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Review Article

Direct Hemoperfusion with Polymyxin B Immobilized Fiber for Abdominal Sepsis in Europe

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Abstract
Since direct hemoperfusion with polymyxin B immobilized fiber (PMX-DHP) received its product certification for use in Europe in 1998, several prospective randomized controlled trials (RCTs) have been conducted in European countries. The first RCT, performed in six European academic medical centers in 2005, concluded that PMX-DHP is associated with improved hemodynamic status and cardiac function. Subsequently, a meta-analysis of PMX-DHP was presented in Italy in 2007. This systematic review found positive effects of PMX-DHP on mean arterial pressure and dopamine/dobutamine use, PaO_2/FiO_2 ratio, endotoxin removal, and mortality. However, like most trials on extracorporeal therapies, none of the studies was double-blinded. The EUPHAS study, a multicenter RCT performed in ten Italian intensive care units in 2009, found that PMX-DHP improved 28-day survival, blood pressure, vasopressor requirement, and degree of organ failure. However, investigators in Belgium and Canada pointed out that there was no statistical difference in 28-day survival. Two more RCTs, the ABDO-MIX and EUPHRATES studies, the primary end points of which are 28-day mortality, were started in Europe and the United States at the end of 2010. We are hoping that these RCTs will resolve this issue.

Key words Direct hemoperfusion · Polymyxin B immobilized fiber · PMX-DHP · Toraymyxin · Randomized controlled trial · Meta-analysis

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Introduction

Sepsis and septic shock continue to be life-threatening complications and major causes of death in medical and surgical intensive care units (ICUs), with an estimated incidence in the United States of 750 000 cases per year and a mortality rate of 25%–80%.[1] Endotoxin, which exists in the outer membrane of Gram-negative bacteria, interacts with the host during Gram-negative sepsis. Endotoxin triggers the release of cytokines, such as interleukin (IL)-1 and tumor necrosis factor-α, activates complements and coagulation factors, and is considered one of the principal biological substances that cause Gram-negative septic shock.[2,3] Polymyxin B is an antibiotic effective against Gram-negative bacteria, which also has an extremely high affinity for endotoxin and counteracts the activation of endotoxin by binding to it. However, the intravenous administration of polymyxin B is impossible because of its significant nephrotoxic and neurotoxic effects.[3–5] The polymyxin B cartridge (Toraymyxin; Toray Industries, Tokyo, Japan) is an extracorporeal hemoperfusion device that uses polymyxin B fixed to α-chloroacetamide-methyl polystyrene-derived fibers packed in the cartridge. Polymyxin B is chemically immobilized in the polystyrene-derived fiber through covalent bonds and does not leach from the fiber. Polymyxin B, bound and immobilized to polystyrene fibers (PMX-F), has been reported to effectively bind endotoxin in both in vitro and in vivo studies.[6] Since 1994, direct hemoperfusion with PMX-F (PMX-DHP) has been listed as a blood purification device in Japan and is reimbursed by Japanese national health insurance[6]. PMX-DHP can be administered to patients with endotoxemia or suspected Gram-negative infection, who fulfill the conditions of systemic inflammatory response syndrome (SIRS) and have septic shock requiring vasoactive agents. Since 1994 more than 60 000 patients have received this treatment.[6–23] Open-label clinical trials using PMX-DHP have been conducted in

Japan, demonstrating the safety of PMX-DHP in the treatment of septic shock and its capacity to decrease endotoxin levels.[24] More recent studies have shown improvement of hemodynamic status,[23,25,26] pulmonary oxygenation,[25,26] systemic inflammatory response,[27] and survival[28,29] in patients with sepsis treated by PMX-DHP.

High-mobility-group box chromosomal protein 1 (HMGB1) was recently identified as a late mediator of systemic inflammation.[30] HMGB1 is associated with various infectious and noninfectious conditions related to SIRS, such as sepsis, hemorrhagic shock, trauma, ischemia/reperfusion injury, disseminated intravascular coagulation, and major surgery.[31] In relation to infectious conditions, HMGB1 is released systemically in murine models of endotoxemia and sepsis induced by cecal perforation.[32,33] Moreover, in one study, serum HMGB1 levels were significantly higher in 25 sepsis patients than in healthy volunteers, and were higher in patients who succumbed to disease than in survivors.[30] HMGB1 is associated with acute lung injury (ALI), ventilator-induced lung injury (VILI), and occult lung injury. Elevated HMGB1 levels are found in the plasma and lung epithelial lining fluid of patients with ALI and in mice instilled with lipopolysaccharide.[34] It has been suggested that direct hemoperfusion using a polymyxin B immobilized fiber column reduces HMGB1 levels in septic patients.[35]

The PMX device received its product certification (CE mark) for use in Europe, according to the directives of the European Community, in 1998, and is classified as a Class IIb medical device. In this systematic review, we analyze prospective randomized controlled trials (RCTs) and meta-analyses of PMX-DHP conducted in Europe, on patients with sepsis, and present new RCTs of PMX-DHP for Europe and the United States.

A Pilot-Controlled Study of PMX-DHP in European Academic Medical Centers

This study, titled "A pilot-controlled study on a polymyxin B immobilized hemoperfusion cartridge in patients with severe sepsis secondary to intra-abdominal infection," was reported in the journal *Shock*[36] in 2005. It was the first prospective RCT of the PMX device in patients with sepsis. Six European academic medical centers, in Belgium, the United Kingdom, Germany, the Netherlands, and Spain, were involved in the study.

The study population consisted of surgical patients with severe sepsis (with or without shock) presumed to be caused by Gram-negative infection, arising from the abdominal cavity, and still present after surgery. Patients who had initially undergone elective surgery were eligible if PMX-DHP could be started within 24 h of the diagnosis of severe sepsis. Patients admitted to hospital for an intra-abdominal infection requiring emergency surgery were also eligible, provided that PMX-DHP was started within 48 h of the diagnosis of severe sepsis.

Patients who fulfilled the study criteria were randomized, using sealed envelopes, to receive standard therapy plus PMX-DHP (PMX group) or standard therapy only (control group). All patients in the PMX group received PMX-DHP only once, on day 0, after severe sepsis had been diagnosed. Each individual session of hemoperfusion lasted for 2 h.

Patients were followed up for 28 days. For each patient, the following hemodynamic and blood gas variables were assessed: heart rate (HR), mean arterial pressure (MAP), right atrial pressure (RAP), pulmonary arterial pressure (PAP), pulmonary capillary wedge pressure (PCWP), cardiac index (CI), systemic vascular resistance (SVR), left ventricular stroke work index (LVSWI), oxygen delivery index (DO_2I), oxygen consumption index (VO_2I), and arterial partial pressure of oxygen/fraction of inspired oxygen (PaO_2/FiO_2) ratio. Organ dysfunction was assessed using the Sequential Organ Failure Assessment (SOFA) and Goris scores. Endotoxin and IL-6 levels were also measured. Endotoxin was measured with the modified limulus amebocyte lysate (LAL) assay[37] using a commercial kit (COATEST; Diapharma, West Chester, OH, USA). The LAL assay is highly sensitive (0.005 EU/ml in saline; 0.05 EU/ml in serum or plasma) and is virtually specific for endotoxin. The primary end point was improvement of organ dysfunction in patients and was assessed by changes in the SOFA score and Goris score. Secondary end points were reduction in plasma endotoxin and IL-6 concentrations, patient survival for over 28 days after PMX-DHP, and length of ICU stay.

A total of 36 patients were randomly assigned to the study over 2 years: 17 in the PMX group and 19 in the control group. Gram-negative bacteria were detected in 13 (72%) control and 14 (82%) PMX patients. Endotoxin levels in both groups did not change significantly between baseline and 120 min. There were no significant differences in the endotoxin levels between the two groups at any time point. Interleukin-6 levels in both groups tended to decrease after 24 h, but not significantly. There were no significant differences between groups in the doses of each inotropic or vasopressive agent. There were no significant differences in the SOFA and Goris scores between the control and the PMX groups. Five (28%) of 18 control patients and five (29%) of 17 PMX patients died during the study period. The survival analysis of time to death showed no significant difference between the two groups. There was no significant difference in the mean ICU stay between

the two groups (PMX, 13.2 ± 9.4 days; control, 17.0 ± 9.4 days; not significant). The MAP increased significantly from baseline to day 2 ($P = 0.006$) in the PMX group, and the CI was significantly greater in the PMX than in the control group on days 1 and 2 ($P = 0.012$ and $P = 0.032$). In the PMX group, the LVSWI increased from baseline to day 2 and was greater than that in the control group on day 2 ($P = 0.015$). The DO_2I increased in the PMX group, to become significantly greater than that in the control group on day 2 ($P = 0.007$).

During this pilot study there was no significant change in the primary end points of the SOFA score and the Goris score, nor in the secondary end points of plasma endotoxin, IL-6 concentrations, patient survival over 28 days, and length of ICU stay between the two groups. However, the results indicated that CI, LVSWI, and DO_2I improved significantly more in the PMX group than in the control group.

Although this pilot study failed to demonstrate lower blood endotoxin levels than the control group, the results show that PMX treatment is safe and is associated with improved hemodynamic status and cardiac function. The findings of this study justified the need for larger, adequately powered, multicenter clinical trials to confirm the effects of PMX treatment on hemodynamics and patient outcomes.

Meta-Analysis of PMX-DHP

This study, titled "Effectiveness of polymyxin B immobilized fiber column in sepsis; a systematic review," was reported in the journal *Critical Care*[38] in 2007. It was an extensive meta-analysis of the PMX device in patients with sepsis. Six medical centers, in Italy, the Philippines, the United States, and Australia, were involved in the study.

PubMed and the Cochrane Collaboration Database were searched up until the end of April 2006, using the following search terms: hemoperfusion, hemadsorption, hemodiafiltration, hemofiltration or hemodialysis and polymyxin, polymyxin B, Toraymyxin, PMX-DHP, or DHP-PMX, without language restrictions. Bibliographies of retrieved articles were also reviewed. Published English, Japanese, and Italian language full-text case series, cohort studies, and RCTs of PMX-DHP were eligible. The trials that were included had at least one of the following outcome measures: MAP, doses of vasoactive agents, PaO_2/FiO_2 ratios, endotoxin levels, and mortality. The primary end points were change in MAP, use of vasoactive agents, PaO_2/FiO_2 ratio, and mortality. A secondary end point was the change in endotoxin levels after PMX-DHP.

This study reviewed 159 abstracts, from which 106 articles were deemed worthy of further exploration and review. Finally, 28 publications relevant to this review were identified. Of these, 16 parallel trials (9 RCTs and 7 non-RCTs) and 12 pre–post cohort studies reported at least one of the necessary outcome measures and were included in the analysis. The 28 trials included 1425 patients: 978 in the PMX group and 447 in the conventional medical therapy group. Gram-negative infections were identified in 71% of the patients. PMX-DHP was given over 2 h at a blood flow rate of 50–150 ml/min, once or a maximum of two or three times, depending on the clinical response of the patient. Each subsequent PMX treatment, if necessary, was performed 24 h after the previous treatment.

All studies that provided sufficient data reported improvement in MAP after PMX-DHP.[14,17,20–22,36,39–44] The pooled estimate showed that PMX-DHP was associated with a significant increase in MAP (weighted mean difference, 19 mmHg; $P < 0.001$). This represented a 26% mean increase in MAP (range, 14%–42%). Four studies reported the dose of dopamine or dobutamine or the average of the sum of the two.[14,21,23,43] All studies showed a trend toward a decrease in the dose after PMX-DHP. Overall, the dose was decreased by 1.8 μg/kg per minute ($P = 0.01$). The effect of PMX therapy on PaO_2/FiO_2 was ascertained in a pooled analysis of seven studies (151 patients),[17,21,36,41,43–45] only one of which was an RCT. Overall, the PaO_2/FiO_2 ratio increased by 32 units ($P < 0.001$) after PMX-DHP. Data on mortality, variably reported as 14-day,[20] 28-day,[13,14,18,36,40,46] 30-day,[9,41,47] and 60-day mortality,[11] were available from 11 studies. Pooled mortality rates were 61.5% in the conventional therapy group and 33.5% in the PMX group. In the pooled estimate, PMX-DHP appeared to reduce mortality significantly compared with conventional medical therapy. In 19 studies, data on endotoxin levels were available.[7–23,36,43] The pooled estimate showed that endotoxin levels decreased by 21.2 pg/ml after PMX-DHP, representing a decrease of 33%–80% from pre-PMX levels.

Putting these data into perspective, this systematic review of the published literature found positive effects of PMX-DHP on MAP and dopamine/dobutamine use, PaO_2/FiO_2 ratio, endotoxin removal, and mortality. However, the study quality was poor, with Jadad scores[48] of less than 3. Among the randomized studies, allocation concealment was deemed adequate in three trials[7,8,36] and uncertain in six.[9–14] Randomization was not performed in seven of the parallel-design studies.[18,20,39–41,46,47] Like most trials on extracorporeal therapies, none of the studies was double-blinded. The analyzed studies were of suboptimal quality, which may have exaggerated the magnitude of these effects. These putative benefits remain to be determined definitively in a prospective trial with appropriate clinical end points.

EUPHAS Randomized Controlled Trial

This study, titled "Early use of PMX-DHP in abdominal septic shock (EUPHAS)," was reported in the *Journal of the American Medical Association (JAMA)*[49] in 2009. Its trial registration is clinicaltrials.gov identifier: NCT00629382. This study was a multicenter prospective RCT of the PMX device in patients with severe sepsis or septic shock. Ten Italian ICUs were involved in the study. The hypothesis was that PMX-DHP would be associated with better patient outcomes, such as improved survival, better hemodynamic and oxygenation status, and mitigation of organ dysfunction.

The EUPHAS study was conducted between December 2004 and December 2007. Patients were eligible for enrollment if they had severe sepsis or septic shock caused by an intra-abdominal cavity infection requiring emergency abdominal surgery. Eligible patients were randomly assigned within 6 h of surgery to treatment with either conventional medical therapy alone, according to the Surviving Sepsis Campaign[50] guidelines (conventional therapy group), or direct hemoperfusion therapy with polymyxin B in addition to standard therapy (PMX-DHP group). Hemoperfusion with polymyxin B was performed for 2 h, within 24 h after abdominal surgery, followed by a second PMX-DHP treatment 24 h after the end of the first treatment.

The severity of organ dysfunction or failure was expressed using the SOFA score.[51] The dose of vasoactive/vasopressor agents was expressed as the inotropic score.[52,53] This score has also been referred to as the vasopressor score[54] or catecholamine index.[55] The primary end points were changes from baseline to 72 h in MAP and vasopressor requirement. The secondary end points included the PaO_2/FIO_2 ratio, change in organ dysfunction (measured by delta-SOFA scores), and 28-day mortality. The time in ICU, hospital stay, and all-cause hospital mortality were also reported.

There were 64 patients: 34 in the PMX-DHP group and 30 in the conventional therapy group. Abdominal surgery was performed for bowel perforation ($n = 41$), intestinal occlusion/resection ($n = 13$), complicated cholecystitis ($n = 7$), intra-abdominal abscess ($n = 2$), and peritonitis not otherwise specified ($n = 1$). At 72 h, the MAP had increased significantly from 76 mmHg at the baseline to 84 mmHg ($P = 0.001$), while the inotropic score had decreased significantly from 29.9 at the baseline to 6.8 in the PMX-DHP group ($P < 0.001$). This was not observed in the conventional therapy group. A borderline significant improvement in the PaO_2/FIO_2 ratio was also observed in the PMX-DHP group, from 235 at the baseline to 264 at 72 h ($P = 0.049$). The PaO_2/FIO_2 ratio remained unchanged in the conventional therapy group. At 72 h, the PMX-DHP group had a greater reduction than the conventional therapy group in terms the total SOFA (mean delta-SOFA score, –3.4 vs –0.1; $P < 0.001$), cardiovascular SOFA (mean delta-SOFA score, –1.7 vs –0.7; $P = 0.04$), and renal SOFA (mean delta-SOFA score, –0.3 vs 0.6; $P = 0.01$). The 28-day crude mortality was 32% (11/34 patients) in the PMX-DHP group and 53% (16/30 patients) in the conventional therapy group. Likewise, there was no significant difference in the mean stay in ICU or in hospital.

In this RCT of surgical patients with septic shock and severe sepsis induced by abdominal sepsis, PMX-DHP therapy proved effective for improving 28-day and hospital survival, blood pressure, vasopressor requirements, and degree of organ failure, as indicated by the delta-SOFA score, when added to conventional medical treatment. These findings are in agreement with those of other studies in diverse populations, as summarized in a recent meta-analysis.[38] This preliminary RCT demonstrates that PMX-DHP, when added to conventional medical therapy, was effective in improving clinical outcomes in a targeted population with severe sepsis and septic shock caused by intra-abdominal infections. Larger multicenter studies are needed to confirm these encouraging findings in other patient populations.

Reviews of the EUPHAS Study

The EUPHAS study proved the superiority of PMX-DHP therapy over conventional therapy; however, there were several problems with this study. First, although one of its strengths was its highly targeted patient population, this contributed to slow patient accrual; second, owing to the nature of the study intervention, it was not feasible for physicians to blindly treat patients in the allocation groups; and third, the trial was stopped early based on the results of the interim analysis, following accepted standards for stopping. Initially, a sample size of 120 patients (60 in each group) was calculated to detect an absolute difference of 5 mmHg in delta-MAP between the PMX-DHP group and the conventional therapy group, with a power of 80% and two-sided $\alpha = 0.05$. However, only 64 patients (34 in the PMX-DHP group and 30 in the conventional therapy group) were entered in the study, because the analysis triggered the stopping rule, due to a significant reduction in mortality in the PMX-DHP group. The results were discussed with the president of the ethics committee (Internal Institutional Ethical Commission of the coordinating center), who declared it unethical to deprive a potentially beneficial therapy to a group of patients at a high risk of mortality. Thus, the study was terminated based on "early termination for benefit." Moreover, early termination itself tends to cause over-

estimation of the true effect for the factor used in the stopping rule; in this case, mortality.

From these findings, well-grounded arguments were presented in the *JAMA* in 2009. Vincent[56] stated the following:

> In their randomized controlled trial, Dr Cruz and colleagues[49] reported that polymyxin B hemoperfusion reduced 28-day mortality in patients with severe sepsis or septic shock. The statistically significant difference in mortality rates reported in this study was based on the analysis of a hazard ratio generated by a Cox proportional hazards regression survival model. However, the odds ratio (OR) of the crude 28-day mortality rates did not reach statistical significance (11/34 vs 16/30; OR, 0.42; 95% confidence interval [CI], 0.13–1.29; $P = 0.13$). This observation concerns an unblinded trial, in which investigators could forgo life support later in patients in the treatment group, creating a false appearance of improved survival in that group early in the study. Although the study by Cruz et al. remains interesting because it confirmed the hemodynamic improvements seen with this approach,[36,38] it is unfortunate that it was interrupted so early, in the absence of statistically significant differences in mortality, thus leaving unanswered the important question of whether this therapeutic approach can improve outcomes in patients with severe sepsis.

Amaral,[57] of the Department of Critical Care Medicine, Sunnybrook Health Sciences Centre, Toronto, Ontario, Canada, stated the following:

> In their randomized controlled trial, Dr Cruz and colleagues[49] assessed the early use of polymyxin B hemoperfusion in abdominal sepsis. There did not appear to be a statistically significant difference in 28-day or hospital mortality in this study ($P = 0.13$ by Fisher exact test). Therefore, the correct interpretation of the results would be that polymyxin B hemoperfusion prolongs time to death without a statistically significant effect on 28-day or hospital mortality. Survival analysis may offer increased statistical power over the more correct comparison of 28-day, 60-day, or hospital mortality and may be a useful pilot trial end point but should not be confused with clinical efficacy data and should not be used to stop trials early. In addition, this study lacked a direct comparison of primary outcomes. A statistically significant change only in treated patients was reported, but with no actual between-group comparison, which was the relevant research question.

In response to these comments Antonelli,[58] co-author of the EUPHAS study, wrote:

> For 28-day mortality, the OR was 0.42 (95% CI, 0.15–1.15; $P = 0.13$) in a Fisher exact test. For hospital mortality, however, the OR was 0.35 (95% CI, 0.13–0.97; $P = 0.049$), consistent with a statistically significant decrease in risk. For between-group comparison of the primary end point, namely, change in mean arterial pressure from baseline to 72 hours, there was a mean increase of 8 mmHg in the polymyxin B group and 2 mmHg in the conventional group ($P = 0.15$). The change in inotropic score was –23.4 in the polymyxin B group compared with –3.4 in the conventional group ($P = 0.009$). This indicates a significantly greater reduction in vasopressor use in the polymyxin B group without compromising blood pressure.

Planned Prospective Randomized Control Trials for PMX-DHP in Europe and the United States

ABDO-MIX Study

This study is a multicenter prospective RCT of 20 ICUs in France. The study population consists of patients with septic shock arising from the abdominal cavity. The sample size is 220 patients (110 in each group) and the primary end point is 28-day mortality. The chief investigator is Dr Payen of the Lariboisière University Hospital, Paris, France. This study commenced at the end of 2010.

EUPHRATES Study

The official title of this study is "Evaluating the Use of Polymyxin B Hemoperfusion in a Randomized Controlled Trial of Adults Treated for Endotoxemia and Septic Shock (EUPHRATES)." This study is presented on the website of the US National Institutes of Health and the clinical trials.gov identifier is NCT01046669. This study is a multicenter prospective randomized control trial in the United States. The study population consists of patients with septic shock arising from not only the abdominal cavity but also other organs (e.g., the respiratory organs or the urinary tract). The sample size is 360 patients (180 in each group), and the primary end point is 28-day mortality and secondary end points are 90-day, 6-month, and 12-month mortalities after the initiation of treatment. The inclusion criteria are hypotension requiring vasopressor support, and that each subject must have received intravenous fluid resuscitation, with documented or suspected infection, and

evidence of at least one new-onset organ dysfunction. The Endotoxin Activity Assay ≥0.6 EAA units is another important feature. The chief investigator is Dr Dellinger of the Cooper University Hospital, NJ, USA. This study started at the end of 2010.

Conclusions

The polymyxin B immobilized fiber column (Toraymyxin) is sent from Japan to countries all over the world. It has been adopted not only by European countries such as Italy, but also by the United States, Canada, and some Asian countries, based on solid evidence of its usefulness described in many articles. However, the studies we reviewed in this article have some issues with regard to 28-day mortality. Two prospective randomized control trials, the primary end points of which are 28-day mortality, are under way in Europe and the United States, and we earnestly hope that these RCTs resolve the issue.

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