

## The Effects of Polymyxin B-immobilized Fiber Hemoperfusion on Respiratory Impairment in Endotoxemic Pigs

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### Abstract

**Purpose:** This study investigated the effects of direct hemoperfusion with polymyxin B-immobilized fibers (PMX-DHP) on respiratory impairment in endotoxemic pigs.

**Materials and Methods:** Thirteen anesthetized, mechanically ventilated pigs were divided into PMX-DHP (n=7) and control (n=6) groups. All pigs were hemodynamically monitored with the pulse index contour cardiac output (PiCCO) system (Pulsion Medical Systems, Munich, Germany) and infused intravenously with live *Escherichia coli* (LD50). In the PMX-DHP group, an arteriovenous extracorporeal circuit with a PMX column was applied for 30 to 150 minutes after endotoxin injection. We analyzed the laboratory data, arterial blood gas levels, and PiCCO variables (extravascular lung water [EVLW] and pulmonary vascular permeability index [PVPI]). Furthermore, we performed computed tomography of the chest in all pigs. The data were statistically analyzed with Student's *t*-test, the chi-square test, and the Mann-Whitney U-test.

**Results:** With PMX-DHP endotoxemia significantly decreased and blood pressure increased 150 minutes after endotoxin injection. PiCCO revealed more cases of decreased EVLW in the PMX-DHP group. PVPI increased after endotoxin infusion in both groups. Computed tomography showed improvements in the PMX-DHP group. The survival rate was greater in the PMX-DHP group (100%) than in the control group (71%).

**Conclusion:** PMX-DHP is effective for treating respiratory impairment and contributes to the decreased mortality rate in the endotoxemic pigs.

(J Nippon Med Sch 2014; 81: 130–138)

**Key words:** polymyxin B-immobilized fiber hemoperfusion, sepsis, pulse index contour cardiac output system, extravascular lung water, pulmonary vascular permeability index

## Introduction

Sepsis is a life-threatening complication induced by serious Gram-negative bacterial infections. Acute respiratory distress syndrome (ARDS), the most severe form of acute lung injury (ALI), is a serious complication in patients with sepsis and is characterized by acute respiratory failure with severe hypoxemia and diffuse pulmonary infiltrates. Moreover, sepsis-related ARDS is associated with higher disease severity and worse outcomes than is non-sepsis-related ARDS<sup>1</sup>.

The main cause of septic complications is the lipopolysaccharide endotoxin. Direct hemoperfusion with polymyxin B-immobilized fiber (PMX-DHP) is a blood purification therapy developed to adsorb endotoxin in patients with sepsis. Recent multicenter, randomized, controlled trials have found that PMX-DHP is more effective than conventional therapy in treating patients with severe sepsis and septic shock due to intra-abdominal infections<sup>2</sup>. In Japan, PMX-DHP treatment has been covered by health insurance since 1994 and has been applied to patients with septic shock. Although the effects of PMX-DHP for patients with ARDS have been reported<sup>3-5</sup>, whether this treatment is effective against lung impairment remains controversial. A study in sheep has shown that PMX-DHP improves endotoxin-induced shock and increases oxygenation, and a study in a neonatal piglet model of abdominal sepsis has shown that PMX-DHP increases oxygen saturation and improves outcome<sup>6,7</sup>. However, no studies with animal models have shown that PMX-DHP improves physiological variables or morphology.

On the other hand, the pulse index contour cardiac output (PiCCO) system (Pulsion Medical Systems, Munich, Germany) allows monitoring of the intravascular volume status and may be used to guide volume therapy in patients who have severe sepsis or are critically ill. The extravascular lung water (EVLW) and the pulmonary vascular permeability index (PVPI) have been shown to be strongly correlated with the severity of pulmonary edema<sup>8</sup>.

Therefore, the aim of the present study was to investigate the effects of PMX-DHP on respiratory impairment with respect to physiological variables, including the EVLW and PVPI, and computed tomography (CT) findings in a pig model of endotoxemia.

## Materials and Methods

### Setup of Animal Models

All experimental procedures were conducted after obtaining the approval of the ethics committee for animal experiments of Rakuno Gakuen University. The care and handling of the animals were in accordance with the guidelines of the National Institutes of Health. Adult pigs weighing 9.0 to 11.9 kg (N=13) were used. The pigs were premedicated with medetomidine hydrochloride (40.0 µg/kg), midazolam (0.2 mg/kg), and butorphanol tartrate (0.2 mg/kg), after which anesthesia was maintained with intravenous injection of propofol in intubated animals. The ventilation settings were 11 to 18 mL/kg of tidal volume with oxygen (FiO<sub>2</sub> = 0.9 to 1.0) at 27 to 40 breaths/minute with a respirator (Nuffield 200 Ventilator; Penlon Ltd., Oxford, UK). A silicon tube was passed into the left femoral artery to measure systemic arterial pressure and analyze arterial blood gases. A central venous catheter was inserted into the left cervical vein to set up the PiCCO system (**Fig. 1**).

### Measurements and Observations

Thirteen pigs were assigned to either the PMX-DHP group (n=6) or the control group (n=7). The pigs received intravenous infusions of live *Escherichia coli* (LD50). In addition, all pigs underwent hemodynamic monitoring with the PiCCO system. The PiCCO system is a less invasive type of advanced hemodynamic monitoring that employs transpulmonary thermodilution and an arterial pulse contour analysis. By using the PiCCO system, it is easy to evaluate lung edema, as well as the cardiac output, cardiac function, and cardiac load. In the PMX-DHP group, the right jugular vein was cannulated with silicon tubes connected to the direct hemoperfusion systems to remove and return the

## Experimental system (Endotoxin injection pig model)

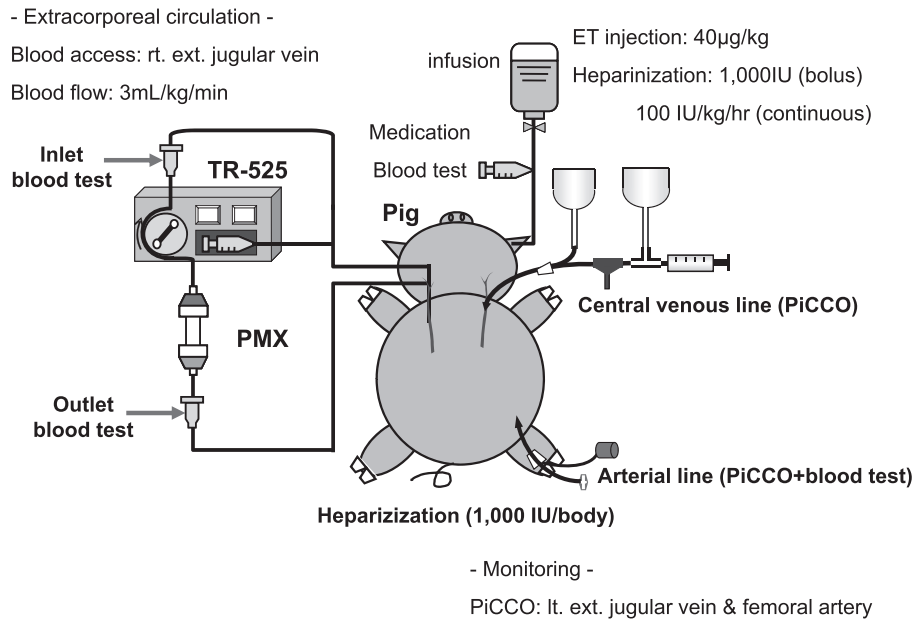


Fig. 1 The development of the endotoxin injection model

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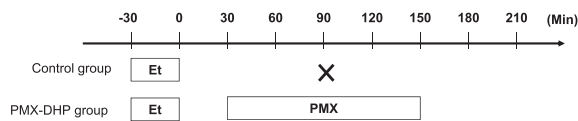


Fig. 2 The time course of the experiment

blood. An arteriovenous extracorporeal circuit with a PMX column (Toraymyxin PMX-05R, Toray Medical Co., Ltd., Tokyo, Japan) was applied for 30 to 150 minutes after *E. coli* injection (**Fig. 2**). Hemodynamic PiCCO variables, blood biochemical values, and arterial blood gases were measured 9 times every 30 minutes starting 30 minutes before injection of endotoxin. We analyzed laboratory data (white blood cells, red blood cells, hemoglobin, hematocrit, platelet count, Na, K, glucose, lactate, prothrombin time, activated partial thromboplastin time, fibrinogen, the endotoxin activity assay [EAA]), arterial blood gases (pH, PaO<sub>2</sub>, PaCO<sub>2</sub>, HCO<sub>3</sub>, base excess), and PiCCO variables (EVLW and PVPI). Furthermore, we performed chest CT scans every 30 minutes in all pigs.

### Statistical Analysis

To compare differences between the PMX-DHP

and control groups, Student's *t*-test, the chi-square test, and the Mann-Whitney U test were used. The data were statistically analyzed using IBM SPSS Statistics for Windows version 19 (IBM Corp., Armonk, NY, USA). The data are expressed as numbers of patients and ratios (%) or the means ± standard error. Values of *p*<0.05 were considered to indicate significance.

### Results

In the PMX-DHP group, systolic blood pressures increased more than in the control group 180 minutes after endotoxin injection (**Fig. 3A**). However, PMX-DHP had no significant effect on oxygenation (**Fig. 3B**). In the PMX-DHP group EAA increased 30 minutes after endotoxin administration, gradually decreased, and was significantly reduced after 210 minutes (**Fig. 3C**). On the other hand, PMX-DHP decreased platelet counts (**Fig. 3D**) and significantly decreased K values (**Fig. 3E**). The markedly high activated partial thromboplastin time at the start of PMX-DHP treatment may be associated with the use of heparin (**Fig. 3F**). There were no significant differences in other vital signs or hematologic values.

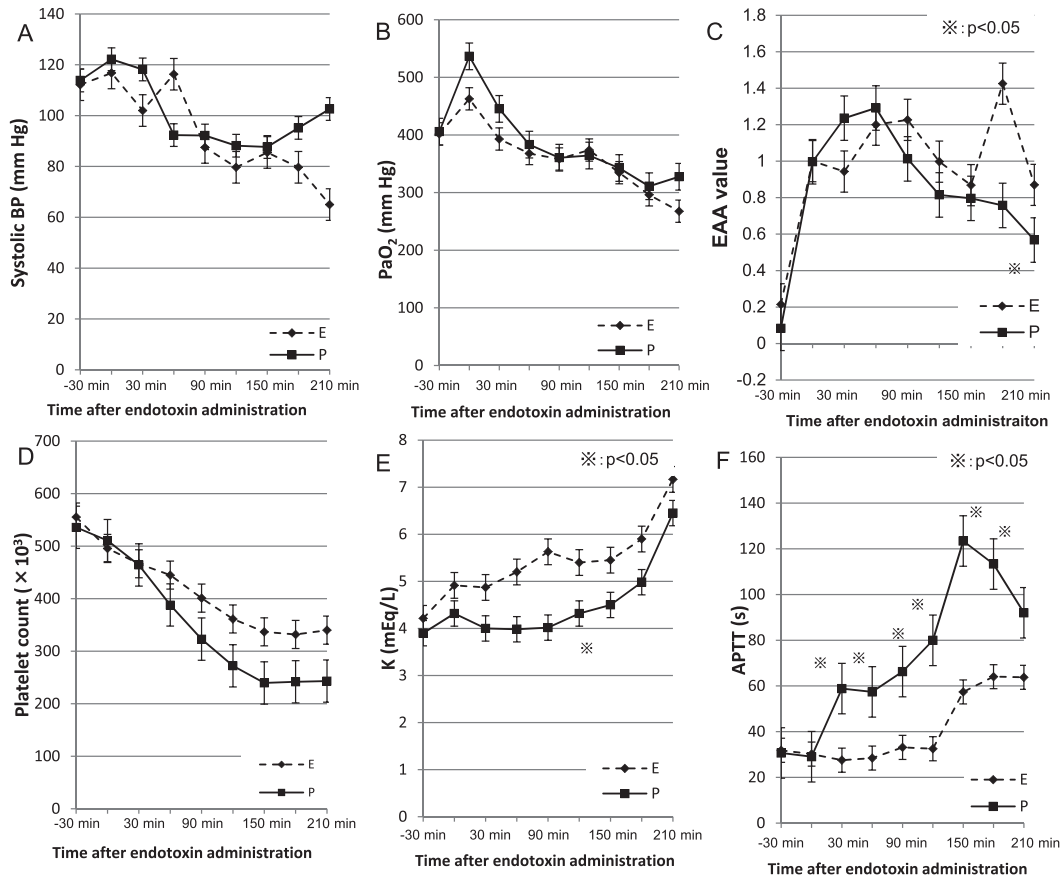


Fig. 3 The changes in variables, which included the systolic blood pressure (BP) (A), PaO<sub>2</sub> (B), EAA value (C), platelet count (D), K (E), and activated partial thromboplastin time (APTT) (F) after endotoxin administration.

With respect to PiCCO variables, the EVLW decreased in more cases of the PMX-DHP group than of the control group but did not differ significantly between the groups (Fig. 4A). The PVPI increased significantly after endotoxin infusion in both groups but did not differ between the groups (Fig. 4B).

Examination with CT showed that pulmonary edema gradually worsened after the administration of endotoxin (Fig. 5A). However, some pigs of the PMX-DHP group showed improvements in pulmonary edema 150 minutes after endotoxin injection (Fig. 5B). A relationship was observed between the findings of PiCCO and pulmonary edema 30 to 150 minutes after the administration of endotoxin (Table 1). Pulmonary edema showed improvements only in the PMX-DHP group and progressed more in the control group than in the PMX-DHP group. Interestingly, the severity of pulmonary edema was positively correlated with

EVLW in the control group. In contrast, all pigs with improved pulmonary edema in the PMX-DHP group showed decreased EVLW.

Of the 7 pigs of the control group, 2 died of sepsis, 90 minutes and 200 minutes after endotoxin injection. The survival rate was higher in the PMX-DHP group (100%) than in the control group (71%; p=0.27).

## Discussion

In this study, the PMX-DHP group included more cases of decreased EVLW and showed improvements in lung edema on CT. Therefore, we believe that PMX-DHP is effective for treating respiratory impairment with sepsis-induced lung edema.

Many reports have indicated that PMX-DHP is effective in patients with sepsis due to Gram-negative bacteria. Tani et al. have reported that

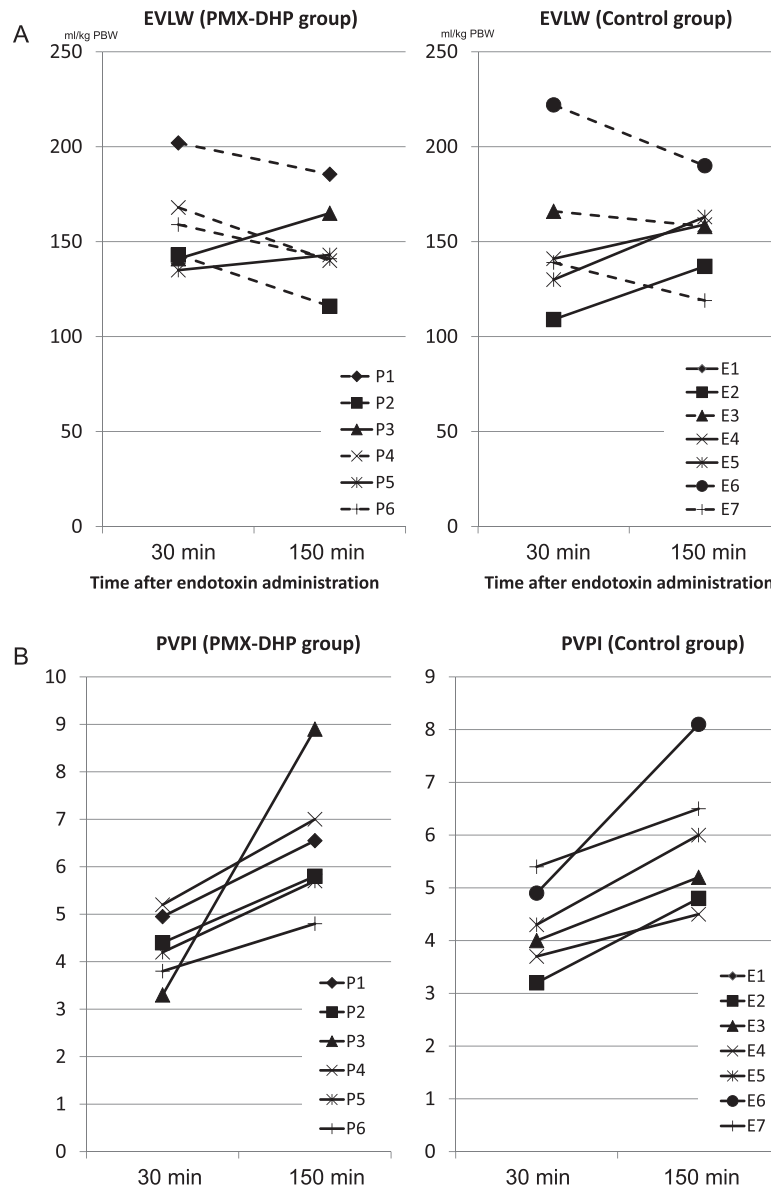


Fig. 4 The changes in the EVLW after endotoxin administration (A). The changes in the PVPI after endotoxin administration (B).

PMX-treated patients, despite having been in a more severe condition before treatment, had significantly higher survival rates than did non-PMX-treated patients<sup>9</sup>. In Japan, PMX-DHP has been covered by health insurance for use against septic shock since 1994, and more than 50,000 patients with sepsis have been treated<sup>10</sup>. Cruz et al have concluded, on the basis of a review of the literature, that PMX-DHP has significant effects, including increasing the mean arterial pressure, reducing the need for dopamine use, improving the PaO<sub>2</sub>/FiO<sub>2</sub> ratio, and decreasing the mortality rate<sup>11</sup>. However, most studies were not randomized control trials, and PMX-DHP was not

recommended by the Surviving Sepsis Campaign Guidelines 2008<sup>12</sup>.

In the present study, 2 pigs of the control group died of sepsis 90 and 200 minutes after endotoxin injection, and the other pigs showed decreased systolic blood pressure. On the other hand, in the PMX-DHP group systolic blood pressure increased after treatment and no pigs died. The EAA level in the PMX-DHP group decreased significantly compared with that in the control group. The EAA value of patients with sepsis correlates with the severity and the mortality rate<sup>13</sup>, and in the present study, severe bacteremia and sepsis developed in all



pigs receiving injections of endotoxin. Therefore, we conclude that PMX-DHP effectively removes endotoxin and tends to improve hemodynamic performance.

The beneficial effects of PMX-DHP on morbidity and mortality have also been demonstrated in patients with ALI and ARDS. Yamamoto et al. have reported that PMX-DHP reverses endotoxin-induced shock and improves the deteriorated oxygenation in anesthetized sheep, probably through the suppression of nitric oxide production<sup>6</sup>. Kushi et al have reported that PMX-DHP significantly decreases blood levels of plasminogen activator inhibitor type 1, neutrophil elastase, and interleukin (IL) 18 and improves the PaO<sub>2</sub>/FiO<sub>2</sub> ratio<sup>14</sup>. Furthermore, the absorption of N-arachidonylethanolamine with PMX-DHP reportedly improves hypotension and outcomes in patients with sepsis<sup>15,16</sup>. Moreover, some papers have reported effects of PMX-DHP on the expression of high mobility group box-1, which is associated with sepsis, in patients with severe septic shock<sup>17-19</sup>. In addition, several studies have found that PMX-DHP is able to increase the PaO<sub>2</sub>/FiO<sub>2</sub> ratio; reduce the levels of plasma cytokines, such as IL-1 $\beta$ , IL-6, IL-10, IL-18, and tumor necrosis factor  $\alpha$ ; and improve plasma levels of thrombomodulin and von Willebrand factor in patients with septic shock<sup>20-23</sup>. Ebihara et al. have suggested that angiopoietin 1 and 2 play roles in the pathogenesis of ALI, and PMX therapy ameliorates the angiopoietin balance in patients with ALI associated with sepsis<sup>24</sup>.

In our study, we did not observe any significant effect of PMX-DHP on oxygenation. This may be due to the different severities of sepsis in each model, and because there was not enough time for oxygenation to become impaired. Moreover, the effect of ventilation might have overshadowed that of the PMX-DHP. The extracorporeal circulation in the PMX-DHP group might have had some effect on the hemodynamics.

The PiCCO system is a useful and less invasive advanced hemodynamic monitoring system. Ritter et al have reported that the PiCCO system is an alternative to the use of a pulmonary artery catheter and identified cardiac dysfunction in

patients with acute heart failure and in patients with severe sepsis or septic shock<sup>25</sup>. Moreover, this system is a reliable and accurate method for assessing changes in the cardiac index induced by volume expansion and norepinephrine in patients with sepsis<sup>26</sup>.

Various hemodynamic variables of the PiCCO system could be clarified in this pig model. The EVLW can be used to estimate fluid in the pulmonary interstitial and alveolar spaces<sup>27</sup>. An increased EVLW indicates increased amounts of extravascular lung water and worsening of lung edema and has also been demonstrated to be well correlated with the oxygenation level in patients who have septic shock with ALI and ARDS<sup>28</sup>. Chew et al. have reported that the EVLW is correlated with respiratory function and that an increased EVLW is related to the severity of lung disease and to mortality<sup>29</sup>. In the present study, although the differences were not significant, the PMX-DHP group had more cases of decreased EVLW and tended to show improvements in pulmonary edema, which contributed to the decreased mortality rate.

On the other hand, the increased pulmonary vascular permeability can be estimated with the PVPI. A recent study has shown that the pulmonary vascular permeability is higher in patients with ALI/ARDS than in with cardiogenic edema and pleural effusion with atelectasis and might be a useful as a quantitative diagnostic tool for ARDS in patients with hypoxemic respiratory failure and radiographic infiltrates<sup>30</sup>.

The present study revealed a strong correlation between the parameters of PiCCO and lung edema (**Table 1**). We confirmed that worsening of lung edema was associated with an increased EVLW because all pigs in the control group with exacerbation of pulmonary edema showed an increased EVLW. Moreover, 2 pigs in the PMX-DHP group with improvement of pulmonary edema showed lower EVLW values, which indicated that PMX-DHP reduced pulmonary edema. Although a correlation between pulmonary edema and EVLW values was not found in some pigs in the PMX-DHP group, such a lack of correlation might be associated with the effects of extracorporeal circulation and

individual difference. The increasing PVPI levels observed in both groups indicate that the effects of various stressors (e.g., endotoxin, intubation, and PMX-DHP) were stronger than the effects of PMX-DHP.

PMX-DHP is clearly effective in treating pulmonary impairment in a pig model of endotoxemia. However, we have not found earlier articles showing, with CT findings and PiCCO system, the effectiveness of PMX-DHP in treating pulmonary impairment. Large-scale studies are needed in the future.

In conclusion, PMX-DHP was found to be effective in treating respiratory impairment and contributed to the decreased mortality in a pig model of endotoxemia.

**Conflict of Interest:** The authors have no conflicts of interest to disclose.

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- (Received, September 19, 2013)  
(Accepted, December 13, 2013)
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