SHOCK, Vol. 34, No. 5, pp. 461–466, 2010

APHERESIS OF ACTIVATED LEUKOCYTES WITH AN IMMOBILIZED POLYMYXIN B FILTER IN PATIENTS WITH SEPTIC SHOCK

Tuneyasu Kumagai,* Naoshi Takeyama,† Teru Yabuki,* Makoto Harada,* Yasuo Miki,* Hideki Kanou,† Sousuke Inoue,* Takashi Nakagawa,* and Hiroshi Noguchi†

**Department of Emergency & Critical Care Medicine, Aichi Medical University, Nagakute; and †Department of Emergency & Critical Care Medicine, Fujita Health University School of Medicine, Toyoake, Aichi, Japan*

Received 20 Oct 2009; first review completed 11 Nov 2009; accepted in final form 16 Mar 2010

ABSTRACT—In this study, we examined the effects of direct hemoperfusion through filters with immobilized polymyxin B (PMX-DHP) on leukocyte function and plasma levels of cytokines in patients with septic shock. We found that PMX-DHP caused increased expression of C-X-C chemokine receptor 1 (CXCR1) and CXCR2, along with decreased expression of CD64 and CD11b, by circulating neutrophils in patients with septic shock. Plasma levels of cytokines, including interleukin 6 (IL-6), IL-8, IL-10, and high-mobility group box 1, were elevated in patients with septic shock compared with healthy controls, but cytokine levels were not altered by PMX-DHP. These results suggest that PMX-DHP influences neutrophils via a mechanism that does not involve cytokine. *Ex vivo* perfusion of heparinized blood from patients with sepsis and septic shock through PMX filters in a laboratory circuit caused a significant decrease in neutrophil and monocyte counts. After 120 min of perfusion, neutrophils, monocytes, and lymphocytes were decreased by 78%, 70%, and 10%, respectively, compared with baseline values. Flow cytometric analysis indicated that activated neutrophils with high levels of CD11b/CD64 expression and low levels of CXCR1/CXCR2 expression showed preferential adhesion to PMX filters. Neutrophils isolated from the blood after *ex vivo* PMX perfusion caused less damage to an endothelial cell monolayer than cells from sham-treated blood, whereas neutrophil phagocytosis of opsonized *Escherichia coli* was unaffected. These results indicate that PMX-DHP selectively removes activated neutrophils and reduces the ability of circulating cells to cause endothelial damage. Selective removal of activated neutrophils using PMX-DHP may improve the systemic inflammatory response in patients with septic shock.

KEYWORDS—Chemokine receptors, CD11b, CD64, endothelial damage, HLA-DR, monocytes, neutrophil activation

INTRODUCTION

Polymyxin B (PMX) has both antibacterial and antiendotoxin effects because it destroys the bacterial outer membrane and binds endotoxins to neutralize their toxicity (1). Cartridges containing PMX immobilized to fibers (Toraymyxin; Toray Industries, Tokyo, Japan) have been developed for selective adsorption of circulating endotoxin in patients with gram-negative bacterial infections, and this treatment has been proven to be highly effective (2). Several studies have also found that direct hemoperfusion through PMX (PMX-DHP) is beneficial in patients with gram-positive (3) or endotoxin-negative bacterial infections (4). Interestingly, PMX-DHP has also been shown to improve pulmonary oxygenation in patients with acute respiratory distress syndrome (ARDS) (4–6), suggesting that the therapeutic effects of PMX-DHP on sepsis and ARDS involve other mechanisms in addition to endotoxin removal.

The pathophysiology of sepsis and acute lung injury/ARDS is complex and has yet to be fully elucidated, although it is generally accepted that activated macrophages and dendritic cells, as well as neutrophils, play important roles. The initial response to infection and tissue damage involves the release of proinflammatory and anti-inflammatory cytokines and chemokines through activation of macrophages and dendritic cells, then acting locally to stimulate chemotaxis and activate neutrophils (7). Activated neutrophils release oxygen radicals, proteases, leukotrienes, and other proinflammatory molecules such as platelet-activating factor (8). Activation of neutrophils is an essential part of the innate immune response. However, when local defense mechanisms are unable to eliminate an infectious agent, the inflammatory response becomes systemic, and this systemic inflammatory response can lead to widespread organ damage and is associated with increased mortality (8).

In the present study, we theorized that pathological activation of neutrophils and monocytes is associated with sepsis, acute lung injury/ARDS, and multiple organ failure, and that removal of these cells from the circulation could reduce leukocyte-dependent tissue injury. To address this issue, we evaluated the effect of PMX-DHP on circulating leukocytes in patients with septic shock through changes of neutrophil and monocyte surface antigen expression after PMX-DHP. In another experiment, we passed heparinized blood from patients with sepsis and septic shock through PMX filters in a laboratory circuit and assessed changes of the cell count and surface antigen expression by neutrophils and monocytes. After perfusion, neutrophils were isolated and the capacity of these cells to damage cultured endothelial monolayers was also determined.

MATERIALS AND METHODS

Subjects

This study was conducted in accordance with the Declaration of Helsinki and was approved by the Aichi Medical University Institutional Review Board. After obtaining informed consent from each subject, we studied 33 consecutive critically ill patients (Table 1), who were divided into a sepsis group and a septic shock group. The diagnosis of septic shock was made in accordance with the modified 2001 criteria from the consensus statement of the Society

Address reprint requests to Tuneyasu Kumagai, MD, Department of Emergency & Critical Care Medicine, Aichi Medical University, Nagakute-cho, Aichi, Japan. E-mail: kuma@aichi-med-u.ac.jp.

DOI: 10.1097/SHK.0b013e3181e14ca0

TABLE 1. **Demographic data**

	Septic shock group	Sepsis group
No. patients	18	15
Age (median [IQR]), years	67 (52–77)	53 (40–65)
Sex ratio (male/female)	11/7	10/5
APACHE II score (median [IQR])	24 (21–31)	8 (6–11)
SOFA score (median [IQR])	14 (11–19)	3 (2–5)
Mortality, %	54	0
Site of infection (no.)		
Abdominal	12	4
Urinary tract	2	1
Others	4	10

APACHE II–Acute Physiology and Chronic Health Evaluation II; IQR–interquartile range; SOFA–Sequential Organ Failure Assessment.

of Critical Care Medicine/European Society of Intensive Care Medicine/American College of Chest Physicians/American Thoracic Society/Surgical Infections Society (9). Briefly, septic shock was defined by the presence of systemic inflammatory response syndrome (SIRS), known or suspected infection, and the onset of shock within the previous 72 h (systolic blood pressure <90 mmHg despite fluid replacement or the requirement for at least 1 h of vasopressor infusion). Infection was diagnosed from clinical signs of infection and/or by culture of microorganisms from suspected infectious foci. *Systemic inflammatory response syndrome* was defined as being present if two or more of the diagnostic criteria of Bone et al. (10) were positive. The severity of illness was assessed by calculating the Acute Physiology and Chronic Health Evaluation II score (11) and the Sequential Organ Failure Assessment score (12). The PMX-DHP was performed in 18 patients who were admitted to our department with septic shock or developed it during their stay in the intensive care unit. The PMX-DHP was commenced within 12 h after the onset of septic shock in 16 patients and more than 12 h after the onset of septic shock in 2. The *ex vivo* PMX perfusion study was performed with 15 other patients who met the criteria for sepsis (SIRS with infection) and 18 patients with septic shock.

PMX-DHP

The PMX-DHP procedure involved the insertion of a double-lumen catheter into a femoral vein, and hemoperfusion for 2 to 4 h at a flow rate of 80 to 100 mL/min. Blood samples were collected in heparinized tubes before and immediately after hemoperfusion to evaluate changes in leukocyte surface antigen expression and serum cytokine levels.

Immunofluorescence studies

For determination of neutrophil surface antigen expression, 100 μL of heparinized whole blood was reacted directly with fluorescein isothiocyanate (FITC)–conjugated antibodies directed against C-X-C chemokine receptor 1 ([CXCR1] clone 42705; R & D Systems, Minneapolis, Minn), CXCR2 (clone 48311; R & D Systems), CD11b (clone BEAR1; BD Biosciences, San Diego, Calif), CD64 (clone 22; Beckman Coulter Immunotech, Fullerton, Calif), or the respective isotype controls for 10 min at room temperature. This was followed by lysis of erythrocytes and fixation of cells using FACS Lysing Solution (BD Biosciences) according to the manufacturer's instructions. For determination of human leukocyte antigen (HLA)-DR expression, peripheral blood mononuclear cells (PBMCs) were separated from blood samples by Ficoll Hypaque gradient density centrifugation. After washing twice with phosphate-buffered saline (PBS), PBMCs were suspended in PBS containing 3% fetal bovine serum and 0.01% sodium azide for flow cytometric analysis. Then phycoerythrin-cyanin 5.1–conjugated anti-CD14 (clone RMO52; Beckman Coulter Immunotech), FITC-conjugated anti–HLA-DR (clone L243; BD Biosciences), or the respective isotype controls were reacted under saturating conditions with a suspension of PBMCs for 10 min at room temperature, followed by fixing of the cells with FACS Lysing Solution. After the cells were washed once with cold PBS, immunofluorescence was analyzed using a FACSCanto II flow cytometer with CellQuest software (BD Biosciences). Granulocytes (10,000 events) or monocytes (2,000 events) were studied by gating according to forward- and side-scatter properties. Results were expressed as the mean fluorescence intensity (MFI) index, which was calculated as the MFI of cells stained by each specific antibody divided by the MFI of cells stained by the respective isotype-matched control antibody.

Measurement of cytokine levels

Heparinized blood was centrifuged at 800*g* for 5 min at 4°C and stored at −80°C until analysis. Plasma concentrations of cytokines were measured using enzyme-linked immunosorbent assay kits from BioSource International (Camarillo, Calif) for interleukin 8 (IL-8), IL-6, and IL-10, and from Sino Test Corporation (Sagamihara, Kanagawa, Japan) for high-mobility group box 1 (HMGB1). The detection limits of these kits were 0.7 pg/mL, 3 pg/mL, 1 pg/mL, and 1 ng/mL for IL-8, IL-6, IL-10, and HMGB1, respectively.

Laboratory model of an extracorporeal circuit

Peripheral blood was collected into heparinized tubes from patients with sepsis and septic shock and used in a laboratory model of an extracorporeal circuit. Small modules containing PMX (internal dimensions, 5 mm × 13 mm; capacity 1 mL; PMX content approximately 3 mg) were provided by Toray Medical Corp (Tokyo, Japan). Closed loop hemoperfusion was performed through a circuit formed by connecting the small PMX module to a polyvinylchloride tube and a peristalsis pump (Atto, Tokyo, Japan). The tube and module were filled with 6 mL of heparinized blood, after which the tube was connected to the exit from the module to complete a closed circuit. A similar circuit with an empty module was used for sham perfusion. Each circuit was perfused at a flow rate of 0.6 mL/min, and blood samples were collected every 40 min until 120 min to determine cell counts. After 2 h of perfusion, the PMX filter was removed from the module and washed with PBS. Leukocytes adherent to the filter were removed and fixed by incubation with FACS Lysing Solution for 10 min. After washing with PBS, the leukocytes were stained with labeled monoclonal antibodies or the appropriate isotype controls for 10 min at room temperature. The stained cells were then washed once with PBS, resuspended, and analyzed using flow cytometry.

Determination of leukocyte counts

Leukocyte counts were measured using BD TruCOUNT Tubes (BD Biosciences). A 50-μL sample of heparinized whole blood was reacted with phycoerythrin-cyanin 5.1–conjugated CD45 (clone 22; Beckman Coulter Immunotech) for 15 min at room temperature, followed by lysis of erythrocytes and fixation of cells with FACS Lysing Solution. The lyophilized pellet in the tube then dissolved and released a known number of fluorescent beads. During analysis, the number of positive cells in whole blood (cells/μL) was determined by comparing cellular events with bead events.

Assessment of neutrophil phagocytosis

Neutrophils obtained after perfusion of blood through the laboratory circuit for 2 h with or without a PMX module were taken and used for neutrophil phagocytic assay. Flow cytometry was used to determine the amount of ingested FITC-labeled opsonized *Escherichia coli* per neutrophil (Phagotest; Opregen Pharma, Heidelberg, Germany). Results were expressed as the MFI ratio, calculated as the MFI of cells incubated at 37°C divided by the MFI of cells incubated at 0°C.

Isolation of neutrophils

Neutrophils obtained after perfusion of blood through the laboratory circuit for 2 h, with or without a PMX module, were isolated using density gradient centrifugation (13). Blood was placed onto a two-layer Histopaque gradient comprising Histopaque 1119 (bottom layer) and 1077 (upper layer; Sigma-Aldrich, St Louis, Mo) and was centrifuged at room temperature for 5 min at 300 g followed by 20 min at 800 g. The neutrophil suspensions were then washed twice in Ca^{2+} and Mg^{2+} free Hanks' balanced salt solution and resuspended in LHC-9 complete growth medium. This procedure yielded neutrophils with more than 90% purity and more than 98% viability using the trypan blue dye exclusion test.

Coculture of BEAS-2B cells with neutrophils

BEAS-2B human bronchial epithelial cells purchased from ATCC (Rockville, Md) were cultured with LHC-9 complete growth medium in BD Biocoat T25 tissue flasks (BD Biosciences) at 37°C under 5% CO_2. Cells were maintained at between 30% and 90% confluence and were passaged every 2 to 4 days by trypsinization. Cells (5×10^4 per well) were seeded into fibronectin-coated 96-well flat-bottomed culture plates containing LHC-9 complete growth medium. After the cells reached confluence, the monolayer was washed and treated with bacterial lipopolysaccharide (LPS; 100 ng/mL). Next, 5×10^4 neutrophils were added to each well (14), and cell viability was evaluated after 4 h using a Dojindo cell counting kit-8 (Dojindo Laboratories, Japan) according to the manufacturer's recommendations (15). Briefly, 100 μL of WST-8 was added, followed by incubation for 1 h, and absorbance at 450 nm was measured

TABLE 2. **Leukocyte surface markers and cytokines**

Variable	Septic shock (n = 18)		Healthy controls (n = 10)
	Before PMX-DHP	After PMX-DHP	
Cytokines			
IL-6, pg/mL	2,170 (1,379–3,210)*	2,347 (1,437–3,892)*	19 (13–29)
IL-8, pg/mL	342 (210–541)*	371 (166–598)*	23 (16–30)
IL-10, pg/mL	320 (123–468)*	308 (219–549)*	12 (8–18)
HMGB1, ng/mL	17 (11–31)†	15 (13–25)†	8 (5–14)
Surface antigen, MFI index			
HLA-DR	18 (13–30)*	17 (11–33)*	45 (30–71)
CXCR1	43 (31–67)*	98 (76–189)†‡	155 (102–277)
CXCR2	12 (8–17)*	19 (13–32)§	35 (27–60)
CD64	33 (24–55)*	22 (17–39)*§	4 (3–6)
CD11b	12 (8–24)†	9 (5–15)§	8 (6–14)

Values are medians (interquartile range). The MFI index was calculated as the MFI of cells stained by the specific antibody divided by the MFI of cells stained by the respective isotype-matched antibody.
*$P < 0.01$ vs. healthy controls.
†$P < 0.05$ vs. healthy controls.
‡$P < 0.01$ vs. before PMX-DHP.
§$P < 0.05$ vs. before PMX-DHP.

in a 96-well spectrophotometer. WST-8 is a tetrazolium salt that is reduced by cellular NAD^+- and $NADP^+$-dependent dehydrogenases to an orange formazan product, which is soluble in culture medium. The amount of the formazan product detected at 450 nm is directly proportional to the number of living cells. Results are expressed as the percentage of viable cells relative to untreated control cells, calculated from the absorbance ratio. All experiments were performed in triplicate.

Statistical analysis

Clinical data were expressed as medians with the interquartile range, and results from *ex vivo* perfusion experiments were given as the mean ± SEM. One-way ANOVA and the Holm-Sidak test were used for comparisons between groups, and differences were considered to be significant at $P < 0.05$. Calculations were performed using SigmaStat software (version 3.5; Systat Software Inc, Point Richmond, Calif).

RESULTS

Plasma cytokine levels and leukocyte surface antigen expression in septic shock patients

Levels of IL-6, IL-8, IL-10, and HMGB1 were significantly higher in patients with septic shock than in healthy controls (Table 2). CD11b, CXCR1, and CXCR2 were expressed by neutrophils from all subjects, although neutrophils from septic shock patients showed a higher level of CD11b expression and a lower level of CXCR1/CXCR2 expression than neutrophils from healthy controls. Table 2 also shows that CD64 was expressed by more neutrophils in the septic shock group than the healthy control group. The MFI of peripheral blood monocytes expressing HLA-DR was significantly lower in patients with septic shock than in healthy controls.

Leukocyte surface antigen expression and serum cytokine levels after PMX-DHP

As shown in Table 2, PMX-DHP did not alter levels of either proinflammatory or anti-inflammatory cytokines. The mean percentage of neutrophils expressing CXCR1/CXCR2 was significantly increased after PMX-DHP, whereas CD11b/CD64 expression was significantly decreased. HLA-DR expression by monocytes was not altered after PMX-DHP.

Effect of in vitro *perfusion through PMX on cells*

Blood samples from 15 patients with sepsis and 18 patients with septic shock were perfused through the laboratory circuit with or without a PMX filter. Figure 1 shows that perfusion through the PMX filter for 40 min resulted in removal of 30% of neutrophils, 32% of monocytes, and 9% of lymphocytes. Further perfusion led to a progressive decline in the number

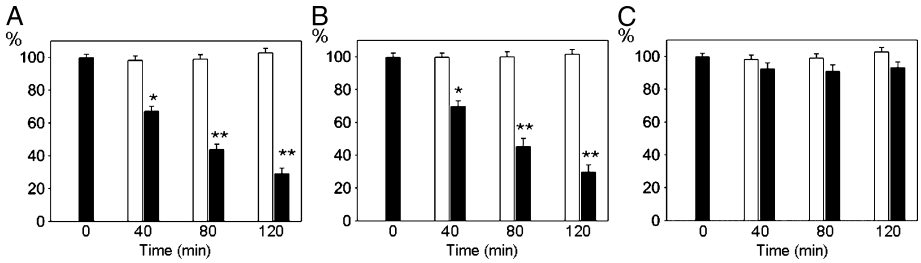

FIG. 1. **Loss of leukocytes during perfusion through the laboratory extracorporeal circuit**. Perfusion was conducted through a circuit with PMX (filled bar) or without PMX (open bar). Numbers of neutrophils (A), monocytes (B), and lymphocytes (C) was measured using BD TruCOUNT tubes. Results are expressed as a percentage of the relevant cell count obtained before hemoperfusion. Bars represent the mean ± SEM of 33 experiments. *$P < 0.05$; **$P < 0.01$ vs. sham.

TABLE 3. **Surface antigen expression by leukocytes after *ex vivo* perfusion**

	Sham filter (n = 33)	PMX filter (n = 33)	*P*
HLA-DR	43 ± 6	48 ± 9	0.37
CD11b	8 ± 2	12 ± 2	0.039
CD64	4 ± 0.6	6 ± 0.9	0.044
CXCR1	134 ± 19	85 ± 11	0.019
CXCR2	43 ± 5	33 ± 3	0.034

Surface antigen expression by leukocytes adherent to PMX filters and leukocytes from the sham filter was analyzed using FACS. Values are mean ± SEM. Results are expressed as the MFI index, calculated as the MFI of cells stained by the specific antibody divided by the MFI of cells stained by the respective isotype-matched antibody.

of neutrophils but no significant change in lymphocyte numbers. After 120 min, 78% of neutrophils and 70% of monocytes were depleted. Perfusion through the circuit without a PMX filter did not lead to any significant reduction in leukocyte numbers.

Surface antigen expression by leukocytes adherent to the PMX filter and leukocytes from the sham circuit were analyzed using FACS. CD11b/CD64 expression by neutrophils adherent to the PMX filter was significantly higher than that of neutrophils obtained from sham perfusion, whereas CXCR1/CXCR2 expression by neutrophils from the filter was lower than that after sham perfusion (Table 3). No differences in HLA-DR expression by monocytes were seen after PMX or sham perfusion.

Phagocytic functions of neutrophils obtained after 2 h of perfusion with or without PMX were analyzed using FACS. No significant difference was seen in the number of *E. coli* taken up per neutrophil, as expressed by the MFI ratio, when neutrophils were obtained either after PMX (19 ± 3, n = 10) or sham (17 ± 3, n = 10) perfusion.

We also investigated whether neutrophils obtained after 2 h of perfusion with or without PMX caused damage when cocultured for 4 h with preactivated endothelial cells. Neutrophils from blood perfused without PMX induced damage to 20% ± 6% of cells in the endothelial monolayer versus 12% ± 3% of cells for neutrophils from blood perfused with PMX ($P < 0.01$). When endothelial cell monolayers were not preactivated with LPS, the level of cell damage was below the detection limit of the WST-8 assay in both cases.

DISCUSSION

This study is the first to report that PMX-DHP rapidly alters surface antigen expression by neutrophils in patients with sepsis. We found that PMX-DHP altered neutrophil expression of surface receptors, including CXCR1, CXCR2, CD11b, and CD64, but did not alter monocyte expression of HLA-DR. Plasma levels of various cytokines (IL-6, IL-8, IL-10, and HMGB1) were elevated in patients with septic shock compared with healthy controls, but these cytokines were not altered after PMX-DHP. These results suggest that PMX-DHP influences neutrophils, but not monocytes, through a mechanism unrelated to cytokines. In the *ex vivo* experiments, a large number of neutrophils and monocytes adhered to the PMX filters and were removed from circulation. Activated neutrophils with increased expression of CD11b/CD64 and low expression of CXCR1/CXCR2 selectively showed adhesion to the PMX filters. In addition, neutrophils obtained after *ex vivo* perfusion through PMX filters showed less toxicity for endothelial monolayers than neutrophils obtained after sham perfusion, although neutrophil phagocytosis of opsonized *E. coli* was unaffected. These results indicate that PMX-DHP selectively removes activated neutrophils from the blood and therefore could improve the systemic inflammatory response in patients with septic shock.

In our patients with septic shock, plasma levels of IL-8 were increased, along with reduced expression of CXCR1/CXCR2 by neutrophils. As reported in patients with sepsis (16), trauma (17), and cardiac surgery (18), downregulation of IL-8 receptors indicates *in vivo* activation of neutrophils. After IL-8 stimulation, CXC receptors are rapidly internalized and are subsequently recycled to the plasma membrane or degraded (17). Increased expression of CD64 and CD11b by neutrophils con firmed neutrophil activation in our septic shock patients. Increased CD64 expression by neutrophils is a sensitive marker of early infection (19), and CD64 is rapidly upregulated in response to LPS and proinflammatory cytokines such as interferon-γ and granulocyte colony-stimulating factor (20). In contrast to increased expression of CD64 and CD11b by neutrophils, HLA-DR expression by monocytes is decreased in patients with severe sepsis and septic shock (21). Reduced monocyte HLA-DR expression and increased plasma IL-10 levels indicate the occurrence of immunoparalysis in patients with septic shock.

The use of PMX-DHP led to a reduction in activated circulating neutrophils but did not increase monocyte expression of HLA-DR, and cytokine levels were also unaffected by PMX-DHP. These results agree with the earlier report by Tsushima et al. (6) that plasma levels of tumor necrosis factor-α, IL-6, IL-10, and IL-8 were unaffected by PMX-DHP. However, Ono et al. (22) reported that IL-10, but not IL-6, was significantly decreased after PMX-DHP. Further investigations are needed to clarify the effects of PMX-DHP on circulating cytokine levels.

Our findings suggest that alterations of surface antigen expression by neutrophils after PMX-DHP depend on mechanisms other than the removal of humoral mediators such as cytokines and endotoxin. Recently, Nishibori et al. (23) reported that a large fraction of circulating monocytes became adherent to PMX filters and were removed from circulation. Ono et al. (22) also reported that about 20% of peripheral monocytes and neutrophils adhered to the PMX column after PMX-DHP. Because lymphocytes showed less adhesion to PMX filters, not all blood cells were trapped nonspecifically, but rather there was an interaction between leukocytes and the fibers of the PMX filter. The pattern of surface antigen expression by leukocytes adherent to PMX filters confirmed that it was a selective process, as CD11b/CD64 expression was significantly higher in neutrophils collected after PMX perfusion than after sham perfusion, whereas CXCR1/CXCR2 expression showed the opposite changes. These results indicate that activated neutrophils were selectively adherent to PMX filters.

The detailed mechanisms of the leukocyte adherence to PMX-DHP are unknown. Polymyxin B is covalently grafted on to the polystyrene fiber surface. The average surface concentration of fiber-grafted PMX molecules is 0.25 molecules/nm^2 or an average linear concentration of one PMX molecule every 2 nm (24). The length of the PMX molecule is estimated to be about 2.6 nm by molecular analysis (25), suggesting that the polystyrene fiber surface is fully covered by PMX molecules. Neutrophils and monocytes would therefore adhere to the polymyxin B molecule itself rather than the polystyrene fiber or other constituents. This suggests that the interaction between polymyxin B and leukocytes would involve both hydrophobic and hydrophilic forces because both leukocytes and polymyxin B have an amphiphilic nature.

The exact mechanisms by which PMX-DHP selectively absorbs activated neutrophils are obscure. Primed and activated neutrophils display altered membrane expression of receptors that mediate adhesion, chemotaxis, and other functions (26). These changes in the membrane environment are accompanied by altered characteristics of hydrophobic and hydrophilic residues (27). Selective absorption of activated neutrophils may be accounted for by changes in the membrane environment after neutrophil activation.

This raises the question of whether a PMX filter preferentially removes a subset of neutrophils that causes increased vascular permeability, microvascular occlusion, and damage to blood vessel walls in the heart, lungs, and other organs. We also evaluated the activity of neutrophils that remained in the blood after *ex vivo* PMX or sham perfusion and found that neutrophils obtained after PMX perfusion were less cytotoxic than those obtained after sham perfusion, although neutrophil phagocytosis of opsonized *E. coli* was unchanged. These results suggest that PMX-DHP removes toxic activated neutrophils from the blood, whereas bacterial phagocytosis by neutrophils would be unaltered.

Preferential removal of activated neutrophils has also been reported with a leukocyte-depleting filter (LG6, Pall, Portsmouth, UK) that reduced the number of neutrophils adherent to blood vessel walls, thereby alleviating the manifestations of SIRS (28).

In patients with SIRS, the proinflammatory cytokine response causes widespread activation of circulating phagocytes, particularly neutrophils, which release oxygen radicals and a wide range of proteolytic enzymes that cause tissue damage (8). In addition to tissue injury, activated neutrophils cause vascular congestion, hemorrhage, microthrombi, stagnant hypoxia, and increased vascular permeability leading to edema. Removal of activated neutrophils in SIRS patients could therefore be a useful strategy (28). It is known that inhibition of white blood cell function significantly reduces mortality in animal models of SIRS (29), but total pharmacological blockade of white blood cell function may also be deleterious in patients with severe sepsis (30). In patients undergoing cardiac surgery, perioperative leukocyte depletion reduces the subsequent inflammatory response, including acute lung injury (31, 32).

Although our study did not evaluate the effects of PMX-DHP on clinical outcomes, reported improvements in hemodynamics (2) and pulmonary oxygenation (6) during PMX-DHP may be in part caused by removal of activated neutrophils from the blood.

In conclusion, we demonstrated that PMX-DHP leads to an increase of CXCR1/CXCR2 expression and a decrease of CD64/CD11b expression by circulating neutrophils. *Ex vivo* perfusion experiments showed that activated neutrophils adhered preferentially to PMX filters, and that the remaining neutrophils caused less endothelial damage, although neutrophil phagocytic function was not affected. Selective removal of activated neutrophils by PMX-DHP may improve the systemic inflammatory response in patients with septic shock.

ACKNOWLEDGMENTS

The authors thank Ms Sanae Masuko, Mr Toru Arakane, and Hisataka Shoji for their valuable comments and advice.

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