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Hemoperfusion with an Immobilized Polymyxin B Column Reduces the Blood Level of Neutrophil Elastase

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Key Words
Neutrophil elastase · Immobilized polymyxin B fiber column · Direct hemoperfusion · Sepsis · Systemic inflammatory response syndrome

Abstract
Background: We investigated whether direct hemoperfusion with an immobilized polymyxin B column (DHP with PMX) could reduce the blood level of neutrophil elastase. ***Methods:*** 20 sepsis patients were enrolled in the study. DHP with PMX was performed twice within a 24-hour period. Neutrophil elastase was measured 7 times. ***Results:*** Neutrophil elastase was 468 ± 75.1 μg/l, while it was 1,531 ± 201.7 μg/l immediately after the first session, declined to 351 ± 73.9 μg/l before the second session of DHP with PMX, and increased again to 599.3 ± 112.7 μg/l immediately after the second session, 328 ± 73.7 μg/l at 24 h, 264 ± 39.3 μg/l at 48 h, and 230 ± 36.1 μg/l at 72 h after DHP with PMX. The levels from 48 h onwards were significantly lower compared with that before treatment. ***Conclusion:*** DHP with PMX has an overall effect that reduces circulating neutrophil elastase levels.

Introduction

Severe sepsis [1] is currently a major cause of multiple organ failure (MOF) [2] and the mortality rate exceeds 70% [3]. Endotoxins produced by Gram-negative bacteria and lipoteichoic acid [4] produced by Gram-positive bacteria act on macrophages and monocytes to stimulate the production of pro-inflammatory cytokines, which then induce a series of inflammatory responses that are observed in sepsis [1]. Both neutrophils and macrophages produce an abundance of arachidonic acid metabolites, platelet-activating factor, and cytokines such as IL-1 [5], TNF [6], and IL-8 [7]. The fact that neutrophils produce cytokines indicates involvement in the early stages of the immune response. Neutrophil elastase is one of the proteinases that are abundant in human neutrophils, and is released by activated neutrophils. It was isolated and purified in 1976 by Baugh and Travis [8]. It is an important enzyme involved in the digestion or degradation of bacteria or foreign matter, and is released at the time of phagocytosis. However, neutrophil elastase has a low substrate specificity and thus readily degrades various host molecules, such as plasma proteins, tissue elastin, or tissue collagen so that it becomes a mediator of tissue destruction [9] in inflammatory conditions associated with organ failure.

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Methods of eliminating pathogenic toxins that cause inflammatory reactions have been tried in recent years to prevent the occurrence of the sequential inflammatory cascade. An immobilized polymyxin B column (PMX; Toray Industries Inc., Tokyo Japan) was developed in Japan in 1994 and it has been used for the treatment of endotoxemia. Elimination of pathogenic toxins is not only relevant to lipopolysaccharide produced by Gram-negative bacteria, but also to lipoteichoic acid produced by Gram-positive bacteria [4]. It has been reported that elimination of pathogenic toxins by clinical application of the PMX significantly improves the survival rate [10] but the detailed mechanism of action is yet to be elucidated.

We have previously reported [11] that the PMX prevents the activation of vascular endothelial cells, but no studies have been performed to assess the effect of PMX on the circulating neutrophil elastase level. Therefore, we investigated the time course of the blood level of neutrophil elastase to determine whether PMX altered the circulating amount of this enzyme.

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 treatment with the PMX, global oxygen metabolism was measured. A thermodilution catheter (Edwards Life Sciences LLC, Irvine, Calif., 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. The criteria for inclusion in the study were the following findings within the previous 24 h: (1) signs of the systemic inflammatory response syndrome due to infection (including fever or hypothermia (temperature >38 or <36°C, respectively), tachycardia (>90/min), tachypnea (>20/min) or a $PaCO_2$ <32 mm Hg or mechanical ventilation, and a white blood cell count $>12.0 \times 10^4$/l or $<4.0 \times 10^4$/l or at least 10% immature neutrophils); (2) mean arterial pressure >60 mm Hg irrespective of the use of catecholamines; (3) stable global oxygen metabolism (DO_2I >500 ml/min/m^2 and VO_2I >120 ml/min/m^2), and (4) antibiotic therapy to minimize lipopolysaccharide release by Gram-negative rods. Exclusion criteria were an age under 18 years and a mean blood pressure ≤60 mm Hg irrespective of the use of catecholamines. The Acute Physiology and Chronic Health Evaluation II (APACHE-II) index [12] was employed for assessment of severity before performing direct hemoperfusion with the PMX (DHP with PMX). Blood urea nitrogen and serum creatinine levels were determined before the first session of DHP with PMX, as well as 24, 48, and 72 h afterwards as an index of renal function. The treatment was given to 20 patients who had sepsis due to Gram-positive or Gram-negative bacteria and fulfilled the inclusion criteria given above.

Direct Hemoperfusion

To perform DHP with PMX, a double-lumen catheter was inserted into a femoral vein of each patient and DHP was carried out for 3 h at a flow rate of 80–100 ml/min. Treatment was performed twice within a 24-hour period. Nafamostat mesylate (Torii Co. Ltd, Tokyo, Japan) was used as the anticoagulant.

Measurement of Neutrophil Elastase

The blood level of neutrophil elastase was measured by using a commercial enzyme immunoassay kit (Sanwa Chemical Institute, Nagoya, Japan). Two forms of neutrophil elastase (free neutrophil elastase and neutrophil elastase-α_1-antitrypsin complex) could be detected by the kit, if they possessed antigenicity. The normal neutrophil elastase level measured by this kit was 29 ± 2.7 μg/l (mean ± SD) in 139 healthy persons (SRL Inc., Tokyo Japan; unpubl. data).

Peripheral blood was sampled at the following 7 times for measurement of neutrophil elastase: before DHP with PMX, immediately after the first session of DHP with PMX (3 h after the start), before and immediately after the second session of DHP with PMX, and 24 h (at least 3 h after the second session), 48 h, and 72 h after the first session. As DHP with PMX was used in patients with Gram-negative and/or Gram-positive bacterial infection, neutrophil elastase values were stratified as follows: (1) the value for all 20 patients was determined, (2) the value for patients with Gram-negative infection was determined, (3) the value for patients with Gram-positive infection was determined, and (4) the value for patients with mixed infection (Gram-negative, Gram-positive, and/or fungal) was determined.

Statistical Analysis

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

Results

DHP with PMX was performed 40 times in 20 patients (15 men and 5 women) between the ages of 33 and 85 years (mean 60 ± 13.8). Treatment was performed twice (for 3 h per session) within a 24-hour period. As shown in table 1, the underlying diseases varied and patients had multiple conditions. With respect to the prognosis, 16 patients survived and were discharged from hospital whereas the other 4 patients died. The cause of death was hepatic failure in 1 patient, cardiac failure in 1 patient, and bacterial endocarditis in 2 patients. Table 2 shows the types of infection. Pneumonia was the most frequent primary focus and 29 bacterial strains were detected in the 20 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 an antibiotic to which each isolated microorganism was sensi-

Table 1. Demographic characteristics and underlying diseases of the 20 patients studied

Patients	20
Age, years	60 ± 13.8 (33–85)
Male:female	15:5
Underlying disease	
Respiratory disease	7
Cardiovascular disease	4
Diabetes mellitus	3
Neurological disease	3
Recent trauma	2
Chronic liver disease	1

Table 2. Location of infection

Respiratory system	8
Abdominal cavity	3
Urinary tract	2
Biliary tract	2
Cardiovascular system	2
Central nervous system	2
Other	1

Table 3. Causative organisms

Gram-negative bacteria	
Escherichia coli	4
Pseudomonas aeruginosa	5
Klebsiella pneumoniae	3
Neisseria	3
Vibriocitrobactor	1
Gram-positive bacteria	
Staphylococcus aureus	4
Staphylococcus epidermidis	2
Corynebacterium	1
α-*Streptococcus*	2
Enterococcus faecalis	1
Fungi	
Candida albicans	3

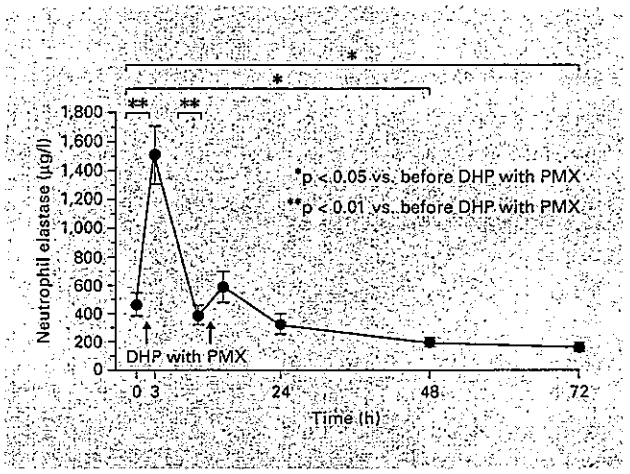

Fig. 1. Changes in the blood level of neutrophil elastase. The blood level of neutrophil elastase level was 468 ± 75.1 μg/l before DHP with PMX, 1,531 ± 201.7 μg/l immediately after the first session, 351 ± 73.9 μg/l before the second session of DHP with PMX, 599.3 ± 73.7 μg/l immediately after the second session, 328 ± 73.7 μg/l at 24 h, 264 ± 39.3 μg/l at 48 h afterwards, and 230 ± 36.1 μg/l at 72 h after the first session. A transient, but significant, increase was observed immediately after the first and the second session of DHP with PMX, while a significant decrease was observed from 48 h onwards compared with the pretreatment level.

tive. The APACHE-II score was 20 ± 1.5 at the start of DHP with PMX. The mean neutrophil elastase level of all 20 patients was 468 ± 75.1 μg/l before DHP with PMX, while it increased to 1,531 ± 201.7 μg/l immediately after the first session of this treatment. It declined to 351 ± 73.9 μg/l before the second session of DHP with PMX, but increased to 599.3 ± 73.7 μg/l immediately after the second session. The neutrophil elastase level was 328 ± 73.7 μg/l at 24 h afterwards, 264 ± 39.3 μg/l at 48 h afterwards, and 230 ± 36.1 μg/l at 72 h afterwards. A transient, but significant, increase was observed immediately after the first and the second session of DHP with PMX, while a significant decrease was observed from 48 h onwards compared with the pretreatment level (fig. 1). There were 6 patients with Gram-negative infection, in whom the neutrophil elastase level was 617 ± 177.7 μg/l before DHP with PMX, 1,779 ± 496.3 μg/l immediately after the first session, 417 ± 216.5 μg/l before the second session of DHP with PMX, 780 ± 272.9 μg/l immediately after the second session of DHP with PMX, 362 ± 102.0 μg/l at 24 h afterwards, 309 ± 106.6 μg/l at 48 h, and 310 ± 112.1 μg/l at 72 h after the first session. In the 4 patients with Gram-positive infection, the neutrophil elastase level was 282 ± 40.1 μg/l before DHP with PMX, 1,471 ± 423.8 μg/l immediately after the first session, 231 ± 108.0 μg/l before the second session, 779 ± 352.5 μg/l immediately after the second session, and 328 ± 109.0 μg/l at 24 h, 301 ± 117.9 μg/l at 48 h, and 205 ± 131.5 μg/l at 72 h after the first session. Ten patients had mixed infection with Gram-negative bacteria, Gram-positive bacteria, and fungi. Their

Table 4. Changes of neutrophil elastase (μg/l) stratified by the type of infection

Type of infection	TM1	TM2	TM3	TM4	TM5	TM6	TM7
Gram-negative (6 cases)	617 ± 177.7	1,779 ± 496.3	417 ± 216.5	780 ± 272.9	362 ± 102.0	309 ± 106.6	310 ± 112.10
Gram-positive (4 cases)	282 ± 40.1	1,471 ± 423.8	231 ± 108.0	779 ± 352.5	328 ± 109.0	301 ± 117.9	205 ± 131.5
Combined (10 cases)	428 ± 139.5	1,653 ± 287.6	270 ± 128.9	434 ± 148.6	203 ± 38.1	178 ± 36.4	203 ± 74.2
Total, average (20 cases)	468 ± 75.1	1,531 ± 201.7	351 ± 73.9	599 ± 73.7	328 ± 73.7	264 ± 39.3	230 ± 36.1

TM1 = Before the first session of DHP with PMX; TM2 = immediately after the first session of DHP with PMX; TM3 = before the second session of DHP with PMX; TM4 = immediately after the second session of DHP with PMX; TM5 = 24 h afterwards; TM6 = 48 h afterwards; TM7 = 72 h afterwards.

neutrophil elastase level was 428 ± 139.5 μg/l before DHP with PMX, 1,653 ± 287.6 μg/l immediately after the first session, 270 ± 128.9 μg/l before the second session of DHP with PMX, 434 ± 148.6 μg/l immediately after the second session, 203 ± 38.1 μg/l at 24 h, 178 ± 36.4 μg/l at 48 h, and 230 ± 74.2 μg/l at 72 h after the first session (table 4).

The blood urea nitrogen level was 33 ± 4.7 mg/dl before the first session of DHP with PMX, while it was 34 ± 4.9, 33 ± 5.3, and 37 ± 6.8 mg/dl at 24, 48, and 72 h afterwards, respectively. Serum creatinine was 1.8 ± 0.3 mg/dl before the first session of DHP with PMX, while it was 1.6 ± 0.3, 1.5 ± 0.3, and 2.2 ± 0.6 mg/dl at 24, 48, and 72 h afterwards, respectively. The urea nitrogen and creatinine levels remained within the normal range, showing no evidence of renal impairment.

Discussion

Patient Selection

No standard criteria for the use of DHP with PMX have been established, so some medical institutions start treatment depending on the APACHE-II score [13, 14], cardiac index [15], or interleukin-6 level [16]. This treatment has generally been reserved for patients with severe sepsis or septic shock. The clinical efficacy of DHP with PMX varies among medical institutions depending on the time of starting the treatment. The criteria employed in this study meant that DHP with PMX was applied to patients in whom organ perfusion and global oxygen metabolism were maintained. Selection of criteria for systemic oxygen metabolism and the mean blood pressure was based on the concept that DHP with PMX should be started before the tissues and cells have been significantly affected even if some organ dysfunction is present. Once shock occurs and organ ischemia progresses, cellular dysfunction at the molecular level becomes severe and this makes it difficult to assess the effect of DHP with PMX. We therefore selected our patients for DHP with PMX according to strict criteria. In the patients who died, infection could not be controlled and MOF developed due to aggravation of dysfunction attributable to the underlying disease.

In recent years, it has been reported that PMX can remove lipoteichoic acids, which are cell wall components of Gram-positive bacteria, from the blood so that TNF-α production is inhibited [4]. Accordingly, DHP with PMX was also performed for patients with Gram-positive infection who met the enrollment criteria.

Forms of Circulating Neutrophil Elastase

Approximately 90 and 10% of the neutrophil elastase released into the blood from neutrophils binds to α_1-antitrypsin (a protease inhibitor) and α_2-macroglobulin, respectively, to form circulating complexes that are inactive [17]. The elimination half-life of free elastase is 0.61 ms and its affinity for α_1-antitrypsin is high. Any elastase that binds to α_1-antitrypsin is deactivated and eliminated from the blood (the elimination half-life is 60 min). The remaining 10% of neutrophil elastase binds to α_2-macroglobulin and is eliminated with a half-life of 10 min. However, the existence of complexes with other inhibitors and the presence of free neutrophil elastase have also been suggested.

We employed a sandwich immunoassay using a neutrophil elastase antibody to detect two forms of neutrophil elastase, which were free elastase and neutrophil elastase-α_1-antitrypsin complex.

Kinetics of Neutrophil Elastase

Neutrophil elastase is a glycoprotein with a molecular weight of approximately 30,000 and it has three isozymes. Its physiological action is proteolysis of bacteria and foreign matter. However, because of its low substrate specificity, neutrophil elastase can also attack various host proteins, such as plasma proteins, coagulation factors, complement, elastin, and collagen [18]. Intact cells are also reported to be damaged [9]. Neutrophil elastase inhibitors exist in the normal tissues to prevent such destruction. However, inflammation and sepsis cause neutrophils to accumulate and secrete elastase in large quantities. Because neutrophils also release myeloperoxidase and superoxide at the same time, which oxidize and deactivate α_1-antitrypsin [19], free neutrophil elastase has the chance to damage proteins such as collagen and elastin, thus destroying the host tissues. When such pathological changes and activation of vascular endothelial cells are extensive, multiple organ dysfunction syndrome arises and progresses to MOF. Duswald et al. [20] reported that an elevated neutrophil elastase level is useful in the diagnosis of sepsis. The circulating level of neutrophil elastase is increased in patients with sepsis, but it has also been reported to increase due to acute renal failure alone [21]. In the present study, the renal function of the subjects was maintained within the normal range, so alteration of renal function did not contribute to the changes of neutrophil elastase.

When we investigated the blood level of neutrophil elastase we found that it was significantly elevated immediately after DHP with PMX, and this change was seen after both the first and second session. Therefore, it seems likely that neutrophils passing through the column were activated by the polystyrene fiber resin [22]. At 24 h after the initial treatment (at least 3 h after the second DHP with PMX session), the neutrophil elastase level was lower than before DHP with PMX, although the difference was not significant. In other words, an increased neutrophil elastase level should be considered as a transient phenomenon that occurs during or at immediately after DHP with PMX. Seyfert et al. [23] tested the effects of a cuprophane membrane (a cellulose membrane), and a polyacrylonitrite (a non-cellulose membrane), and they reported that neutrophil elastase levels peaked from 240 min onwards. The PMX used in this study is a non-cellulose membrane, and we found that neutrophil elastase increased significantly immediately after DHP with PMX, but declined again after 3 or more hours had passed. Such a difference may have been related to differences in biocompatibility between PMX and other membranes. The neutrophil elastase levels at 48 and 72 h after DHP with PMX were significantly lower compared with that before treatment. These results suggest that adsorption of pathogenic toxins by the column prevented the release of inflammatory cytokines [13] and resulted in less stimulation of neutrophils, rather than DHP with PMX having a direct influence on cytokine production or neutrophils being cleared from the blood by the column.

The pattern of change in neutrophil elastase levels was similar in patients with Gram-negative, Gram-positive, and mixed infections. However, the actual changes of neutrophil elastase values were larger in patients with Gram-negative infection than in those with Gram-positive infection. Taken together, these results suggest that DHP with PMX appears to prevent the activation of neutrophils, even if indirectly. The ability of DHP with PMX to prevent activation of vascular endothelial cells [11] and reduce the blood level of neutrophil elastase might decrease the incidence of organ dysfunction.

In conclusion, DHP with PMX shows efficacy for sepsis, and our investigation revealed that circulating neutrophil elastase levels were decreased by this therapy. It is therefore suggested to be an extracorporeal treatment that may be effective even in patients with MOF. Appropriate standards for the use of DHP with PMX should be established, so that it can be performed in the early stage of sepsis and thus contribute to improvement of the prognosis.

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