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Treatment with polymyxin B-immobilized fiber reduces platelet activation in septic shock patients: Decrease in plasma levels of soluble P-selectin, platelet factor 4 and β-thromboglobulin

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Abstract. *Objective*: To clarify whether plasma concentrations of soluble P-selectin, platelet factor-4 (PF-4) and β-thromboglobulin (βTG) are altered in patients with septic shock and whether polymyxin B-immobilized fiber (PMX-F) treatment affects these changes.
Subjects: Thirty patients with septic shock who were treated with PMX-F (group A), 20 such patients who received conventional therapies (group B) and 20 healthy control subjects(group C).
Methods: ELISA using commercial kits. Endotoxin elimination by direct hemoperfusion using PMX-F.
Results: Blood endotoxin levels decreased significantly from 49.4 ± 8.8 pg/ml to 13.0 ± 4.5 pg/ml after PMX-F treatment. The pretreatment plasma concentrations of soluble P-selectin, PF-4 and βTG in patients in groups A and B were significantly higher than those in group C ($p < 0.001$). Plasma concentrations of these factors decreased significantly in group A after PMX-F treatment ($p < 0.01$); however, the concentrations in group B were not altered after conventional treatment. The survival rate of group A (60%) was higher than that of group B (30%).
Conclusions: Our findings suggest that soluble P-selectin, PF-4 and βTG may be associated with septic shock and that PMX-F is effective in reducing these markers in patients with septic shock.

Key words: Hemoadsorption – Sepsis – Platelet – Endotoxin – Polymyxin B

Introduction

In sepsis, microorganisms may be present in the blood stream or may proliferate locally and release various bioactive substances [1]. Although early administration of appropriate antibiotics is associated with improved survival, antibiotics appear to have little immediate effect on the course and outcome of septic shock [2]. Novel therapeutic strategies have attempted to target mediators of sepsis by using agents and/or devices that either inhibit the production and/or activation of these mediators [2]. Recent development of an endotoxin-removal column containing polymyxin B-immobilized fiber (PMX-F) has enabled us to safely use a direct hemoperfusion procedure to treat septic shock [3–5]. Recent multicenter studies have demonstrated that the average number of failed organs, the severity of illness and vasopressor requirement were reduced significantly by PMX-F treatment in patients with sepsis [6, 7]. We reported previously that activated peripheral blood monocytes expressed increased metalloproteinase-9 mRNA in patients with septic shock and that PMX-F treatment reduced these mRNA levels [8].

Platelet activation has been estimated by measuring increases in plasma levels of platelet α-granule proteins including β-thromboglobulin (TG) and platelet factor (PF)-4 [9, 10]. Soluble P-selectin is present in normal circulation, and its plasma levels are elevated under certain pathological conditions including hemolytic-uremic syndrome and hemodialysis [11–13]. Platelet-activating factor (PAF) reportedly plays an important role in the pathogenesis of septic shock [2]; high concentrations of PAF associated with platelets have been observed in sepsis [14]. Thus, the inflammatory response may involve activation of platelets in patients with sepsis. The aim of the present study was to clarify whether plasma β-TG, PF-4 and soluble P-selectin concentrations are altered in patients with septic shock and whether treatment with PMX-F affects these changes.

Patients and methods

Patients

Fifty septic patients treated at the Misato Junshin Hospital and the Koto Hospital between October, 1996 and May, 1998 and ranging in age

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from 20 and 81 years (mean 53.8) were included in the present study. Twenty healthy volunteers between the ages of 20 and 80 years of age (mean 52.2 years) were also included. Patients had an infection focus. The clinical and demographic patient data are summarized in Tables 1 and 2. Sepsis in the majority of the patients was originated from pneumonia, urinary tract infection or bile duct infection. Severe sepsis was diagnosed according to the criteria used by Abel [15]: (a) systolic blood pressure < 90 mmHg, (b) tachycardia (heart rate > 90 bpm), (c) tachypnea (respiratory rate > 20 bpm), (d) temperature > 38.5 °C or < 36.0 °C, (e) leukocytosis (leukocyte count > 12000/μl) or leukocytopenia (leukocyte count < 3500/μl) and (f) acute organ failure. APACHE II score, which is based on 12 biological parameters in addition to age and chronic organ failure, was determined for each patient on the day of admission and prior to initiating hemoperfusion therapy [16]. No patients or volunteers were taking steroids, immunosuppressive agents or nonsteroidal anti-inflammatory agents. Informed consent was obtained from all participating subjects or their families.

Table 1. Patient demographic and clinical data.

		Group A (n = 30)	Group B (n = 20)
Sex	men	18	12
	women	12	8
Age (years)	mean	54.4	52.9
	max	81	78
	min	20	24
Infection site	respiratory system	14	9
	urinary tract	6	4
	bile tract	4	2
	wound	3	2
	intra-abdomen	2	2
	peritonitis	1	1

Group A: patients treated with PMX-F.
Group B: patients who received conventional therapies.

Table 2. The types of bacteria detected and the sources.

Sources	Types of bacteria	Number of patients	
		group A	group B
Blood	*E. coli*	5	3
	P. aeruginosa	5	3
	K. pneumoniae	4	3
	S. marcescens	3	1
	P. vulgaris	2	1
	P. mirabillis	2	1
Sputum	*K. pneumoniae*	6	5
	H. influenzae	3	2
	E. cloacae	2	1
	P. aeruginosa	2	1
	P. vulgaris	1	0
Urine	*E. coli*	4	3
	P. aeruginosa	1	1
	S. marcescens	1	0
Galle	*E. coli*	3	2
	P. aeruginosa	1	0
Skin	*P. aeruginosa*	3	2
Ascites	*E. coli*	1	2
	E. cloacae	1	0
	P. aeruginosa	1	1

Group A: patients treated with PMX-F.
Group B: patients received conventional therapies.

Conventional treatments including antibiotics and γ-globulin, hemodynamic monitoring and organ support in the intensive care unit were continued for 5 days in all patients with septic shock. On the sixth day, patients were randomly assigned to one of two treatment modalities. Thirty patients were treated with polymyxin B-immobilized fiber (PMX-F) (group A) and the other 20 patients were treated conventionally by vasopressors including catecholamines (group B).

PMX-F and the hemoperfusion column have been described previously [3–8]. In 1990, the Critical Network Group in Japan reported that hemoperfusion with PMX-F was a safe and effective treatment for sepsis [4]. PMX-F is now commercially available in Japan (Toraymyxin, Toray Medical Co., Tokyo, Japan). PMX-F therapy was repeated twice in all patients with a 24-hour interval. Access to blood for direct hemoperfusion with PMX-F was obtained via a double-lumen catheter (Arrow International Inc., Reading, PA, USA) inserted into the femoral vein by Seldinger's method. Direct hemoperfusion was carried out for 2 h at a flow rate of 80 to 100 ml/min. Peripheral blood samples (30 ml) were collected before and immediately after PMX-F treatment. Each sample was kept at –20 °C prior to assay. Blood endotoxin levels were determined by Endospecy test [4]. The detection limit of endotoxin using this method was 0.5 pg/ml and the upper limit of normal subjects was 9.8 pg/ml. The samples for βTG and PF4 were transferred into precooled tubes containing 0.27% theophylline, 0.1% adenosine, 0.01% dipyridamole, 2.43% sodium citrate and 0.58% citrate and allowed to cool in an ice bath for 30 min as previously described [13]. The blood was centrifuged at 2000 g for 30 min at 4 °C, then frozen and stored at –20°C. Plasma PF-4 and βTG concentrations were measured using a commercially available ELISA kit (Diagnostic Stago, Asnieres, France). The samples for soluble P-selectin were transferred into precooled tubes containing 3.8% sodium citrate, immediately centrifuged at 3000 g for 10 minutes at 4 °C, and then frozen and stored at –20 °C. Plasma soluble P-selectin concentration was measured by double-sandwich ELISA using anti-human selectin murine monoclonal antibodies (GMP-140 EIA kit, Takara, Kyoto, Japan) [11, 12]. Data are expressed as mean values ± SEM. Statistical analyses were performed using the Wilcoxon signed-rank test for paired data and the Mann-Whitney U-test for unpaired data. In addition, one-way analysis of variance with Scheffe's multiple linear regressions and rank-sum test were used for the statistical analysis. $p < 0.05$ was considered to be statistically significant.

Results

Gram-negative bacteria were detected in all 50 septic patients. The survival rate in the PMX-F-treated group (60%) was significantly higher than that in conventionally-treated group (30%). All survivors were discharged from our hospital within 30 days after PMX-F treatment. The mean APACHE II score was improved from 24.8 ± 1.3 before PMX-F treatment to 10.6 ± 0.4 14 days after treatment ($p < 0.01$). The number of failed organs was reduced from 4.2 ± 0.3 before PMX-F treatment to 2.3 ± 0.4 after treatment ($p < 0.01$). The systolic blood pressure was increased significantly from 84 ± 8 mmHg before treatment to 96 ± 12 mmHg after the first PMX-F treatment ($p < 0.01$) and to 116 ± 14 mmHg after the second PMX-F treatment ($p < 0.001$). The mean concentration of endotoxin was decreased significantly from 49.4 ± 8.8 pg/ml before PMX-F treatment to 21.6 ± 4.8 pg/ml after the first treatment ($p < 0.01$) and to 13.0 ± 4.5 pg/ml after the second treatment ($p < 0.001$) (Fig. 1A). The mean concentration of endotoxin was 4.6 ± 1.8 pg/ml in group C. No significant alteration in platelet count was detected during PMX-F treatment (pretreatment, $21.5 \pm 4.6 \times 10^4/mm^3$; after the first treatment, $20.6 \pm 4.2 \times 10^4/mm^3$; after the second treatment, $19.8 \pm 3.8 \times 10^4/mm^3$). Before treatment, the plasma

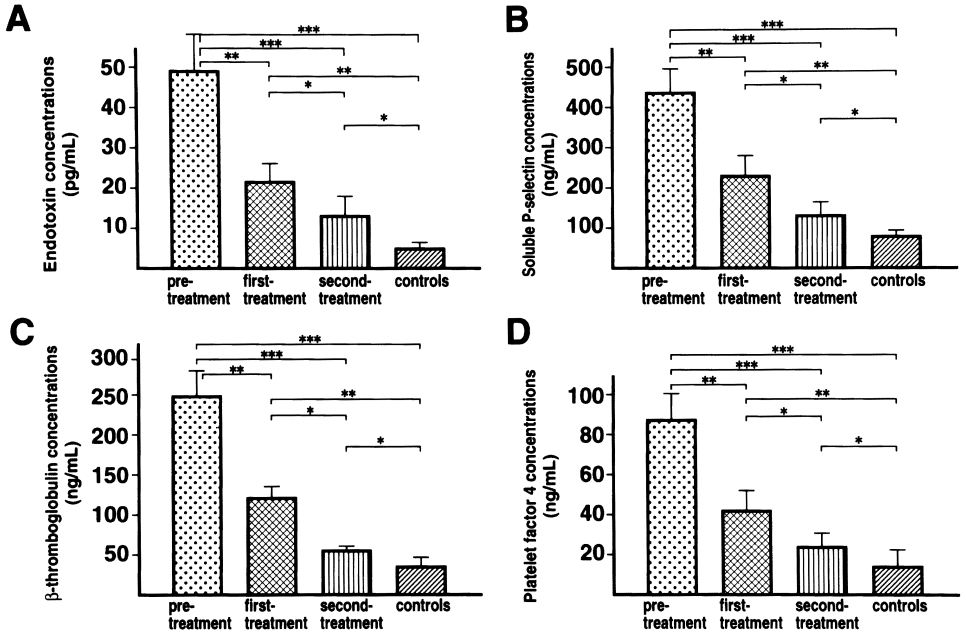

Fig. 1. Effect of PMX-F treatment on plasma concentrations of endotoxin (A), soluble P-selectin (B), β-thrombomodulin (C) and platelet factor 4 (D) in patients with septic shock, *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$.

soluble P-selectin concentrations were elevated significantly in group A (440 ± 68 ng/ml) compared with those in group C (72 ± 14 ng/ml) ($p < 0.001$). These concentrations decreased significantly after PMX-F treatment (first treatment, 230 ± 50 ng/ml, $p < 0.01$, and second treatment, 130 ± 36 ng/ml, $p < 0.001$) (Fig. 1B). Both plasma βTG and PF-4 concentrations also were elevated significantly before treatment in group A (250.6 ± 16.8 ng/ml and 88.6 ± 14.2 ng/ml, respectively) ($p < 0.001$) compared with those in group C (36.8 ± 8.4 ng/ml, $p < 0.001$ and 14.6 ± 8.2 ng/ml, $p < 0.001$, respectively). These concentrations were also significantly decreased after PMX-F treatment: βTG; first treatment, 122.6 ± 12.2 ng/ml ($p < 0.01$) and second treatment, 52.6 ± 4.8 ng/ml($p < 0.001$); PF-4, first treatment, 42.6 ± 9.8 ng/ml ($p < 0.01$) and second treatment, 24.4 ± 6.8 ng/ml ($p < 0.001$) (Figs. 1C and D). In contrast, plasma P-selectin, βTG and PF-4 concentrations showed little change during conventional treatments in group B (before vs. after the treatment; P-selectin, 420 ± 56 ng/ml vs. 408 ± 50 ng/ml; βTG, 240.8 ± 17.8 ng/ml vs. 234.2 ± 16.8 ng/ml; PF-4, 86.4 ± 13.6 ng/ml vs. 84.8 ± 13.2 ng/ml).

The endotoxin concentrations in group A paralleled the P-selectin, βTG and PF-4 concentrations over the course of measurement. There was a strong correlation between patient outcome and APACHE II score ($p < 0.01$), and plasma concentrations of endotoxin ($p < 0.001$), P-selectin ($p < 0.001$), βTG ($p < 0.001$) and PF-4 ($p < 0.001$).

Discussion

Recently, Shoji et al. [5] reported that PMX-F is effective for the treatment of septic shock and is without any critical adverse effects. The PMX Clinical Study Group in Japan has reported that PMX-F improved the patient outcome in case of severe sepsis or septic multiple organ failure [7]. In the present study, we showed that PMX-F therapy is superior to conventional therapies in terms of patient outcome, that plasma levels of soluble P-selectin, βTG and PF-4 were significantly increased in patients with septic shock and that these levels were significantly reduced by PMX-F treatment.

In sepsis, neutrophils adhere to vascular endothelial cells, then pass through the intercellular space and migrate to tissues [17]. Infection activates macrophages and fibroblasts, producing endotoxin and inflammatory cytokines. In septic shock, some investigators have reported increased levels of E-selectin and adhesion molecules in both plasma and in the supernatant of cultured vascular endothelial cells [17, 18]. We have shown previously that plasma levels of von Willebrand factor and thrombomodulin, which are thought to be indicators of endothelial cell injury, were significantly increased in patients with septic shock [19]. In addition, we have shown that plasma MMP-9 concentrations and monocyte MMP-9 mRNA levels may be useful prognostic markers in septic shock [8].

P-selectin is a membrane glycoprotein located in α-granules of platelets and Weibel-Palade bodies of endothelial cells. When platelets or endothelial cells are activated, P-selectin is rapidly translocated to the cell surface and can be a receptor for leukocytes at sites of inflammation [20].

Soluble forms of adhesion molecules have received much attention as markers reflecting the presence of inflammatory mediators, cellular activation or damage and disease activity [21]. P-selectin plays a fundamental role in mediating the inflammatory responses of leukocytes [22]. The plasma levels of P-selectin have been reported to increase in collagen diseases and in proliferative glomerulonephritis [22–24]. The majority of soluble P-selectin in blood is the alternatively spliced form lacking a transmembrane domain with the N-terminus intact, and the remainder

is proteolytically derived from the membrane-bound form of P-selectin [23]. Though it is unclear what stimuli induce the alternative splicing, the plasma levels of P-selectin can reflect either platelet activation and/or vascular endothelial perturbation in septic shock. βTG and PF-4 are released from α-granules of activated platelets [25]. Our data implies that platelets were activated in patients with septic shock. However, the precise mechanisms are unclear. The increased soluble P-selectin, βTG and PF-4 levels may not only be indicators of platelet activation but also play further roles in septic shock syndrome.

We observed a decrease in blood endotoxin concentration with PMX-F treatment that paralleled that of plasma concentrations of P-selectin, βTG and PF-4, and suggested that endotoxin may stimulate platelets to release P-selectin, βTG and PF-4 into the circulation. We reported previously that PMX-F reduced levels of humoral mediators in patients with septic shock [26]. A decrease in plasma P-selectin, βTG and PF-4 concentrations with PMX-F may be, in part, due to a reduction of humoral mediators including various kinds of cytokines. PMX-F therapy improved many clinical signs and symptoms including the APACHE II score, the number of failed organs and blood pressure. These improvements parallel the changes in P-selectin, βTG and PF-4.

Septic shock causes various activation reactions including blood coagulation and fibrinolysis in addition to platelet activation. Activation of the coagulation system was significantly attenuated in patients with sepsis treated with epinephrine [27]. Recently, Baudo et al. [28] have reported that replacement of antithrombin III reduces mortality in patients with septic shock. We are now studying the effect of PMX-F treatment on blood coagulation and fibrinolysis in patients with septic shock.

In summary, plasma P-selectin, βTG and PF4 levels, which are markers of platelet activation, were increased in patients with septic shock. PMX-F therapy decreases elevated plasma endotoxin and platelet activation markers in these patients.

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