram-positive bacteria, and its effect is considered to be based on the inhibition of pro-inflammatory cytokines produced by either Gram-negative rods or Gram-positive bacteria. Tani et al. (5) performed a multicenter clinical study and found that tumor necrosis factor (TNF)- α , IL-6, IL-10, and PAI-1 levels were lower at 2 h after DHP with PMX. Patients with Gram-negative as well as Gram-positive infection were also enrolled in this study, and it was suggested that DHP with PMX prevented the production of humoral mediators such as cytokines stimulated by toxins in addition to its endotoxin-adsorbing effect. Therefore, it is not ethically problematic to perform DHP with PMX in patients with non-Gram-negative infection.

Changes of plasminogen activator inhibitor-1

Plasminogen activator inhibitor-1 is a protein with a molecular weight of 50 kDa, produced by vascular endothelial cells, that plays a central role in activation of the fibrinolytic system (18). It has recently attracted attention as an index of vascular endothelial cell activation (9). The levels of PAI-1 are reported to be very high in patients with severe sepsis and are not lowered by DHP plus PMX in patients who have a fatal outcome (5). Plasminogen activator

inhibitor-1 is known to have various forms in the blood, including an active form, an inactive form, a latent form, a tissue plasminogen activator/PAI-1 complex, and vitronectin/PAI-1 complex. We measured total PAI-1 in this study, which is the total amount of PAI-1 produced by vascular endothelial cells. Organ failure in patients with severe sepsis is induced by an excessive increase of humoral mediators such as pro-inflammatory cytokines and the resulting activation of neutrophils, followed by accumulation of activated neutrophils in the target organs. Pro-inflammatory cytokines such as TNF- α and IL- β stimulate vascular endothelial cells, induce adhesion molecules, and target activated neutrophils to stimulate PAI-1 production by vascular endothelial cells to inhibit fibrinolysis (9). In this study, PAI-1 was significantly lower at 48 and 72 h after DHP with PMX compared with the baseline value. It may be considered that, rather than direct inhibition of PAI-1 production by DHP with PMX, adsorption of pathogenic bacteria prevented the release of inflammatory cytokines and lessened the stimulation of vascular endothelial cells to lower the PAI-1 level. In this study, nafamostat mesilate was used for some of the patients during direct hemoperfusion. Vascular endothelial injury in rats was reported to be decreased by nafamostat mesilate (19), but the dose administered to the rats was much higher than the normal dose administered to humans. Thus, there is no report about improvement of vascular endothelial injury when the normal dose is used in humans. Direct hemoperfusion with PMX is therefore considered to contribute to preventing the activation of vascular endothelial cells, although indirectly. Prevention of the activation of vascular endothelial cells might have improved organ failure and thus lowered the SOFA score in our patients. In conclusion, the present data indicate that DHP with PMX indirectly inhibits vascular endothelial cell activation.

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