Research article

Direct hemoperfusion with polymyxin B-immobilized fiber improves shock and hypoxemia during endotoxemia in anesthetized sheep

Hiroshi Yamamoto, Tomonobu Koizumi, Toshimichi Kaneki, Keisaku Fujimoto, Keishi Kubo, Takayuki Honda

First Departments of Medicine and Laboratory Medicine, Shinshu University School of Medicine, Matsumoto, Japan

This study evaluates the effect of direct hemoperfusion (DHP) using polymyxin B-immobilized fibers (PMX-F) as an extracorporeal blood filter on systemic hypotension and lung injury during endotoxemia. Sheep were anesthetized, intubated, mechanically ventilated with 50% oxygen and connected to the DHP system between the right femoral artery and left jugular vein. Group 1 ($n = 6$) sheep were infused with 10 μg/kg *Escherichia coli* endotoxin over a 30 min period. At the same time, sheep underwent DHP with PMX-F (Toraymyxin®: PMX-20R) for 2 h at a flow rate of 60 ml/h. Group 2 ($n = 6$) sheep were infused with the same dose of endotoxin and treated with a sham column, in the same manner as those in group 1. DHP with PMX-F significantly improved and restored systemic pressure and arterial oxygen tension in group 1 sheep, although these values never returned to the baseline levels of group 2 sheep. Pulmonary hypertension and leukocytopenia were observed after endotoxin infusion in both groups, but there were no significant differences between these values. DHP with PMX-F significantly decreased the elevation of plasma nitric oxide products. The treatment with PMX-F improves shock and deteriorated oxygenation during endotoxemia, probably through the suppression of nitric oxide production.

Introduction

Sepsis, a life-threatening complication induced by serious Gram-negative infections, is a major cause of irreversible hypotension and multiple organ failure, especially acute respiratory distress syndrome (ARDS).[1] Mortality in patients with ARDS is high (48–71%).[2–8] Endotoxin, a lipopolysaccharide, is a central initiator of Gram-negative sepsis or ARDS.[9,10] In the past few decades, various approaches have been attempted for the treatment of sepsis or ARDS according to its proposed pathophysiology.[11] The human IgG antibody to *Escherichia coli* J5 (J5-IVIG) did not reduce the number of systemic complications and the occurrence of death in sepsis.[12] In addition, Bone *et al.*[13] showed that the monoclonal antibody to endotoxin did not improve mortality in non-shock patients with Gram-negative sepsis. In an awake-sheep model, Wheeler *et al.*[14] reported that anti-endotoxin antibody did not neutralize infused endotoxin effectively. In addition, although several inflammatory cytokines have been involved in sepsis or ARDS, the receptor antagonists or monoclonal antibodies to the cytokines have failed to improve the parameters associated with sepsis.[15,16] More recently, Carraway *et al.*[17] reported that antibody to E- and L-selectin did not prevent lung injury or mortality in septic baboons. Thus, these new therapeutic approaches showed insufficient improvement of sepsis or ARDS.

The removal or detoxification of circulating endotoxin using an extracorporeal perfusion system has emerged as a new treatment for sepsis or ARDS. Cheadle *et al.*[18] showed efficacy of polymyxin B-immobilized fibers (PMX-F) in rats given intravenous, live *E. coli*. Hanasawa *et al.*[19] also showed that PMX-F treatment increased the survival rate in septic dogs. In clinical trials, Aoki *et al.*[20] reported that treatment with PMX-F of

Received 31 August 2001
Revised 28 January 2002, 18 June 2002
Accepted 5 July 2002

Correspondence to: Tomonobu Koizumi MD, First Department of Internal Medicine, Shinshu University School of Medicine, 3-1-1 Asahi, Matsumoto 390-8621, Japan
Tel: +81 263 35 4600 (ext 5252); Fax: +81 263 36 3722; E-mail: tomonobu@hsp.md.shinshu-u.ac.jp

DOI 10.1179/096805102125001019

patients with sepsis decreased plasma endotoxin levels and improved systemic blood pressure. Thus, the removal of endotoxin can produce a positive outcome in conditions of endotoxemia. However, few studies have focused on the effect of PMX-F on endotoxin-induced lung injuries. The present study was designed to clarify the efficacy of PMX-F on endotoxin-induced fatal shock and lung injury in anesthetized sheep.

MATERIALS AND METHODS

The study protocol was approved by the Institutional Review Board for the care of animals in Shinshu University. Care and handling of animals were in accordance with the guidelines of the National Institutes of Health. The animals had free access to commercial chow.

Adult sheep weighing 30–38 kg (n = 12) were used. Animals were injected intravenously with pentobarbital sodium (12.5 mg/kg) and intubated. Anesthesia was maintained with 0.5–1.0% halothane. Ventilation was set at 10 ml/kg tidal volume with oxygen (FiO_2 = 0.5) and at 15 breaths/min using a Harvard-type respirator (Model SN-480-3; Shinano Co., Tokyo, Japan). A silicon tube was passed into the right carotid artery to measure systemic arterial pressure and for analyses of arterial blood gas. A 7 F thermodilution Swan-Ganz catheter was inserted via a right cervical vein into the pulmonary artery to measure the pulmonary artery pressure and cardiac output. The right femoral artery and left jugular vein were cannulated with silicon tubes. These were connected to the direct hemoperfusion system to remove and return blood.

Measurements

All measurements were made in the supine position. Systemic arterial pressure and pulmonary artery pressure were continuously measured using calibrated pressure transducers (Statham P50; Statham Instruments, Hato Rey, PR, USA) and an 8-channel recorder (WT-685G; Nihon Koden Co., Tokyo, Japan). The position of the left atrium was estimated as the zero point, and the transducers were attached to the same point during each experiment. Cardiac output was measured every 30 min by the thermodilution method using a cardiac output computer (model 9520A; Edward Laboratories, Santa Ana, CA, USA). Circulating blood leukocytes were quantitated with a microcell counter (CC-108; Toa Co., Kobe, Japan). Arterial and pulmonary arterial (mixed venous) blood gases were analyzed using an ABL-2 blood gas analyzer (Radiometer, Copenhagen, Denmark). Capillary (CcO_2), arterial (CaO_2) and venous blood oxygen contents (CvO_2) were calculated, assuming a hemoglobin–oxygen binding capacity of 1.39 ml/g. The intrapulmonary shunt ratio (Qs/QT) was calculated using a standard shunt equation: $(CcO_2 - CaO_2)/(CcO_2 - CvO_2)$. The plasma levels of endotoxin were measured by Endospecy® (Seikagaku Kogyo Co. Ltd, Tokyo, Japan) after pretreatment with the new perchloric acid (PCA) method.[21] Endospecy® is a factor G-depleted lysate that does not react with (1,3)-β-glucan and does not promote reactions in the factor G systems, another pathway in the reaction process of endotoxin and *Limulus* lysate. The sensitivity of this assay was 5 pg/ml. The plasma concentration of nitric oxide (NO) as nitrate plus nitrite (NOx) was measured using the Cayman Chemical Nitrate/Nitrite Assay Kit (Cayman Chemical Company, Ann Arbor, MI, USA). It provides an accurate and convenient method for measurement of total nitrate/nitrite concentration in a simple two-step process. The first step is the conversion of nitrate to nitrite utilizing nitrate reductase. The second step is the addition of Griess reagents,[22] which converts nitrite into a deep purple azo-compound.

Polymorphonuclear leukocyte accumulation in lung tissue

The animals were sacrificed by pentobarbital sodium overdose after the experiment. The left lower lobe was immediately excised through a left intercostal incision for histological examination. The lobe was inflated to 40 cmH_2O, and fixed with 10% buffered formalin. The samples were embedded in paraffin, sectioned into 6 μm pieces, and stained with hematoxylin-eosin and the periodic acid Schiff reaction. Samples were analyzed by two pathologists who had no knowledge of the animal groupings. The number of alveoli and polymorphonuclear leukocytes (PMNs) in the peripheral lung tissue were quantitated and the mean values were calculated. The magnification was x400, averaging a total of 100 alveoli for each biopsy specimen.

Experimental protocols

Anesthesia was maintained during the operation and experiments. Oxygen concentrations were maintained using an oxygen monitor (OM-25ANL; Houeisyoukou Co., Tokyo, Japan). Sheep were connected to the direct hemoperfusion (DHP) system between the right femoral artery and left jugular vein. The polymyxin B-immobilized fiber (Toraymyxin®, PMX-20R) was purchased from Toray Co. (Tokyo, Japan). It was propelled by an ambulatory infusion pump (model TR-27; Sanyodenki, Gifu, Japan). The total extracorporeal volume containing PMX-F was 200 ml. We measured all variables over more than a 2 h-baseline period. Endotoxin (10 μg/kg; lipopolysaccharide) from *E. coli* O127:B8 (Difco Laboratories,

St Louis, MO, USA) was diluted in sterile normal saline and infused via the right jugular vein for a 30 min period using an infusion pump for the following experiments.

Group 1 ($n = 6$) sheep were treated with endotoxin + DHP with PMX-F. At the same time as the start of endotoxin addition, DHP with PMX-F was started at a flow rate of 60 ml/h and performed for 2 h.

Group 2 ($n = 6$) sheep were treated with endotoxin + DHP without PMX-F. Sheep were infused with endotoxin and received the DHP with a sham column filled with the same glass beads and volumes. Endotoxin infusion and DHP were performed in the same manner as for group 1 sheep. In both groups, Ringer's lactate solution was continuously infused at a rate of 60 ml/h from 1 h before endotoxin administration, and 50 units/kg of heparin sodium was used as an anticoagulant while DHP was performed. After endotoxin infusion, observation was continued for 5 h.

Statistical analysis

All values are expressed as mean ± SD. Comparisons among each experimental group were carried out with the analysis of variance (Fisher's LSD test). In comparing the numbers of PMNs/100 alveoli, Student's *t*-test was used. Baseline and post-treatment values were compared using the *t*-test for paired data. Probability less than 0.05 was accepted as indicating a significant difference.

Results

There were no significant differences in any of the baseline parameters in both groups. The time courses of systemic and pulmonary hemodynamics in both groups are summarized in Figure 1. Time course and magnitudes in mean pulmonary artery pressure and cardiac output were similar in both groups. Pulmonary artery pressure increased rapidly and reached a 3-fold peak at 30 min after the start of endotoxin infusion. Then, pulmonary artery pressure decreased and reached a plateau, which was slightly higher than the baseline level. Cardiac output dropped significantly at 30 min after endotoxin infusion, returned to the baseline value approximately 2 h after endotoxin administration, and then declined again. There were no significant differences in pulmonary artery pressure and cardiac output between groups 1 and 2.

In group 1, mean systemic arterial pressure dropped immediately from 115.6 ± 6.2 mmHg at baseline to 75.9 ± 15.4 mmHg at 1.5 h after the concomitant start of DHP with PMX-F and endotoxin infusion. After the cessation of DHP, systemic arterial pressure gradually returned to the baseline and was maintained at a similar level. In group 2, systemic arterial pressure also dropped immediately from

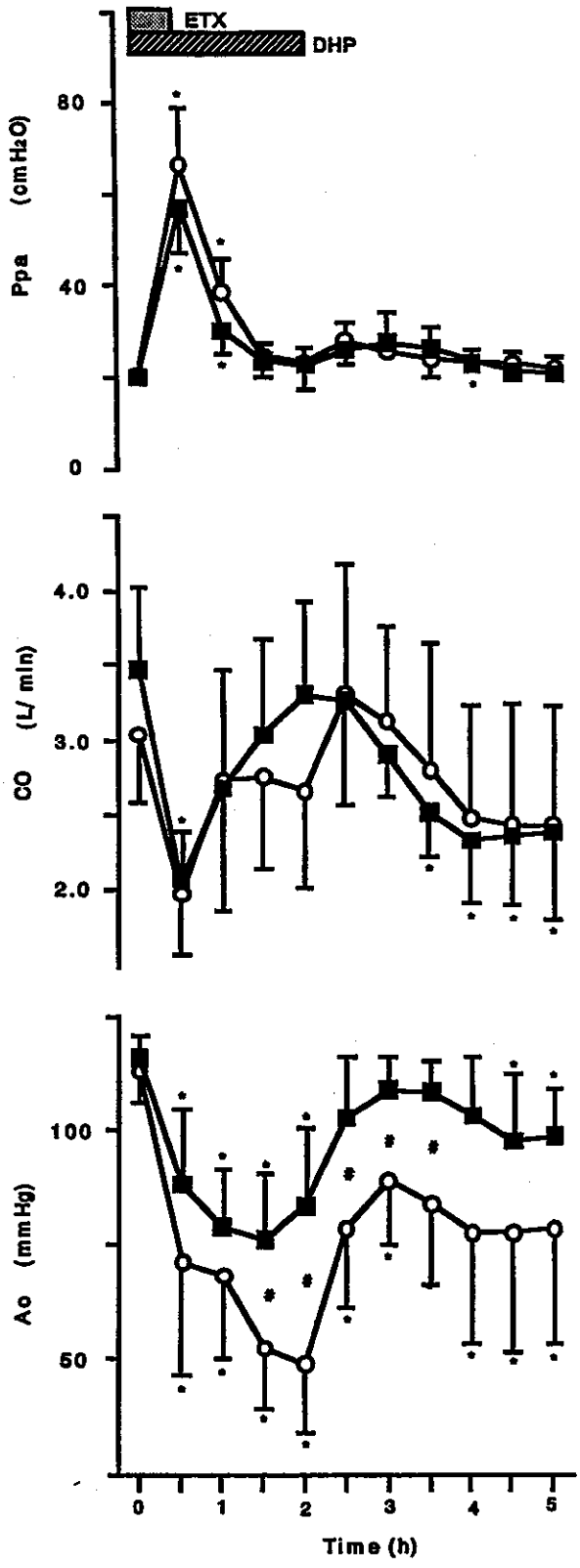

Fig. 1. Effect of direct hemoperfusion (DHP) on endotoxin-induced changes in pulmonary artery pressure (Ppa), cardiac output (CO) and aortic pressure (Ao). Values are expressed as mean ± SD. Values from the endotoxin-plus-sham DHP control experiments ($n = 5$) are represented by open circles; values from the endotoxin-plus-DHP with PMX-F experiments ($n = 6$) are represented by closed squares. *Indicates that the data points are significantly different ($P < 0.05$) from baseline values. #Indicates that the time-matched data points are significantly different ($P < 0.05$) between the two groups.

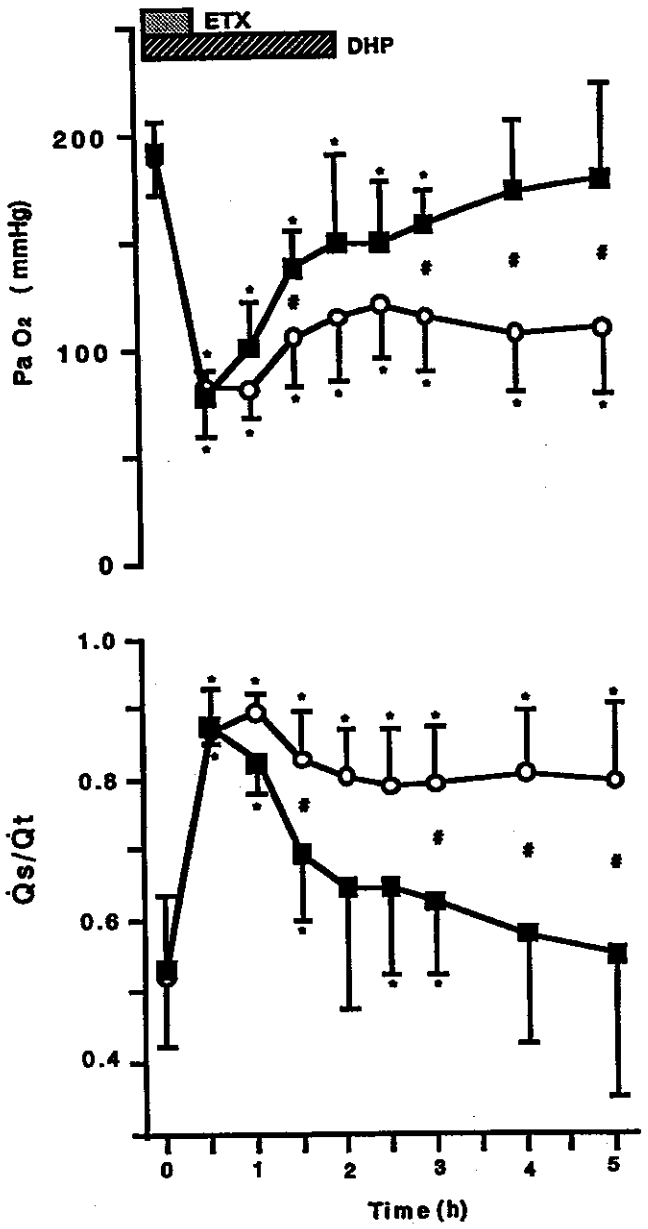

Fig. 2. Effect of direct hemoperfusion (DHP) on endotoxin induced changes in arterial oxygen gas tension (PaO_2), and intrapulmonary shunt ratio (Qs/Qt). Values are expressed as mean ± SD. Values from the endotoxin-plus-sham DHP control experiments ($n = 5$) are represented by open circles; values from the endotoxin-plus-DHP with PMX-F experiments ($n = 6$) are represented by closed squares.
*Indicates that the data points are significantly different ($P < 0.05$) from the baseline values. #Indicates that the time-matched data points are significantly different ($P < 0.05$) between the two groups.

112.6 ± 7.6 mmHg at baseline to 48.8 ± 16.5 mmHg at 2.0 h after the start of sham DHP and endotoxin infusion. After that, systemic arterial pressure increased slightly from the bottom level, but did not reach the baseline value. Thus, systemic arterial pressure values were significantly higher in sheep treated with PMX-F than those in sham-treated sheep.

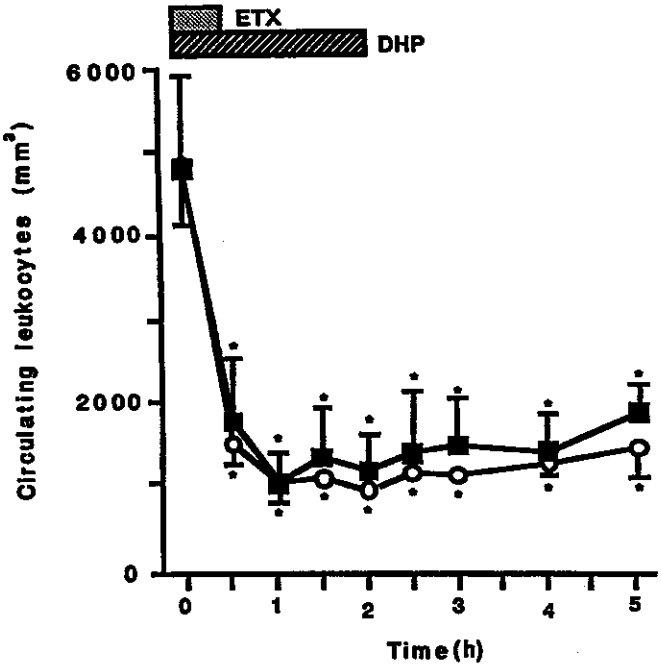

Fig. 3. Effect of direct hemoperfusion (DHP) on endotoxin-induced changes in blood leukocyte counts. All values are represented as mean ± SD. Values from the endotoxin-plus-sham DHP control experiments ($n = 5$) are represented by open circles; values from the endotoxin-plus-DHP with PMX-F experiments ($n = 6$) are represented by closed squares. *Indicates that the data points are significantly different ($P < 0.05$) from baseline values.

The time courses of arterial blood oxygen tension (PaO_2) and intrapulmonary shunt ratio (Qs/Qt) in both groups are summarized in Figure 2. In group 1, PaO_2 dropped immediately from 190.0 ± 18.0 mmHg at baseline to 65.7 ± 13.1 mmHg at 0.5 h and gradually returned to the baseline level approximately 4 h after the start of endotoxin infusion. In group 2, PaO_2 also decreased immediately from 187.3 ± 22.4 mmHg at baseline to 69.4 ± 17.6 mmHg at 1 h after the start of endotoxin infusion. In contrast to group 1, the improvement of PaO_2 was slight. PaO_2 did not return to the baseline value in group 2. Thus, the values in PaO_2 were significantly higher in sheep treated with PMX-F than those in sham-treated sheep during endotoxemia.

In group 1, Qs/Qt increased immediately from 0.53 ± 0.11 at baseline to 0.87 ± 0.24 at 0.5 h and returned to the baseline level approximately 4 h after the start of endotoxin infusion. In group 2, Qs/Qt also increased immediately from 0.52 ± 0.12 at baseline to 0.89 ± 0.04 at 1.5 h after the start of endotoxin infusion and then remained elevated. Thus, Qs/Qt was significantly lower in sheep treated with PMX-F than in sham-treated sheep during endotoxemia.

The time courses of circulating leukocytes in responses to endotoxin in both groups are shown in Figure 3. Circulating leukocytes dropped rapidly after endotoxin infusion and maintained decreased levels during the experiment in both

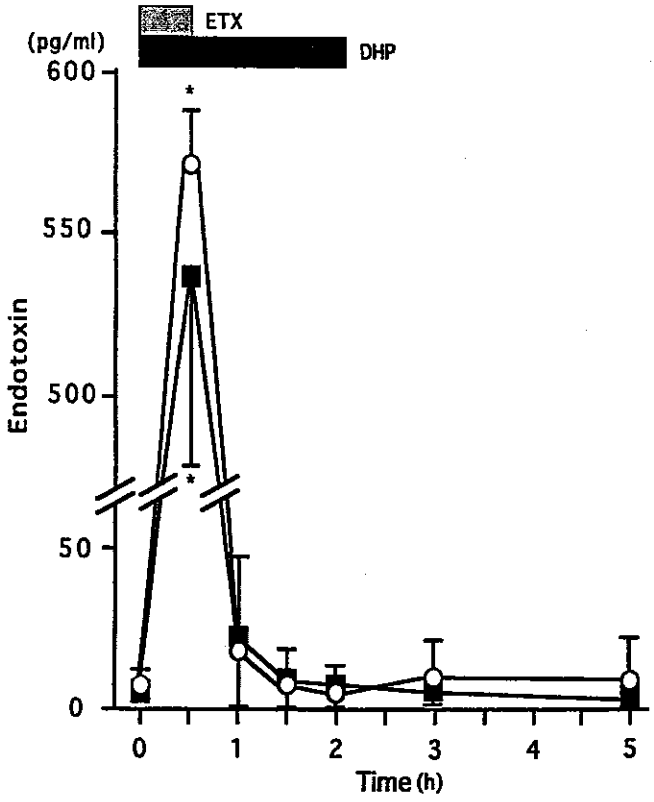

Fig. 4. Effect of direct hemoperfusion (DHP) on endotoxin concentrations after the infusion. All values are represented as mean ± SD. Values from the endotoxin-plus-sham DHP control experiments ($n = 5$) are represented by open circles; values from the endotoxin-plus-DHP with PMX-F experiments ($n = 6$) are represented by closed squares. *Indicates that the data points are significantly different ($P < 0.05$) from baseline values.

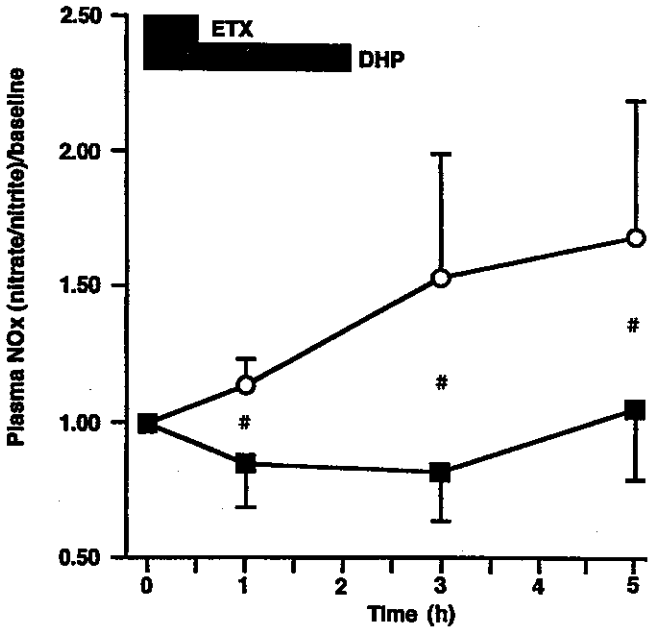

Fig. 5. Effect of direct hemoperfusion (DHP) on endotoxin-induced changes in plasma NOx (nitrate/nitrite). Plasma NOx is estimated as the ratio to the baseline level (NOx after endotoxin/NOx at baseline). Values are expressed as mean ± SD. Values from the endotoxin-plus-sham DHP control experiments ($n = 6$) are represented by open circles; values from the endotoxin-plus-DHP with PMX-F experiments ($n = 6$) are represented by closed squares. #Indicates that the time-matched data points are significantly different ($P < 0.05$) between the two groups.

groups. The degree and the time course of leukocytopenia were similar in both groups. The number of PMNs/100 alveoli in the lungs was 41.3 ± 3.7 in group 1 and 45.1 ± 10.1 in group 2. The number of PMNs/100 alveoli in the lungs in group 2 was slightly higher than group 1, but the difference was not significant.

The time course of plasma endotoxin concentrations in both groups is shown in Figure 4. Plasma endotoxin concentration reached a peak at 30 min after the infusion of endotoxin and then returned to the baseline value at approximately 1.0 h in both groups (from a baseline of 9.7 ± 3.1 pg/ml to a peak of 577.9 ± 11.9 pg/ml in group 1, and from a baseline of 5.0 ± 2.0 pg/ml to a peak of 536.6 ± 59.8 pg/ml in group 2). The peak plasma endotoxin concentration in group 1 at 30 min was slightly lower than in group 2, but did not achieve a significant difference.

Since there were wide variations in the value of plasma total nitrate/nitrite concentration (NOx) levels at the baseline, we estimated plasma NOx as the ratio to the baseline level (the values of plasma NOx after endotoxin/the value of plasma NOx at baseline), as shown in Figure 5. Plasma NOx in group 2 was gradually and significantly elevated after endotoxin administration, whereas the NOx was significantly suppressed over the time after endotoxin infusion in group 1, compared with those in group 2.

Discussion

Endotoxemia leads to pulmonary hypertension, systemic hypotension, circulating leukopenia, hypoxemia, and deteriorated oxygenation in sheep. We examined the effect of the DHP with PMX-F on these cardiopulmonary disorders during endotoxemia. The most novel and striking findings in the present study were: (i) treatment with PMX-F significantly improved systemic hypotension and hypoxemia during endotoxemia; and (ii) PMX-F significantly suppressed NO production in response to endotoxin in anesthetized sheep.

Polymyxin B (PMX-B) is a cyclic cationic polypeptide that detoxifies endotoxin.[23] PMX-B has been tested intravenously in dogs where it neutralizes the hemodynamic effects on systemic pressure and vascular resistance caused by Gram-negative endotoxin.[24] However, its intravenous use is limited because of its severe nephrotoxicity. To solve this problem, PMX-B was bound to an insoluble polystyrene fiber (PMX-F).[19] PMX-F is safe, non-toxic and available for experimental and clinical studies. In an *in*

vitro study, PMX-F removed endotoxin from bovine serum and hemoglobin solution.[19,25] DHP with PMX-F could improve the survival rate in animals subjected to lethal doses of intravenous Gram-negative endotoxin[18] or live *E. coli*[19] *in vivo*. In human studies, DHP with PMX-F was effective treatment for shock in patients with sepsis.[20,26]

In the present study, both PMX-F and sham treatments were started at the same time as endotoxin administration. The decrease in systemic pressure was probably due to the net effects of endotoxin administration and the decrease in circulating blood volume by extracorporeal circulation. When DHP ended, systemic pressure rose gradually in both groups, suggesting that the recovery was partly due to the return of extracorporeal blood volume. However, the decreased systemic pressure was significantly higher in PMX-F-treated sheep than that in sham-treated sheep during DHP. In addition, systemic pressure in PMX-F-treated sheep returned to the baseline value after the cessation of DHP, although those of sham-treated sheep did not return to baseline levels. Thus, PMX-F could attenuate the systemic hypotension during endotoxemia.

Transient pulmonary hypertension occurred after endotoxin infusion. PMX-F did not change the process. Transient pulmonary hypertension following endotoxin is mainly due to thromboxane A_2 release.[27] Although we did not measure the prostanoid products, the present study suggested that PMX-F failed to suppress the release of thromboxane A_2 in the early phase of endotoxemia.

In both groups, the value and time course of decreased circulating leukocytes after endotoxin infusion were similar. In addition, postmortem examination in both groups revealed similar PMN accumulation in the lung. Several studies have suggested that PMNs play an important role in the development of acute lung injury or ARDS.[28,29] The present study suggests that improvements by PMX-F in shock, hypoxemia and deteriorated oxygenation induced by endotoxin are independent of leukocytes and/or PMN-related pathways.

Hypoxemia is thought to be primarily due to ventilation-perfusion (V/Q) mismatch with physiological shunting through collapsed alveoli in patients with ARDS.[30] The abrupt development of hypoxemia after endotoxin administration, coincident with pulmonary hypertension and marked changes in lung mechanics, suggests a combination of vascular and air-space abnormalities contributing to V/Q mismatch. In the present study, PaO_2 dropped and Qs/Qt increased immediately after endotoxin infusion. These parameters in sheep treated with PMX-F returned to the baseline level, although those in sham-treated sheep did not return to the baseline level. PMX-F clearly improved hypoxemia and deteriorated oxygenation induced by endotoxemia. It appears that the degree of pulmonary edema was a major determinant of Qs/Qt. In the present study, the time course and magnitude of cardiac output and pulmonary hypertension were similar in both groups. Although we did not measure extravascular lung water, we think that DHP with PMX-F might decrease extravascular lung water resulting in an improvement of oxygenation. However, there are several contributing factors that affect oxygenation during endotoxemia. Brigham *et al.*[31] reported that gas exchange in humans during ARDS did not correlate with lung water. In a sheep study, Esbenshade *et al.*[32] concluded that endotoxin-induced respiratory failure was not due to pulmonary edema alone. Thus, hypoxemia of endotoxin-induced lung injury is probably due to complex interactions of air spaces and pulmonary blood flow, including chemical mediators as well as the mechanical effects of interstitial and alveolar edema.

It has been reported that PMX-F can remove endotoxin in *in vitro*[19,25] and *in vivo* studies.[18,20,26] However, PMX-F failed to reduce the peak plasma endotoxin level in the present study. The precise explanation for this discrepancy remains uncertain. It may be due to the time schedules of endotoxin infusion and/or sampling time for endotoxin measurements. In addition, plasma endotoxin has been measured by several different methods – toxinometer,[25] endospecy,[33] and PCA or new PCA treatment.[21,34] In the present study, we measured plasma endotoxin concentration by an endospecy method after pretreatment with the new PCA method. It is unclear whether the technique might be suitable for sheep. Since there are no studies of what measurement of endotoxin is typical for animals, further studies are necessary to acquire reasonable measurements of endotoxin.

The efficacy of PMX-F may be due to reasons other than the removal of endotoxin. We speculate that PMX-F suppresses the expression of mediators, which are related to the development of shock and acute lung injury. NO is one of the major mediators suspected of precipitating the cardiopulmonary vascular collapse associated with septic shock.[35-38] Endotoxin leads to the induction of an inducible isoform of NO synthase (iNOS) in a variety of tissues, including macrophages, vascular smooth muscle cells, cardiac monocytes, and endothelial cells.[39] Nitrate plus nitrite (NOx) concentration was increased in septic patients[35-37] in plasma and in broncho-alveolar fluid in patients with ARDS.[38] The increased NOx level during endotoxemia was mainly produced via the iNOS pathway.[40] Several NO synthase inhibitors including a selective iNOS inhibitor prevented septic shock[41,42] and acute lung injury.[43] Furthermore, it is well known that endotoxemia impairs hypoxic pulmonary vasoconstriction (HPV).[44] The attenuated HPV during endotoxemia results in increased V/Q mismatching, augments right-to-left shunting of venous blood, and reduces arterial oxygenation,[44] as observed in the present study. Inducible NO contributes to the impairment of HPV during endotoxemia.[45] We show here that plasma NOx levels in PMX-F were significantly suppressed compared to those in sham-treated sheep, suggesting less production of NO by PMX-F. Thus, a possible explanation for the better

outcome in oxygenation and systemic circulation during endotoxemia shown in the present study is a reduced production of NO by PMX-F. However, it remains unclear whether the suppressive effect of NO was due to removal of endotoxin by PMX-F or to other pathways.

Conclusions

The treatment of DHP with PMX-F as an extracorporeal blood filter improves shock and deteriorated oxygenation during endotoxemia in anesthetized sheep. This therapy might detoxify endotoxins and suppress the production of NO during endotoxemia. DHP with PMX-F is an effective therapeutic strategy for patients with shock and lung injury during endotoxemia. PMX-F therapy is worthy of further clinical investigation in patients with septic shock and ARDS.

Acknowledgements

Supported in part by Grant-in-Aids for Scientific Research (B) 09470539 and (C) 10672261, from the Ministry of Education, Science, Sports and Culture of Japan.

References

1. Kreger BE, Craven DE, McCabe WR. Gram-negative bacteremia. IV. Re-evaluation of clinical features and treatment in 612 patients. *Am J Med* 1980; **68**: 344–355.
2. Zilberberg MD, Epstein SK. Acute lung injury in the medical ICU: comorbid conditions, age, etiology, and hospital outcome. *Am J Respir Crit Care Med* 1998; **157**: 1159–1164.
3. Bone RC, Balk R, Slotman G *et al.* Adult respiratory distress syndrome: sequence and importance of development of multiple organ failure. *Chest* 1992; **101**: 320–326.
4. Suchyta MR, Clemmer TP, Elliot CG *et al.* The adult respiratory distress syndrome: a report of survival and modifying factors. *Chest* 1992; **101**: 1074–1079.
5. Sloane PJ, Gee MH, Gottlieb JE *et al.* A multicenter registry of patients with acute respiratory distress syndrome. *Am Rev Respir Dis* 1992; **146**: 419–426.
6. Montgomery AB, Stager MA, Carrio CJ *et al.* Causes of mortality in patient with the adult respiratory distress syndrome. *Am Rev Respir Dis* 1985; **132**: 485–489.
7. Seidenfeeld JJ, Pohl DF, Bell RC *et al.* Incidence, site and outcome of infections in patients with adult respiratory distress syndrome. *Am Rev Respir Dis* 1986; **134**: 12–16.
8. Bone RC, Mauder R, Slotman G *et al.* An early test of survival in patients with ARDS: the PaO_2/FiO_2 ratio and its differential response to conventional therapy. *Chest* 1989; **96**: 849–851.
9. van Deventer SJ, Buller HR, ten Cate JW *et al.* Endotoxemia: an early predictor of septicaemia in febrile patients. *Lancet* 1988; **2**: 605–609.
10. Ziegler EJ, McCutchan JA, Fierer J *et al.* Treatment of Gram-negative bacteremia and shock with human antiserum to a mutant *Escherichia coli*. *N Engl J Med* 1982; **307**: 1225–1230.
11. Artigas A, Bernard GR, Carlet J *et al.* The American–European Consensus Conference on ARDS, Part 2. *Am J Respir Crit Care Med* 1998; **157**: 1332–1347.
12. Clandra T, Glauser MP, Schellekens J *et al.* Treatment of Gram-negative septic shock with human IgG antibody to *Escherichia coli* J5: a prospective, double-blind, randomized trial. *J Infect Dis* 1988; **158**: 312–319.
13. Bone RC, Balk RA, Fein AM *et al.* A second large controlled clinical study of E5, a monoclonal antibody to endotoxin: results of a prospective, multicenter, randomized, controlled trial. The E5 Sepsis Study Group. *Crit Care Med* 1995; **23**: 994–1006.
14. Wheeler AP, Hardie WD, Bernard G. Studies of anti-endotoxin antibody in preventing the physiologic changes of endotoxemia in awake sheep. *Am Rev Respir Dis* 1990; **142**: 775–781.
15. Fischer Jr CJ, Slotman GJ, Opal SM *et al.* Initial evaluation of human recombinant interleukin-1 receptor antagonist in the treatment of sepsis syndrome: a randomized, open-label, placebo-controlled multicenter trial. The IL-1 RA Sepsis Syndrome Study Group. *Crit Care Med* 1994; **22**: 12–21.
16. Cohen J, Carlet J. INTERSEPT, an international, multicenter, placebo-controlled trial of monoclonal antibody to human tumor necrosis factor-alpha in patients with sepsis. International Sepsis Trial Study Group. *Crit Care Med* 1966; **24**: 1431–1440.
17. Carraway MS, Welty-Wolf KE, Kantarow SP *et al.* Antibody to E- and L-selectin does not prevent lung injury or mortality in septic baboons. *Am J Respir Crit Care Med* 1998; **157**: 938–949.
18. Cheadle WG, Hanasawa K, Gallinaro RN *et al.* Endotoxin filtration and immune stimulation improve survival from Gram-negative sepsis. *Surgery* 1991; **110**: 785–791.
19. Hanasawa K, Tani T, Kodama M. New approach to endotoxic and septic shock by means of polymyxin B immobilized fiber. *Surg Gynecol Obstet* 1989; **168**: 323–331.
20. Aoki H, Kodama M, Tani T *et al.* Treatment of sepsis by extracorporeal elimination of endotoxin using polymyxin B immobilized fiber. *Am J Surg* 1994; **167**: 412–417.
21. Takahashi K. Study on quantitative measurement of endotoxin in human blood using chromogenic substrate. *J Iwate Med Assoc* 1988; **40**: 67–68.
22. Green LC, Wagner DA, Glogowski J *et al.* Analysis of nitrate, nitrite, and [^{15}N]-nitrate in biological fluids. *Anal Biochem* 1982; **126**: 131–138.
23. Rifkind D, Palmer JD. Neutralization of endotoxin toxicity in chick embryos by antibiotics. *J Bacteriol* 1966; **92**: 815–819.
24. Palmer JD, Rifkind D. Neutralization of the hemodynamic effects of endotoxin by polymyxin B. *Surg Gynecol Obstet* 1974; **138**: 755–759.
25. Tani T, Chang TMS, Kodama M *et al.* Endotoxin removed from hemoglobin solution using polymyxin-B immobilized fiber (PMX-F) followed by a new turbidometric endotoxin assay. *Biomat Art Cells Immob Biotech* 1992; **20**: 457–462.
26. Shoji H, Tani T, Hanasawa K *et al.* Extracorporeal endotoxin removal by polymyxin B immobilized fiber cartridge: designing and anti-endotoxin efficacy in the clinical application. *Therap Apheresis* 1998; **2**: 3–12.
27. Kubo K, Kobayashi T. Effect of OKY-046, a selective thromboxane synthetase inhibitor, on endotoxin-induced lung injury in unanesthetized sheep. *Am Rev Respir Dis* 1985; **132**: 494–499.
28. Snapper JR, Bernard GR, Hinson JM *et al.* Endotoxemia-induced leukopenia in sheep: correlation with lung vascular permeability and hypoxemia but not with pulmonary hypertension. *Am Rev Respir Dis* 1983; **127**: 306–309.
29. Heflin AC, Brigham KL. Prevention by granulocyte depletion of increased vascular permeability of sheep lung following endotoxemia. *J Clin Invest* 1981; **68**: 1253–1260.
30. Lamy M, Fallat JR, Koeniger E *et al.* Pathologic features and

mechanisms of hypoxemia in adult respiratory distress syndrome. *Am Rev Respir Dis* 1976; **114**: 267–283.
31. Brigham KL, Kariman K, Harris TR *et al.* Lung water and vascular permeability-surface area in humans during acute respiratory failure (Abstract). *Am Rev Respir Dis* 1980; **121**: 426.
32. Esbenshade AM, Newman JH, Lams PM *et al.* Respiratory failure after endotoxin infusion in sheep: lung mechanics and lung fluid balance. *J Appl Physiol* 1982; **53**: 967–976.
33. Obayashi T, Tamura H, Tanaka S *et al.* A new chromogenic endotoxin-specific assay using recombined *Limulus* coagulation enzymes and its clinical applications. *Clin Chim Acta* 1985; **149**: 55–65.
34. Inada K, Endo S, Takahashi K *et al.* Establishment of a new perchloric acid treatment method to allow detection of the total endotoxin content in human plasma by the *Limulus* test and clinical application. *Microbiol Immunol* 1991; **35**: 303–314.
35. Krafte JB, Brilli R, Szabo C *et al.* Circulating methemoglobin and nitrite/nitrate concentrations as indicators of nitric oxide overproduction in critically ill children with septic shock. *Crit Care Med* 1997; **25**: 1588–1593.
36. Mehta S, Javeshghani D, Datta P *et al.* Porcine endotoxemic shock is associated with increased expired nitric oxide. *Crit Care Med* 1999; **27**: 385–393.
37. Kilbourn R. Nitric oxide synthase inhibitors – a mechanism-based treatment of septic shock. *Crit Care Med* 1999; **27**: 857–858.
38. Sittipunt C, Steinberg KP, Ruzinski JT *et al.* Nitric oxide and nitrotyrosine in the lungs of patients with acute respiratory distress syndrome. *Am J Respir Crit Care Med* 2001; **163**: 503–510.
39. Morris Jr SM, Billiar TR. New insights into the regulation of inducible nitric oxide synthesis. *Am J Physiol* 1994; **226**: E829–E839.
40. Fujii Y, Goldberg P, Hussain SNA. Contribution of macrophages to pulmonary nitric oxide production in septic shock. *Am J Respir Crit Care Med* 1998; **157**: 1645–1651.
41. Pheng LH, Francoeur C, Denis M. The involvement of nitric oxide in mouse model of adult respiratory distress syndrome. *Inflammation* 1995; **19**: 599-601.
42. Stewart TE, Valenza F, Ribeiro SP *et al.* Increased nitric oxide in exhaled gas an early marker of lung inflammation in a model of sepsis. *Am J Respir Crit Care Med* 1995; **151**: 713–718.
43. Numata M, Suzuki S, Miyazawa N *et al.* Inhibition of inducible nitric oxide synthase prevents LPS-induced acute lung injury in dogs. *J Immunol* 1998; **160**: 3031–3037.
44. Hutchison AA, Ogletree ML, Snapper JR *et al.* Effect of endotoxemia on hypoxic pulmonary vasoconstriction in unanesthetized sheep. *J Appl Physiol* 1985; **58**: 1463–1468.
45. Roman U, Kenneth DB, Fumito I *et al.* Hypoxic pulmonary blood flow redistribution and arterial oxygenation in endotoxin-challenged NOS2-deficient mice. *J Clin Invest* 1999; **104**: 1421–1429.