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The Effect of Continuous Veno-Venous Hemofiltration or Direct Hemoperfusion With Polymyxin B-Immobilized Fiber on Neutrophil Respiratory Oxidative Burst in Patients With Sepsis and Septic Shock

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Abstract: Neutrophil activates and injures tissues and organs during sepsis or septic shock. Blood purification therapies such as continuous veno-venous hemofiltration (CVVH) and direct hemoperfusion with polymyxin-immobilized fiber (PMX-DHP) have been used for the treatment of sepsis and septic shock, however, the effects of such therapies on neutrophil activation have previously been poorly understood. We sought to evaluate neutrophil reactive oxygen species (ROS), especially H_2O_2 production, in the pathophysiology of sepsis or septic shock and the effect of CVVH or PMX-DHP on neutrophil ROS. Seven critically ill septic patients requiring CVVH (and 12 matched septic patients who did not require CVVH as control) and seven septic shock patients treated with PMX-DHP were studied. We found that patients with sepsis or septic shock had significantly higher levels of neutrophil ROS compared with normal volunteers (183 ± 42, 292 ± 90, and 103 ± 30) ($P < 0.05$, and <0.005). Neutrophil ROS did not change over time in patients treated either with CVVH or without CVVH. In contrast, neutrophil ROS significantly inhibited PMX-DHP treatment in patients with septic shock (pre-treatment; 292 ± 88 vs. post-treatment; 205 ± 93, $P < 0.05$). In conclusion, neutrophil ROS was significantly enhanced in the sepsis or septic shock affected patients. CVVH did not affect neutrophil ROS while PMX-DHP significant inhibited neutrophil ROS. **Key Words:** CRRT, Direct hemoperfusion, Neutrophil, Polymyxin B, Respiratory oxidative burst, Superoxide.

Sepsis and septic shock are still the leading cause of death in intensive care units. The mortality rate of sepsis or septic shock has been reported to be 30–80% (1,2). Pro-inflammatory cytokines and neutrophils play an important role in this setting. Circulating neutrophils are primed and activated by pro-inflammatory cytokines, they then migrate and injure tissues and organs non-specifically. Activated neutrophils also produce proteases and a large amount of reactive oxygen species (ROS) such as superoxide, hydrogen peroxide (H_2O_2) and hydroxy radicals in a process called a respiratory oxidative burst (3).

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Sepsis and septic shock often result in multiple organ dysfunction syndrome (MODS) because of an inflammatory response by pro-inflammatory cytokines and activated neutrophils. Acute renal failure (ARF) is a common organ failure in MODS. In the past decade, continuous veno-venous hemofiltration (CVVH) has been widely used for the treatment of critically ill patients with ARF (4). Recently, non-renal indication has been proposed for the management of sepsis or septic shock (5). Although previous studies failed to show the effective removal of cytokines and the decrease in plasma cytokine levels with CVVH (6,7), the effect of CVVH on activated neutrophil is poorly understood.

In Japan, direct hemoperfusion with polymyxin B-immobilized fiber (PMX-DHP) that potentially adsorbs plasma endotoxin, was previously widely used for the treatment of septic shock and resulted in decreased plasma endotoxin levels and the

improvement of hemodynamic instability (8). Although several studies failed to show the decrease in plasma cytokine level, there is evidence to show that PMX-DHP improved organ dysfunction such as hypoxemia in patients with acute respiratory distress syndrome (ARDS) (9,10). PMX-DHP might attenuate neutrophil ROS activity. However, the effect of PMX-DHP on the neutrophil ROS is also poorly understood.

In the present study, we sought to evaluate the effect of CVVH or PMX-DHP on the neutrophil ROS in patients with sepsis and septic shock, and we now report our findings.

MATERIALS AND METHOD

Study population

We prospectively examined data from seven septic patients treated with CVVH, 12 similar septic patients treated without CVVH and seven septic shock patients treated with PMX-DHP. Neutrophil ROS activities were compared with the blood samples of 10 healthy volunteers. Sepsis and septic shock were diagnosed according to the criteria of the members of American College of Chest Physician/Society of Critical Care Medicine Consensus Conference Committee (11). The PMX-DHP treatment was initiated if patients had a sustained shock despite fluid resuscitation and vasopressor drugs. Proper informed consent was obtained from each patient or next of kin. All patient records were reviewed to obtain demographic data and details of initial clinical presentation on admission, and then calculated with the APACHE-II score (12) to assess each patient.

Description of continuous veno-venous hemofiltration

CVVH was achieved with a CRRT machine (TR-520 or TR 530; Toray Industries, Inc., Tokyo, Japan). A dual-lumen catheter (Bard Niagara, Vascath, ON, Canada) was inserted into a central vein. Blood flow was kept at 100–120 mL/min. A polyacrylonitrile hollow-fiber hemofilter (PANFRO, Asahi medical, Tokyo, Japan) was used. The bicarbonate-buffered replacement fluid (Sublood-BD; Fuso Pharmaceutical Industries, Osaka, Japan) was given postfilter at 1 L/h. Anticoagulation of the circuit was achieved with nafamostat mesilate (Torii Pharmaceuticals, Co., Ltd, Tokyo, Japan) of 30–35 mg/h to keep an activated coagulation time of 150 s.

Description of polymyxin B-immobilized fiber

PMX-DHP was also achieved with a CRRT machine (TR-520 or TR 530). The PMX column immobilizing polymyxin B (PMX-20R; Toraymyxin, Toray Industries, Inc., Tokyo, Japan) was used for direct hemoperfusion (DHP). The column was washed by perfusion with 4 L of sterile saline. The DHP was carried out at a blood flow rate of 80–100 mL/min for 2 h. PMX-DHP was carried out no more than twice on each patient. In the present study, we evaluated only the data from the first session of PMX-DHP for each patient. Anticoagulation of the circuit was achieved with nafamostat mesilate (Torii Pharmaceuticals, Tokyo, Japan) of 30–35 mg/h to maintain an activated coagulation time of 150 s.

Determination of neutrophil respiratory oxidative burst

Heparinized blood samples were collected before treatment and then daily for CVVH treatment or immediately after treatment of PMX-DHP treatment. The neutrophil ROS was measured immediately after blood sampling by flowcytometry (Coulter Epics System-II, Coulter Co., Miami, FL, USA) using 2,7-dichlorofluorescein diacatete (DCFH-DA; Eastman Kodak, Rochester, NY, USA) as a marker dye by the modified method of Bass et al. (13). Briefly, blood samples were incubated with DCFH-DA at 37°C for 45 min, and erythrocytes were subsequently lyzed with lysis solution (OptiLyse C; Coulter, Miami, FL, USA). Non-fluorescent intracellular DCFH in neutrophil is oxidized to highly fluorescent DCF by H_2O_2 during respiratory oxidative burst (13). Neutrophils were isolated from other cells by forward and side scatter characteristics on flowcytometry, and their histogram according to its fluorescent intensity was constructed. Finally, mean fluorescent intensity channel (MFI) was measured and considered as neutrophil ROS activity. Data are presented as MFI per cell.

Statistical analysis

Statistical analysis was carried out using a commercial statistical package (Statview 5.0; Abacus Concepts, Berkeley, CA, USA). Descriptive statistics for continuous variables are presented as mean with standard deviation. Comparisons of unpaired continuous variables among the groups were assessed using analysis of variance (ANOVA) with Bonferroni correction. Comparisons of paired continuous variables were carried out using Wilcoxon's signed rank test, while comparisons of nominal variables were carried out using Fisher's exact test. The changes over the treatment period were also assessed using ANOVA with Bonferroni correction. To compare the effects of the use of CVVH on neutrophil ROS, we used the

TABLE 1. *Patient characteristics of each group*

	Sepsis treated with CVVH	Sepsis treated without CVVH	Septic shock
Number of patients	7	12	7
Age	73 ± 7	71 ± 8	76 ± 6
Gender (male: female)	4:3	7:5	4:3
APACHE-II score	18.3 ± 2.1	17.5 ± 2.8	22.0 ± 2.6*
Mechanical ventilation	71%	67%	86%
Vasopressor use	86%	67%	100%

*$P < 0.05$, mean ± SD.

CVVH, continuous veno-venous hemofiltration; APACHE-II, acute physiologic and chronic health evaluation—II score.

area under the curve (AUC) method as described by Matthews et al. (14). The difference from pretreatment values was calculated and used for this analysis. In the present study, as the period for each group was the same, the mean value for this difference over the five days was used and presented as the statistical equivalent of the AUC. Comparison of this difference was carried out using Mann–Whitney *U*-test. A $P < 0.05$ was considered as statistically significant.

RESULTS

Seven septic patients treated with CVVH, 12 similar patients treated without CVVH and seven septic shock patients treated with PMX-DHP were recruited to the present study. Patient characteristics such as gender, age, APACHE-II score, mechanical ventilation and vasopressor use were similar between septic patients treated with CVVH and without CVVH (Table 1). Septic shock patients had significantly higher APACHE-II scores (22.0 ± 2.6) compared with others (18.3 ± 2.1, 17.5 ± 2.8, $P < 0.05$) (Table 1). Furthermore, patients with sepsis or septic shock had significantly higher levels of neutrophil ROS compared with healthy volunteers (103 ± 30, 183 ± 42, and 292 ± 90) ($P < 0.05$, and <0.005) (Fig. 1). On admission, septic patients treated with CVVH had a similar neutrophil ROS activities to septic patients treated without CVVH (183 ± 42, 202 ± 40.2, $P = 0.499$). Subsequently, neutrophil ROS did not change over time in septic patients treated either with CVVH or without CVVH (Fig. 2). In contrast, neutrophil ROS significantly inhibited PMX-DHP treatment in patients with septic shock (pretreatment; 292 ± 88 vs. post-treatment; 205 ± 93, $P < 0.05$) (Fig. 3).

DISCUSSION

In the present study, we found several observations that require careful discussion.

First, neutrophil ROS was significantly enhanced in sepsis and septic shock. Previous studies also reported increased neutrophil ROS in a rat endotoxin shock model (15), trauma patients (16), and septic shock patients (17). Our observation was consistent with these reports. Furthermore, several studies have shown that although neutrophil ROS was enhanced in septic shock, the reactivity to chemoattractant was significantly decreased, that is, considered as immunoparalysis (18,19). However, in the present study we highlighted not the immunoparalysis but neutrophil activation.

Second, CVVH of 1 L/h exchange did not attenuate neutrophil ROS. Previously, the effects of CVVH on inflammatory response (especially on pro-inflam-

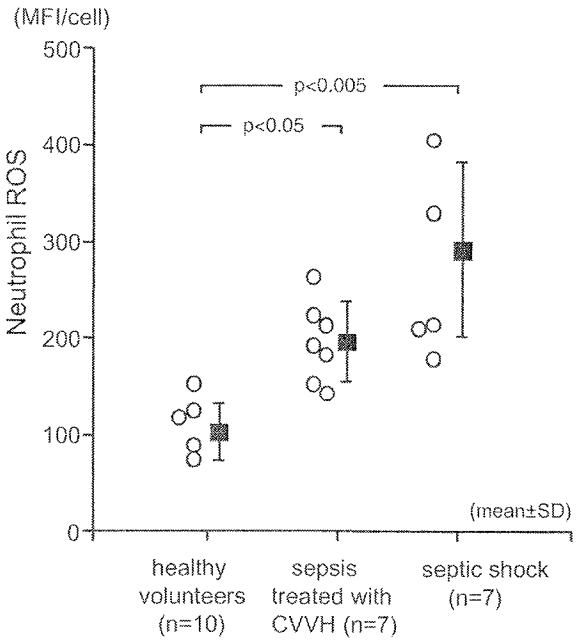

FIG. 1. Neutrophil respiratory burst in healthy volunteers, septic patients treated with CVVH, and septic shock patients. ROS, reactive oxygen species; MFI, mean fluorescent intensity; CVVH, continuous veno-venous hemofiltration.

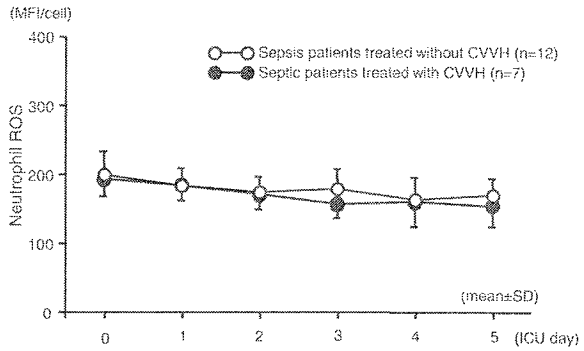

FIG. 2. Daily neutrophil respiratory burst in septic patients treated with CVVH or without CVVH. ROS, reactive oxygen species; MFI, mean fluorescent intensity; CVVH, continuous veno-venous hemofiltration.

matory cytokine kinetics) have been aggressively investigated (5–7). Although circulating inflammatory cytokines were removed by ultrafiltration and adsorption, studies failed to show the decrease in plasma cytokine levels (6,7), even with an aggressive high volume hemofiltration (20). In terms of neutrophil activation, the effect of CVVH on neutrophil ROS is poorly investigated. Yekebas et al. studied neutrophil ROS with CVVH in a porcine animal pancreatitis model (21). Neutrophil ROS activities were initially enhanced and broken down at the end-stage in the control or low dose CVVH (20 mL/kg/h) groups while they were attenuated in the extremely high dose CVVH (100 mL/kg/h) group (21). Our condition of CVVH (1 L/h) was equivalent to low dose CVVH (20 mL/kg/h) in Yekebas's study (21) and resulted in a similar change of neutrophil ROS activity to patients without CVVH treatment. However, the widely used standard ultrafiltration dose for CVVH is 20–25 mL/kg/h and the safety of continuous, long-term use of such extremely high doses of CVVH (100 mL/kg/h exchange) has not been established for the clinical setting.

Third, PMX-DHP resulted in significant inhibition of neutrophil ROS activity. Tani et al. reported that PMX-DHP decreased plasma concentration of endotoxin and improved hemodynamic instability (8), however, Matsuno et al. reported plasma cytokine levels did not change with PMX-DHP treatment (22). Recently, Yamamoto et al. evaluated the effect of PMX-DHP on hypoxemia in an anesthetized sheep model and showed that PMX-DHP significantly decreased intrapulmonary shunt and improved hypoxemia (9). More recently, Tsushima *et al.* studied the effect of PMX-DHP in 20 patients with septic shock and ARDS. Pulmonary oxygenation (PaO_2/FIO_2 ratio) significantly increased with PMX-DHP treatment (125 ± 54 before treatment to 153 ± 73 after treatment) (10). Because PMX-DHP treatment cannot remove inflammatory cytokines from plasma (10), and plasma cytokine levels did not change during the PMX-DHP treatment (22), the improvements in neutrophil ROS were caused by some other mechanism. The mechanism for the inhibition of neutrophil ROS with PMX-DHP is unknown, however, these inhibitions of activated neutrophil ROS could be an explanation for the improvement of respiratory function in septic ARDS patients treated with PMX-DHP (9,10).

Our observations have several limitations. This is not a controlled randomized study, thus it is open to selection bias and error. However data used were prospectively collected and the outcomes are clear, and not subject to interpretation. In the comparison of neutrophil ROS activity between patients treated with CVVH or without CVVH, the characteristics of each patient such as gender, age, and APACHE-II were similar. Furthermore, in terms of neutrophil ROS, neutrophil ROS before the treatment were similar. Thus the only difference was the presence of CVVH.

The dose of ultrafiltration volume for CVVH was relatively low (1 L/h) compared with others (21,23). In 2000, Ronco et al. reported that an increase of ultrafiltration dose (35 mL/kg/h and 45 mL/kg/h) resulted in significant improvement of 15th day mortality rates in critically ill ARF patients compared with a low ultrafiltration dose (20 mL/kg/h) (23). Our ultrafiltration dose (1 L/h) was equivalent to their low ultrafiltration dose (20 mL/kg/h). However, ini-

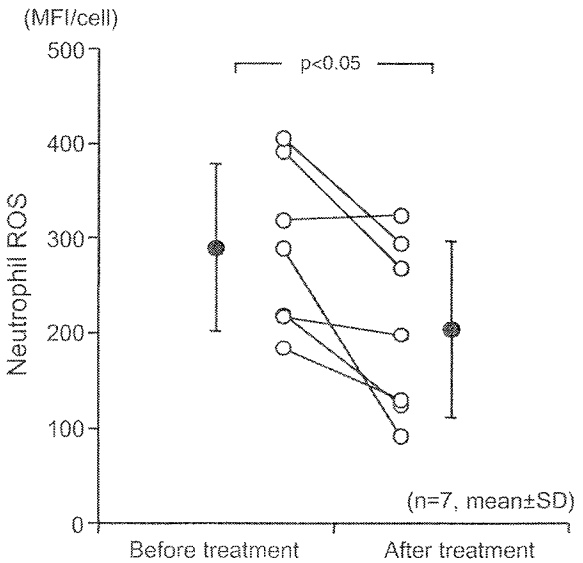

FIG. 3. Neutrophil respiratory burst during PMX-DHP. ROS, reactive oxygen species; MFI, mean fluorescent intensity; PMX-DHP, direct hemoperfusion with polymyxin B-immobilized fiber.

tially, we must evaluate the effect of a standard dose of CVVH on neutrophil ROS activity. As described above, Yekebas et al. reported that high volume hemofiltration (100 mL/kg/h) attenuated neutrophil ROS in an animal model (21). Cole et al. also reported that high volume hemofiltration of 6 L/h exchange significantly improved hemodynamic instability and decreased the vasopressor dose in septic shock patients (20). Increase of ultrafiltration dose might attenuate neutrophil ROS activity. Considering the outcomes of the present study, further investigation is needed.

Finally, to examine the effect of PMX-DHP, we did not have a control population of patients. However, considering the time-course in the CVVH, the neutrophil ROS activities were considered not to decrease over time without PMX-DHP treatment.

In conclusion, we studied neutrophil ROS activity (especially H_2O_2 production) in sepsis or septic shock. Neutrophil ROS was significantly enhanced in sepsis and septic shock. However, low dose (1 L/h) CVVH did not attenuate neutrophil ROS activity while PMX-DHP significant inhibited neutrophil ROS.

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