Polymyxin B-Immobilized Fiber Column Hemoperfusion Therapy for Septic Shock

Chieko Mitaka,[*] Makoto Tomita,[†]

[*]Department of Critical Care Medicine, Tokyo Medical and Dental University Graduate School

[†]Clinical Research Center, Tokyo Medical and Dental University Hospital Faculty of Medicine

Corresponding author:

Dr. Chieko Mitaka

Department of Critical Care Medicine

Tokyo Medical and Dental University Graduate School

1-5-45, Yushima, Bunkyo-ku, Tokyo, 113-8519, Japan

Phone: +81-3-5803-5650

Fax: +81-3-5803-0168

E-mail: c.mitaka.icu@tmd.ac.jp

Running head: PMX therapy for septic shock

ABSTRACT

Endotoxin, an outer membrane component of Gram-negative bacteria, plays an important role in the pathogenesis of septic shock. Endotoxin adsorption therapy by polymyxin B-immobilized fiber column hemoperfusion (PMX) has been used for the treatment of septic shock patients in Japan since 1994. The covalent binding of polymyxin B onto the surface of the polystyrene-based carrier fiber in PMX inactivates the endotoxin in the blood without exerting toxicity. This study was performed as a systematic review to evaluate the efficacy and mechanism of PMX treatment in patients with septic shock. The PubMed database and references from identified articles were used to search and review the literature relating to the efficacy and mechanism of PMX treatment in patients with septic shock. PMX adsorbed monocytes, activated neutrophils, and anandamide as well as endotoxin through direct covalent bond, hydrophobic and ionic interactions, and hydrodynamics, and reduced the blood concentrations of inflammatory cytokines, plasminogen activator inhibitor (PAI)-1 and adhesion molecules. PMX increased blood pressure and reduced the dosage requirements for vasopressive/inotropic agents. The meta-analysis showed that PMX treatment had beneficial effects on the hemodynamics, pulmonary oxygenation, and mortality. These beneficial effects may be attributable to the direct adsorption of endotoxin, monocytes, activated neutrophils, and anandamide, as well as indirect decrease in inflammatory cytokines and other mediators. PMX treatment has additional effects on reducing endothelial damage, proapoptotic activity and immunosupression. Further studies will be needed to confirm the efficacy and mechanism of PMX treatment in septic shock.

Key words – Endotoxin, hemodynamics, hemoperfusion, lipopolysaccharide, sepsis, septic shock, polymyxin B

INTRODUCTION

Septic shock has a high mortality risk despite the availability of various treatments. Endotoxin, an outer membrane component of Gram-negative bacteria, plays an important role in the pathogenesis of septic shock. Endotoxin (lipopolysaccharide, LPS) and LPS binding protein bind together to membrane receptor CD14 on the surfaces of monocytes/macrophages and an accessory protein, myeloid differentiation factor (MD) 2. This, in turn, activates the signaling molecule complex of Toll-like receptor (TLR)-4 to create a signaling complex that activates downstream signaling. This downstream signaling induces an excessive generation of cytokines, which in turn increases systemic inflammatory response and sets the stage for endothelial dysfunction, cellular damage and organ dysfunction (1).

Polymyxin B, a cationic polypeptide antibiotic, has a strong affinity to endotoxin and is able to bind the lipid A portion of endotoxin through ionic and hydrophobic interactions. Intravenous administration of polymyxin B has significant nephrotoxic and neurotoxic effects. However, the covalent binding of polymyxin B onto the surface of the polystyrene-based carrier fiber inactivates the endotoxin in the blood without exerting toxicity. Polymyxin B-immobilized fiber column hemoperfusion (PMX) is a method performed with a clinically applied adsorbent column (Toraymyxin 20-R) containing 5 mg of polymyxin B per gram of polystyrene fiber with a priming volume of 135 ml (Toray Industries, Inc, Tokyo, Japan)(2, 3). PMX has been used for the treatment of septic shock since 1994 in Japan and since 2002 in Italy. Its use continues to widen worldwide year by year. This review evaluates the efficacy and mechanism of PMX treatment in patients with septic shock.

METHODS

A PubMed search was performed to find articles containing the following terms up to February 2011; "PMX" (and/or "polymyxin B hemoperfusion") and "septic shock". The PubMed database and references from identified article were used to search and review the literature relating to PMX treatment in adult patients with septic shock. The quality of each article was

3

assessed. The effects of PMX on hemodynamic status, doses of vasopressive/inotoropic agents, blood concentrations of endotoxin and mediators, pulmonary oxygenations, and mortality in patients with septic shock were evaluated. To provide tabular data, articles that used the sepsis definitions from the American College of Chest Physicians (ACCP)/Society of Critical Care Medicine

(SCCM) consensus conference were selected (4).

Statistical analysis

Concerning meta-analysis, we calculated weighted difference in means and 95% confidence intervals (CI) between PMX treatment and conventional therapy for increase in mean arterial pressure (MAP) and PaO_2/F_IO_2, where it is assumed that 95% confidence level is generally approximated by two standard deviations, or two sigma. We also calculated odds ratio and 95% CI between PMX treatment and conventional therapy for mortality. Difference in means, odds ratio and 95% CI of total were calculated by general variance-based method.

RESULTS

Interventions

The PMX treatment is administered by the following method. A Toraymyxin 20-R is washed by perfusion with 4L of physiological saline. After inserting a double lumen catheter into a central vein of patients, blood is drawn from the proximal port, perfused through Toraymyxin 20-R, and returned to the vein through the distal port of the catheter (V-V method). The blood is perfused at a rate of 80 to 100 ml/min using protease inhibitor nafamostat mesilate (Torii Pharmaceuticals, Co., Ltd., Tokyo, Japan), unfractionated heparin or low-molecular-weight heparin as an anticoagulant. A 2-to 24-hr PMX treatment is administered either once or in some patients up to two, three or four times, depending on the clinical response.

Hemodynamics and dose of vasopressive/inotoropic agents

As shown in Tables 1 and 2, PMX treatment increased blood pressure in almost all studies (5-7, 9, 13-14, 17-19, 25). MAP significantly increased in PMX treatment compared with

4

conventional therapy (Figure 1). In a pilot study in Europe, PMX was found to significantly increase the cardiac index, left ventricular stroke work index, and oxygen delivery index, compared with control (13). The doses of dopamine, dobutamine, and norepinephrine were consistently reported to decrease after PMX treatment (5, 10, 14, 15, 17).

Endotoxin concentrations

In most of the Japanese studies, endotoxin concentrations were measured by endotoxin-specific assay (endospecy; limit of normal is 9.8 pg/ml; Seikagaku Corporation, Tokyo Japan). In one European study (13), endotoxin concentrations were measured by the modified limulus amebocyte lysate (LAL) assay kit (COATEST; DiaPharma Group, Inc., Westchester, OH, USA). This kit is sensitive up to 0.05 endotoxin units [EU]/ml in serum or plasma. In studies by our group and another group (15, 23), endotoxin concentrations were measured by kinetic turbidimetric limulus assay using a Toxinometer (MT-251 or MT-358, Wako Pure Chemicals Industries, LTD, Osaka, Japan)(22, 23), a device theoretically capable of measuring with an accuracy up to 0.01 pg/ml. This limulus assay test is specific to endotoxin and has no cross-reaction to β-glucan, as previously described (24). As shown in Tables 1 and 2, endotoxin concentrations decreased after PMX treatment in most of the studies (5-11, 15, 17, 25) but did not significantly change in the European RCT study (13).

Pulmonary oxygenation

The PaO_2/F_IO_2 ratio increased after PMX treatment in most of the studies (6, 9, 10, 12, 14, 15, 19) (Tables 1 and 2). PMX treatment significantly increased the PaO_2/F_IO_2 ratio compared with conventional therapy (Figure 1).

Mediators

As shown in Tables 1 and 2, PMX decreased blood concentrations of interleukin (IL)-6 (6, 9, 16-18, 25), IL-8 (12), IL-10 (6, 18), tumor necrosis factor (TNF)-α (6, 9), plasminogen activator inhibitor-1 (PAI-1) (6, 10, 12), neutrophil elastase (12, 26), platelet factor-4 (5, 9), β-thromboglobulin (5), soluble P selectin (5), soluble endothelial leukocyte adhesion molecule (ELAM)-1 and soluble intercellular adhesion molecule (ICAM)-1 (15), erythropoietin (20),

5

metalloproteinase-9 (21), anandamide (27), high-mobility group box (HMGB)-1 and soluble receptor for advanced glycation end-products (RAGE) (25). The changes in endotoxin obtained by PMX treatment were significantly correlated with those in HMGB-1, soluble RAGE, and IL-6 (25). However, the other study has reported that HMGB-1 concentrations remained unchanged after PMX treatment (16). In a subset of patients with liver failure (16), HMGB-1 concentrations reduced after PMX treatment in survivors, but HMGB-1 concentrations increased after PMX treatment in non-survivors.

Organ dysfunction and Mortality

PMX treatment decreased SOFA score (14, 15, 19). PMX treatment significantly reduced 28-day mortality compared with conventional therapy (Figure 2).

DISCUSSION

PMX treatment shows beneficial effects compared with conventional therapy in patients with septic shock. PMX treatment increases blood pressure while reducing the dosage requirements for vasopressive/inotropic agents. In one study, the treatment increased the cardiac index (13). In another, it reduced the concentration of cardiac troponin T (8), a potential marker of septic myocardial injury (28). Further, a significant relationship was found between the concentrations of endotoxin and cardiac troponin T in the study in which both were significantly decreased after the treatment (8). On this basis, we speculate that PMX treatment may be effective in reducing myocardial damage by reducing blood endotoxin concentrations. Overall, these findings suggest that PMX treatment improves hemodynamic status.

The beneficial effects of PMX may be attributable to decrease in the concentrations of endotoxin and/or inflammatory cytokines. If this is so, the effect in reducing endotoxins may be capable of modulating the cascade of events in sepsis. Cytokines play an important role in the pathological condition of sepsis. Endotoxin removal by PMX might indirectly inhibit the release of inflammatory cytokines. In fact, PMX treatment decreases plasma concentrations of IL-6 (6, 9, 16, 17, 18), IL-8 (12), IL-10 (6, 18), and TNF-α (6, 9). Elevated nitric oxide (NO) production

6

is a well known phenomenon in septic shock, one that results from the activation of the inducible NO synthase pathway via infection and inflammation (29). PMX treatment decreases NO breakdown products (NOx) in urine (11). As such, the inhibition of NO production by PMX could prevent vasodilation and increase blood pressure. Another potential source of the hemodynamic may be the adsorption of anandamide. Anandamide, an endogenous cannabinoid, is generated by macrophages during endotoxin shock and is thought to contribute to hypotension in a paracrine fashion. PMX has been found to directly bind to anandamide and to be neutralized by bioactivity effects conferred by anandamide such as vasodilation and cytotoxicity (27).

PAI-1, a marker of vascular endothelial cell activation, is elevated by endotoxin and cytokines. The PMX-induced decrease in cytokine release might lower the PAI-1 concentration by reducing the stimulation of vascular endothelial cells (6, 10, 12). PAI-1, one of the fibrinolysis inhibitory factors, plays an important role in regulating fibrinolysis by blocking unnecessary fibrinolysis via its actions as an inhibitor of tissue plasminogen activator. PMX treatment reduces PAI-1 concentration and may thereby inhibit the development of ischemic organ dysfunction in sepsis.

Several studies showed PMX-confirmed improvements in pulmonary oxygenation (6, 9, 10, 12, 14, 15), a condition associated with decreased adhesion molecule in patients with septic shock. A longer-duration PMX treatment in our study improved pulmonary oxygenation associated with decreased adhesion molecule in patients with septic shock (15). We do not know whether PMX treatment removes adhesion molecules directly or weakens their concentration by reducing the stimulation of endothelial cells via its effect in adsorbing pathogenic toxins. One study reported significant negative correlations between the PaO_2/F_1O_2 ratio and the concentrations of IL-8 and neutrophil elastase in patients with septic shock (12). Another study showed PMX-induced decreases of metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 concentrations in patients with acute respiratory distress syndrome (21). The improved PaO_2/F_1O_2 ratio conferred by PMX treatment may therefore be attributable to decreases in adhesion molecules, IL-8, neutrophil elastase, metalloproteinase-9 or the tissue inhibitor of

7

metalloproteinase-1.

The monocyte/macrophage system is involved in acute inflammatory response and plays an important role in host defense. Three of the main functions of monocytes, namely, phagocytosis, antigen expression, and cytokine production, are mediated by surface molecules such as CD14 and human leukocyte antigen (HLA)-DR. CD14 binds LPS and induces cellular activation. Neutrophil receptor CD16 also plays an important role in phagocytosis, cell-mediated cytotoxity and free radicals release. One study revealed decreased expression of surface antigens such as HLA-DR on monocytes and CD16 on granulocytes in patients with septic shock, but PMX increased the expression of these surface antigens on leukocytes in the same patients (18). This suggests that PMX treatment may help septic shock patients recover from immunoparalytic conditions. PMX was also reported to reduce the number of $CD16^+CD14^+$ monocytes and decrease the monocyte expression of TLR-4 in patients with septic shock (30). $CD16^+CD14^+$ monocytes exhibit the features of activated cells and might contribute, in their activated state, to the immunosuppression associated with severe infections. Meanwhile, the adsorption of $CD16^+CD14^+$ monocytes may be the mechanism by which PMX treatment improves the clinical outcome in patients with septic shock. In recent experiments using immunocytochemical and electron microscopic techniques to analyze the cells bound by the PMX column, the column was confirmed to specifically bind monocytes from the peripheral blood leukocytes of septic patients (31). The specific removal of monocytes from septic patients may produce beneficial effects by reducing the interaction between monocytes and functionally associated cells, including vascular endothelial cells. While true mechanism still remains unclear, PMX may remove monocytes by removing the LPS-monocyte complex.

Recently, Kumagai et al. (32) found that activated neutrophils with increased expression of CD11b/CD64 and low expression of CXCR1/CXCR2 preferentially adhered to PMX filters. In addition, neutrophils obtained after ex vivo perfusion through PMX filters showed less endothelial damage without affecting neutrophil phagocytic function. Therefore, PMX selectively removes activated neutrophils and reduces the ability of circulating cells to cause

8

endothelial damage.

PMX treatment reduced acute kidney injury and the proapoptotic activity of the plasma of septic patients on cultured renal cells (33). Apoptosis contributes to organ dysfunction during septic shock, and is activated via endotoxin-initiated signaling pathways. The protective effects of PMX against acute kidney injury may thus be attributable to a reduced systemic proapoptotic activity. PMX treatment seems to decrease mortality. HMGB-1 is a late mediator of endotoxin lethality in mice (34), and RAGE is involved in the HMGB-1 signaling (35). PMX treatment decreased concentrations of HMGB-1 and soluble RAGE in patients with septic shock (25). Therefore, suppression of HMGB-1 and RAGE may be useful in the treatment for septic shock. These effects of PMX treatment may protect patients from multiple organ dysfunction syndrome. In fact, PMX treatment decreased SOFA score (14, 15, 19) and mortality (5,7,9,14,17). The reduction in mortality demonstrated in the EUPHAS trial was especially prominent (14). Yet this beneficial effect of PMX on mortality may depend on the severity of the sepsis. While PMX improved outcome in patients with an APACHE II score less than 30, it failed to improve survival in patients with an APACHE II score of greater than 30 (7). Unlike the EUPHAS trial, the European pilot study on PMX treatment in sepsis with abdominal infection showed no SOFA score improvement (13). The PMX treatment was only administerd once in that study. It may be that a single column hemoperfusion is insufficient to remove endotoxin in septic shock patients under some conditions.

Polymyxin B has both antibacterial and antiendotoxin capability, and it destroys the bacterial outer membrane and selectively binds the lipid A portion of endotoxin, thus neutralizing its adverse effects on cells. Concerning multi-scale analysis of the Toraymyxin column, two studies have been published. Vesentini S, et al. (36) have investigated the interaction energies of polymyxin B-endotoxin complexes and clarified the mechanisms of complex formation. In the short range, the fatty acid chain stabilize the complex and the resulting binding is about 2nN due to both ionic and hydrophobic effects; in the long range, ionic forces become prevalent and the binding force decreases. Fiore GB, et al. (37) have investigated fluid dynamics inside the

9

Toraymyxin column using a computational multi-scale analysis. In macroscale study, the velocity distribution was quite uniform within the sorbent region and peak velocity spots were located immediately below the impact surface. In mesoscale study, wall shear stresses   ranged from homogeneous pattern to uneven pattern depending on the alignment of fiber layers. In microscale study, the fate of an endotoxin particle depends both on its initial distance from the fiber wall and on wall shear stress. They demonstrated that the fiber-grafted polymyxin B was able to capture endotoxin molecules up to distances far beyond the short-range interval. This finding suggests that hydrodynamics strongly affect the adsorption potential.

Various neutralization of endotoxin strategies such as HA-1A human monoclonal antibody against endotoxin, E5 murine monoclonal antiendotoxin antibody and phospholipid emulsion (GR270773) have been investigated (38, 39, 40). However, none of these therapies have been shown to reduce mortality of septic shock. Recently, phase 2 trial of eritotan (E5564), a TLR-4 antagonist, in patients with severe sepsis has shown that mortality was not statistically significant (41). These results are probably due to nonspecific and weak bond achieved in the complex with the lipid A of endotoxin (42). The lack of clinical success with these therapies has led to an interest in extracorporeal therapies to decrease the mediators in septic shock. Suzuki et al. (17) compared cytokine levels, endotoxin levels, and mortality of septic shock patients between PMX plus continuous hemodiafiltration (CHDF) group and CHDF group. CHDF was performed using PMMA dialyzer (Toray Co., Tokyo, Japan). Although CHDF decreased IL-1β and IL-6 levels a little, CHDF alone could not reduce endotoxin levels. In addition, 28-day mortality was 25% in the PMX plus CHDF group and 75% in the CHDF group. These findings show that PMX treatment has more beneficial effects than CHDF. PMX treatment has additional effects on reducing monocytes activity (30, 31), abolishing the diverse negative effects of anandamide such as hypotension, immunosupression, and cytotoxity (27), reducing endothelial damage by removal of activated neutrophils (32) and proapoptotic activity (33), and recovering patients from immunosupression (18, 30). These actions of PMX treatment may be superior to previous neutralization of endotoxin strategies.

10

A fairly stable concentration after peaks of endotoxin have been removed by PMX treatment. Although endotoxin removal from the blood by PMX treatment might promote a dynamic equilibrium between the tissue and blood compartments thus enabling a continuous withdrawal of endotoxin from the tissues during extracorporeal therapies (43), plasma endotoxin levels would be continuously derived from the lysis of bacteria present in sites other than blood. Because, septic shock patients are treated with antimicrobial agents, which induce lysis of bacterial cells and release endotoxin (44). On the other hand, endotoxin is also bound to circulating monocytes (45), to erhythrocyte (46), and to platelets (47) during sepsis. Endotoxin-monocyte complex has been shown to be removed by PMX treatment (31). Since lipid A portion of endotoxin binds to membrane of platelets (48) and erythrocyte (46), PMX theoretically cannot remove the endotoxin which is bound to platelets and erythrocytes. Plasma endotoxin levels have been shown to be significantly higher in platelet-rich plasma than those in platelet-poor plasma (47). Accordingly, endotoxin binding to platelet is present in blood after PMX treatment. Taken together, PMX treatment may not end to a total removal of endotoxin.

All endotoxins from different bacterial origin are biochemically different (49) and thus do not interact similarly with Polymyxin B. Example are provided by the polymyxin B resistant strains. The main mechanism of polymyxin resistance is the modification of the bacterial outer membrane, which is mainly mediated by two-component regulatory systems PhoP-PhoQ and PmrA-PmrB. Polymyxin resistance is encoded by the PmrA-PmrB regulon, whose products modify the LPS core and lipid A portions with ethanolamine and add aminoarabinose to the 4' phosphate of lipid A (50). Therefore, PMX column may not have similar efficiency depending on the nature of the infectious agent.

CONCLUSIONS

These findings suggest that PMX treatment has beneficial effects on hemodynamics, pulmonary oxygenation and mortality in patients with septic shock. These beneficial effects may be attributable to direct adsorption of endotoxin, monocytes, activated neutrophils and

11

anandamide and indirect decreases in inflammatory cytokines and other mediators. PMX treatment has additional effects on reducing endothelial damage, apoptotic activity and immunosupression. Further studies will be needed to confirm the efficacy and mechanism of PMX treatment in septic shock.

12

REFERENCES

1. Peters K, Unger RE, Brunner J, Kirkpatrick CJ. Molecular basis of endothelial dysfunction in sepsis. Cardiovasc Res 60(1):49-57, 2003.

2. Shoji H. Extracorporeal endotoxin removal for the treatment of sepsis: endotoxin adsorption cartridge (Toraymyxin). Ther Apher Dial 7(1):108-114, 2003.

3. Aoki H, Kodama M, Tani T, Hanasawa K. Treatment of sepsis by extracorporeal elimination of endotoxin using polymyxin B immobilized fiber. Am J Surg 167(4):412-417, 1994.

4. American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference: Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. Crit Care Med 20(6):864-874, 1992.

5. Nakamura T, Ebihara I, Shoji H, Ushiyama C, Suzuki S, Koide H. Treatment with polymyxin B-immobilized fiber reduces platelet activation in septic shock patients: Decrease in plasma levels of soluble P-selectine, platelet factor 4 and β-thromboglobulin. Inflamm Res 48(4):171-175, 1999.

6. Tani T, Hanasawa K, Kodama M, Imaizumi H, Yonekawa M, Saito M, Ikeda T, Yagi Y, Takayama K, Amano I, Shimaoka H, Ohta M, Okahisa T, Koga N, Fujita N, Yamasa H. Correlation between plasma endotoxin, plasma cytokines, and plasminogen activator inhibitor-1 activities in septic shock patients. World J Surg 25(5):660-668, 2001.

7. Nemoto H, Nakamoto H, Okada H, Sugahara S, Moriwaki K, Arai M, Kanno Y, Suzuki H. Newly developed immobilized polymyxin B fibers improve the survival of patients with sepsis. Blood Purif 19:361-369, 2001.

8. Nakamura T, Ushiyama C, Shoji H, Koide H. Effects of hemoperfusion on serum cardiac troponin T concentrations using polymyxin B-immobilized fibers in septic patients undergoing hemodialysis. ASAIO Journal 48:41-44, 2002.

9. Nakamura T, Matsuda T, Suzuki Y, Shoji H, Koide H. Polymyxin B-immobilized fiber hemoperfusion in patients with sepsis. Dial Transplant 32(10):602-607, 2003.

10. Ikeda T, Ikeda K, Nagura M, Taniuchi H, Matsushita M, Kiuchi S, Kuroki Y, Suzuki K,

13

Matsuno N. Clinical evaluation of PMX-DHP for hypercytokinemia caused by septic multiple organ failure. Ther Apher Dial 8(4):293-298, 2004.

11. Nakamura T, Kawagoe Y, Matsuda T, Koide H. Effect of polymyxin B-immobilized fiber on bone resorption in patients with sepsis. Intensive Care Med 30(9):1838-1841, 2004.

12. Kushi H, Miki T, Okamoto K, Nakahara J, Saito T, Tanjoh K. Early hemoperfusion with an immobilized polymyxin B fiber column eliminates humoral mediators and improves pulmonary oxygenation. Crit Care 9:R653-R661, 2005.

13. Vincent JL, Laterre PF, Cohen J, Burchardi H, Bruining H, Lerma FA, Wittebole X, De Backer D, Brett S, Marzo D, Nakamura H, John S. A pilot-controlled study of a polymyxin B-immobilized hemoperfusion cartridge in patients with severe sepsis secondary to intra-abdominal infection. Shock 23(5):400-405, 2005.

14. Cruz DN, Antonelli M, Fumagalli R, Foltran F, Brienza N, Donati A, Malcangi V, Petrini F, Volta G, Pallavicini FMB, Rottoli F, Giunta F, Ronco C. Early use of polymyxin B hemoperfusion in abdominal septic shock: the EUPHAS randomized controlled trial. JAMA 301(23):2445-2452, 2009.

15. Mitaka C, Tsuchida N, Kawada K, Nakajima Y, Imai T, Sasaki S. A longer duration of polymyxin B-immobilized fiber column hemoperfusion imporves pulmonary oxygenation in patients with septic shock. Shock 32(5):478-483, 2009.

16. Ueno T, Ikeda T, Ikeda K, Taniuchi H, Suda S, Yeung MY, Matsuno N. HMGB-1 as a useful prognostic biomarker in sepsis-induced organ failure in patients undergoing PMX-DHP. J Surg Res 2009 Dec 10 [Epub ahead of print]

17. Suzuki H, Nemoto H, Nakamoto H, Okada H, Sugawara S, Kanno Y, Moriwaki K. Continuous hemodiafiltration with polymyxin B-immobilized fiber is effective in patients with sepsis syndrome and acute renal failure. Ther Apher 6(3):234-240, 2002.

18. Ono S, Tsujinomoto H, Matsumoto A, Ikuta S, Kinoshita M, Mochizuki H. Modulation of human leukocyte antigen-DR on monocytes and CD16 on granulocytes in patients with septic shock using hemoperfusion with polymyxin B-immobilized fiber. Am J Surg 188:150-156, 2004.

14

19. Novelli G, Ferretti G, Poli L, Pretagostini R, Ruberto F, Perrella SM, Levi S, Morabito V, Berloco PB, Clinical results of treatment of postsurgical endotoxin-mediated sepsis with polymyxin-B direct hemoperfusion. Transplantation Proceedings 42(4):1021-1024, 2010.

20. Nakamura T, Ebihara I, Shimada N, Koide H. Changes in plasma erythropoietin and interleukin-6 concentrations in patients with septic shock after hemoperfusion with polymyxin B-immobilized fiber. Intensive Care Med 24(12):1272-1276, 1998.

21. Nakamura T, Kawagoe Y, Matsuda T, Shoji H, Ueda Y, Tamura N, Ebihara I, Koide H. Effect of polymyxin B-immobilized fiber on blood metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 levels in acute respiratory distress syndrome patients. Blood Purif 22(3):256-260, 2004.

22. Oishi H, Takaoka A, Hatayama Y, Matsuo T, Sakata Y. Automated limulus amebocyte lysate (LAL) test for endotoxin analysis using a new toxinometer ET-201. J Parenter Sci Technol 39(5):194-200, 1985.

23. Kojika M, Sato N, Yaegashi Y, Suzuki Y, Suzuki K, Nakae H, Endo S. Endotoxin adsorption therapy for septic shock using polymyxin B-immobilized fibers (PMX): evaluation by high-sensitivity endotoxin assay and measurement of the cytokine production capacity. Ther Apher Dial 10(1):12-18, 2006.

24. Kambayashi J, Yokota M, Sakon M, Shiba E, Kawasaki T, Mori T, Tsuchiya M, Oishi H, Matsuura S. A novel endotoxin-specific assay by turbidimetry with Limulus amoebocyte lysate containing β-glucan. J Biochem Biophys Methods 22:93-100, 1991.

25. Nakamura T, Sato E, Fujiwara N, Kawagoe Y, Maeda S, Yamagishi S. Supression of high-mobility group box-1 and receptor for advanced glycation end-product axis by polymixn B-immobilized fiber hemoperfusion in septic shock patients. J Crit Care 2011 Jan 25 [Epub ahead of print].

26. Kushi H, Miki T, Nakahara J, Okamoto K, Saito T, Tanjoh K. Hemoperfusion with an immobilized polymyxin B column reduces the blood level of neutrophil elastase. Blood Purif 24(2):212-217, 2006.

27. Wang Y, Liu Y, Sarker KP, Nakashima M, Serizawa T, Kishida A, Akashi M, Nakata M, Kitajima I, Maruyama I. Polymyxin B binds to anandamide and inhibits its cytotoxic effect. FEBS Letters 470(2):151-155, 2000.

28. Fernandes CJ Jr, Akamine N, Knobel H. Cardiac troponin: a new serum marker of myocardial injury in sepsis. Intensive care Med 25(10):1165-1168, 1999.

29. Moncada S, Higgs A. The L-arginine nitric oxide pathway. N Engl J Med 329:2002-2012, 1993.

30. Tsujimoto H, Ono S, Hiraki S, Majima T, Kawarabayashi N, Sugasawa H, Kinoshita M, Hiraide H, Mochizuki H. Hemoperfusion with polymyxin B-immobilized fibers reduced the number of $CD16^+CD14^+$ monocytes in patients with septic shock. J Endotoxin Res 10(4):229-237, 2004.

31. Nishibori M, Takahashi HK, Katayama H, Mori S, Saito S, Iwagaki H, Tanaka N, Morita K, Ohtsuka A. Specific removal of monocytes from peripheral blood of septic patients by polymyxin B-immobilized filter column. Acta Med Okayama 63(1):65-69, 2009.

32. Kumagai T, Takeyama N, Yabuki T, Harada M, Miki Y, Kanou H, Inoue S, Nakagawa T, Noguchi H: Apheresis of activated leukocytes with an immobilized polymyxin B filter in patients with septic shock. Shock 34(5):461-466, 2010.

33. Cantaluppi V, Assenzio B, Pasero D, Romanazzi GM, Pacitti A, Lanfranco G, Puntorieri V, Martin EL, Mascia L, Monti G, Casella G, Segoloni GP, Camussi G, Ranieri VM. Polymyxin-B hemoperfusion inactivates circulating proapoptotic factors. Intensive Care Med 34(9):1638-1643, 2008.

34. Wang H, Bloom O, Zhang M, Vishnubhakat JM, Ombrellino M, Che J, Frazier A, Yang H, Ivanova S, Borovikova L, Manogue KR, Faist E, Abraham E, Andersson J, Andersson U, Molina PE, Abumrad NN, Sama A, Tracy KJ. HMG-1 as a late mediator of endotoxin lethality in mice. Science 285(5425):248-251, 1999.

35. Klune JR, Dhupar R, Cardinal J, Billiar TR, Tsung A. HMGB-1: endogenous danger signaling. Mol Med 14(7-8):476-484, 2008.

36. Vesentini S, Sonicini M, Zaupa A, Silvestri V, Fiore GB, Redaelli A. Multi-scale analysis of the Toraymyxin adsorption cartridge. Part I: Molecular interaction of polymyxin B with endotoxins. Int J Artif Organs 29(2):239-250, 2006.

37. Fiore GB, Soncicni M, Vesentini S, Penati A, Visconti G, Redaelli A. Multi-scale analysis of the Toraymyxin adsorption cartridge. Part II: Computational fluid-dynamic study. Int J Artif Organs 29(2):251-260, 2006.

38. McCloskey RV, Straube RC, Sanders C, Smith SM, Smith CR, the CHESS Trial Study Group: Treatment of septic shock with human monoclonal antibody HA-1A. A randomized double-blind, placebo-controlled trial. Ann Intern Med 121:1-5, 1994.

39. Angus DC, Birmingham MC, Balk RA, Scannon PJ, Collins D, Kruse JA, Graham DR, Dedhia HV, Homann S, MacIntyre N, the E5 Study Investigators. E5 murine monoclonal antiendotoxin antibody in Gram-negative sepsis: a randomized controlled trial. E5 Study Investigators. JAMA 283:1723-1730, 2000.

40. Dellinger RP, Tomayko JF, Angus DC, Opal S, Cupo MA, McDermott S, Ducher A, Calandra T, Cohen J, the Lipid Infusion and Patient Outcomes in Sepsis (LIPOS) Investigators. Efficacy and safety of a phospholipid emulsion (GR270773) in Gram-negative severe sepsis: Results of a phase II multicenter, randomized, placebo-controlled, dose-finding clinical trial. Crit Care Med 37:2929-2938, 2009.

41. Tidswell M, Tillis W, LaRosa SP, Lynn M, Wittek AE, Kao R, Wheeler J, Gogate J, Opal SM, the Eritoran Sepsis Study Group: Phase 2 trial eritoran tetrasodium (E5564), a Toll-like receptor 4 antagonist, in patients with severe sepsis. Crit Care Med 38:72-83, 2010.

42. Opal SM, Glück T. Endotoxin as a drug target. Crit Care Med 21[Suppl.]:S57-S54, 2003.

43. Ronco C. The immunomodulatory effect of extracorporeal therapies in sepsis: A reconciliation of three theories. Int J Artif Organs 30:855-857, 2007.

44. Shenep JL, Flynn PM, Barrett FF, Stidham GL, Westenkirchner DF. Serial quantification of endotoxemia and bacteremia during therapy for Gram-negative bacterial sepsis. J Infect Dis 157(3):565-568, 1988.

45. Takeshita S, Nakatani K, Tsujimoto H, Kawamura Y, Sekine I: Detection of circulating lipopolysaccharide-bound monocytes in children with Gram-negative sepsis. J Infect Dis 182:1549-1552, 2000.

46. Pöschl JM, Leray C, Ruef P, Cazenave JP, Linderkamp O: Endotoxin binding to erythrocyte membrane and erythrocyte deformability in human sepsis and in vitro. Crit Care Med 31:924-928, 2003.

47. Salden HJM, Bas BM: Endotoxin binding to platelets in blood from patients with a sepsis syndrome. Clin Chem 40(8):1575-1579, 1994.

48. Spielvogel AR: An ultrastructual study of the mechanisms of platelet-endotoxin interaction. J Exp Med 126:235-250, 1967.

49. Banoub JH, Aneed AEI, Cohen AM, Joly N. Structural investigation of bacterial lipopolysacharides by mass spectrometry and tandem mass spectrometry. Mass Spectrometry Reviews 29:606, 2010.

50 Gunn JS, Lim KB, Krueger J, Kim K, Guo L, Hackett M, Miller SI. PmrA-PmrB-regulated genes necessary for 4-aminoarabinose lipid A modification and polymyxin resistance. Molecular Microbiology 27(6):1171-1182, 1998.

Figure legends

Figure 1. Effect of PMX treatment on increase in mean arterial pressure (MAP) and PaO_2/F_1O_2. Size of data markers is proportional to the weight of each study in the forest plot. N; number of patients, Horizontal bars = 95% confidence interval (CI).

Figure 2. Effect of PMX treatment on mortality. Size of data markers is proportional to the weight of each study in the forest plot. n; number of non-survivor, N; number of patients, Horizontal bars = 95% confidence interval (CI).

19

Figure 1

MAP

Study	PMX N	Conventional N	Difference in means and 95%CI	Relative Weight(%)	Difference in means	Lower limit	Upper limit	P-value
2005 Vincent, et al. (13)	17	19		0.24	1.8	-5.97	9.57	
2009 Cruz, et al. (14)	34	30		72.10	5.0	4.25	5.75	
2002 Suzuki, et al. (17)	24	24		27.66	6.0	4.67	7.33	
Total	75	73		100.00	5.3	4.59	5.86	<0.0001

-10   0   10

Favours Conventional   Favours PMX

PaO_2/F_IO_2

Study	PMX N	Conventional N	Difference in means and 95%CI	Relative Weight(%)	Difference in means	Lower limit	Upper limit	P-value
2005 Vincent, et al. (13)	17	19		9.26	29.1	-12.6	70.84	
2009 Cruz, et al. (14)	34	30		90.74	18.0	7.57	28.43	
2002 Suzuki, et al. (17)	-	-						
Total	51	49		100.00	18.7	8.74	28.57	0.0002

-20   0   70

Favours Conventional   Favours PMX

20

Figure 2

Mortality

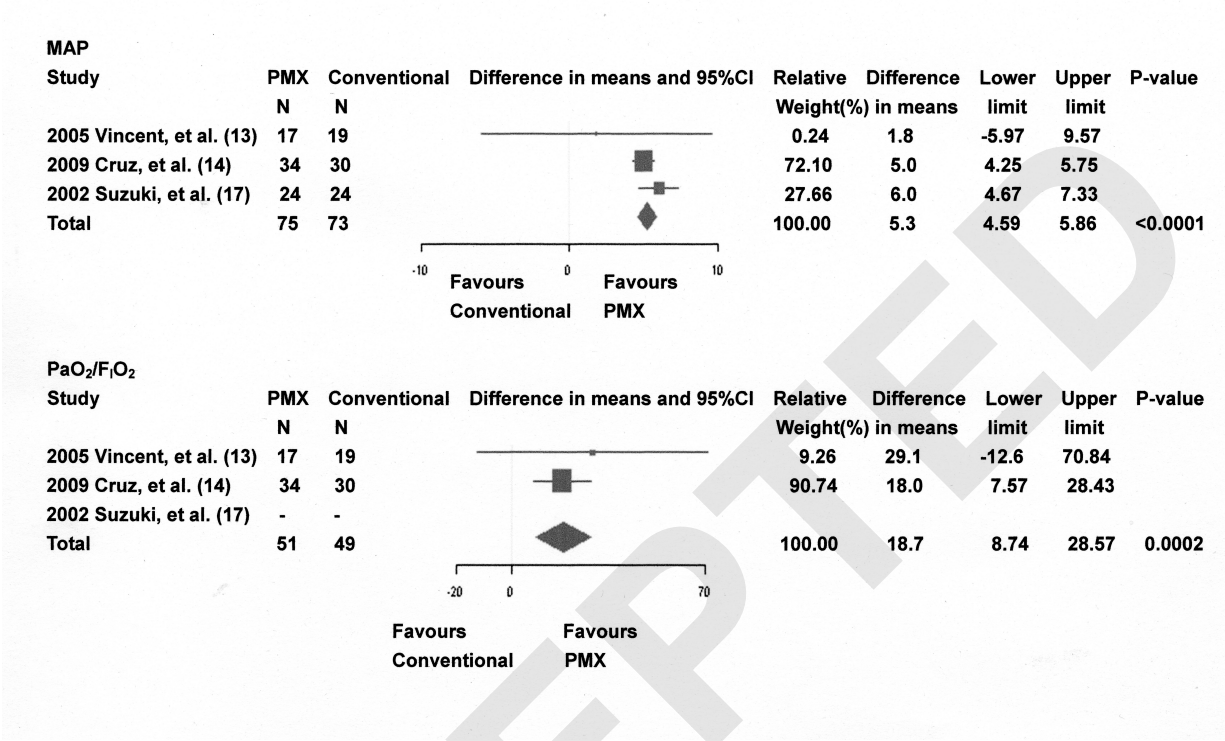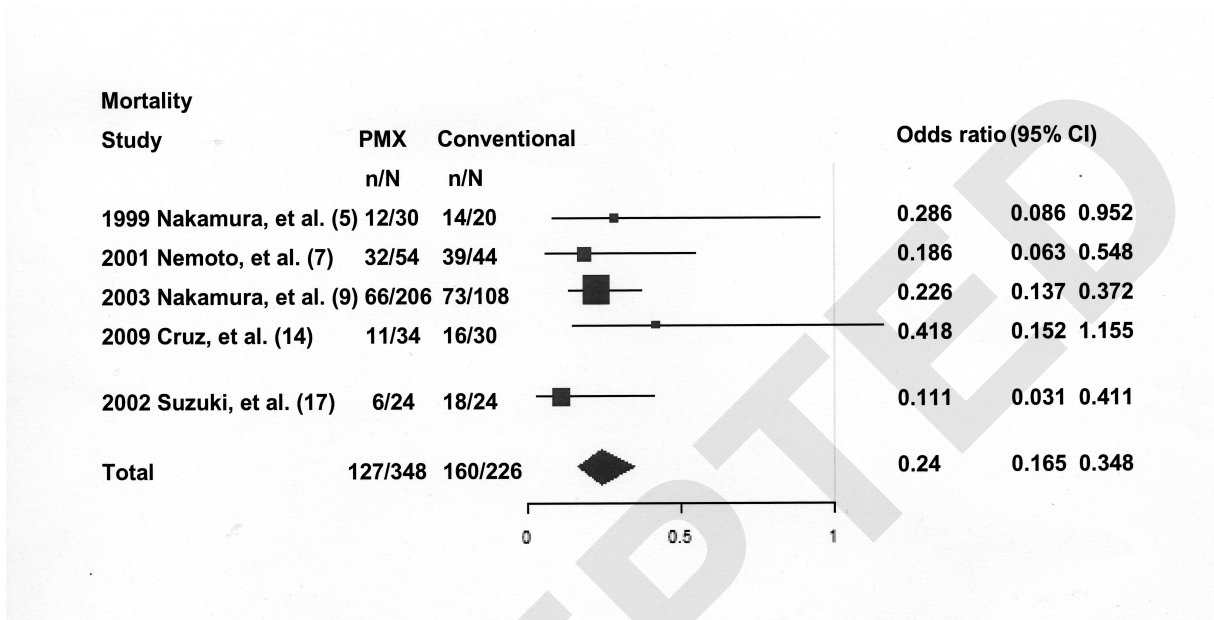| Study	PMX n/N	Conventional n/N		Odds ratio (95% CI)		
1999 Nakamura, et al. (5)	12/30	14/20		0.286	0.086	0.952
2001 Nemoto, et al. (7)	32/54	39/44		0.186	0.063	0.548
2003 Nakamura, et al. (9)	66/206	73/108		0.226	0.137	0.372
2009 Cruz, et al. (14)	11/34	16/30		0.418	0.152	1.155
2002 Suzuki, et al. (17)	6/24	18/24		0.111	0.031	0.411
Total	127/348	160/226		0.24	0.165	0.348

Table 1. Comparison of clinical parameters between PMX treatment and conventional therapy or hemodialysis (HD)/continuous hemodiafiltration (CHDF) in patients with septic shock

Study	PMX							Conventional				
	N	PMX duration	APACHE II score	BP	Vasopressor	Mediator	PaO$_2$/F$_1$O$_2$	N	APACHE II score	BP	Mediator	PaO$_2$/F$_1$O$_2$
1999 Nakamura, et al. (5)	30	2hr x 1	24.8	↑	↓	Endotoxin↓ , PF-4↓ P-selectine↓, β-TG↓		20			Endotoxin→ PF-4→, β-TG→ P-selectine→	
2001 Nemoto, et al. (7)	54	4hr x 1 or 2	22	↑→*		Endotoxin↓		44	23			
2003 Nakamura, et al. (9)	206	2hr x 2	24.6	↑		Endotoxin↓, IL-6↓ TNF-α ↓, sIL-2receptor↓ endothelin↓, PF-4↓(survivor)	↑	108	24			
2004 Nakamura et al. (11)	15	2hr x 2	28.4			Endotoxin↓ Urinary NOx↓		10	28		Endotoxin→ Urinary NOx→	
2005 Vincent, et al. (13)	17	2hr x 1	18.7	↑	→	Endotoxin→ IL-6→		19	16.7	↑	Endotoxin→ IL-6→	
2009 Cruz, et al. (14)	34	2hr x 2	21	↑	↓		↑	30	20	→		→

Study	PMX + HD/CHDF						HD/CHDF			
	N	PMX duration	APACHE II score	BP	Vasopressor	Mediator	N	APACHE II score	BP	Mediator
2002 Nakamura, et al (8)	7	2hr x 2				Endotoxin↓, Troponin T↓	7			Troponin T→
2002 Suzuki et al. (17)	24	4hr	25	↑	↓	Endotoxin↓ IL-1β↓↓, IL-6↓↓	24	25	→	Endotoxin → IL-1β↓ , IL-6↓

N; number of patients, APACHE II score; acute physiology and chronic health evaluation II score, β-TG ; β-thromboglobuline, IL-6; inter leukin-6, IL-1β; inter leukin-1β, TNF-α ; tumor necrosis factor-α , PF-4; platelet factor-4, →* APACHE II score >30,

Table 2. Changes in clinical parameters before and after PMX treatment and mortality in patients with septic shock

Study	N	PMX duration	APACHE II score	BP	Vasopressor	Endotoxin	PaO$_2$/F$_1$O$_2$	Mediators	Mortality (28 day)
2001 Tani, et al. (6)	88	2hr x 2	24.2	↑ *		↓ / ↑ **	↑ *	TNF-α↓*, IL-6↓*,IL-10↓* PAI-1↓ *	48/88(60.2%)
2004 Ikeda, et al. (10)	66	2hr	22.9* 30.7**	↑	↓	↓	↑	TNF-α→, IL-6→, IL-Ira↓, ELAM-1→ ICAM-1→, PAI-1↓*	28/66 (42.4%)
2004 Ono, et al. (18)	10	2hr		↑→**				IL-6↓, IL-10↓, HLA-DR↑, CD14↑, CD16↑	3/10 (30%)
2005 Kushi, et al. (12)	36	3hr x 2	24				↑	PAI-1↓ neutrophil elastase↓, IL-8↓	
2009 Mitaka, et al (15)	16	2hr x 2 3-27hr x 1	23.3	↑	↓	↓→**	↑	ELAM-1↓, ICAM-1↓	8/16 (50%)
2010 Ueno, et al. (16)	60		24.7* 26.7**					IL-6 ↓, HMGB-1→	20/60 (33%)
2010 Novelli, et al. (19)	17	2hr x 2-4		↑		↓	↑		0/17 (0%)
2010 Kumagai, et al. (32)	18	2-4hr	24					IL-6→, IL-8→, IL-10→, HMGB1→ HLA-DR→, CXCR1↑, CXCR2↑, CD64↓, CD11b↓	
2011 Nakamura, et al. (25)	15	2hr x 2		↑		↓		IL-6 ↓, HMGB-1↓, soluble RAGE↓	

N; number of patients, BP; blood pressure, TNF-α; tumor necrosis factor-α, IL-6; inter leukin-6, IL-10; inter leukin-10, PAI-1; plasminogen activator inhibitor-1, ELAM-1; endothelial leukocyte adhesion molecule-1, ICAM-1; intercellular adhesion molecule, CXCR; C-X-C chemokine receptor HMGB-1; high mobility group box-1, RAGE; receptor for advanced glycan end-products, *; survivor, ** ;non-survivor,