



Anti-endotoxin Properties of Polymyxin B-immobilized Fibers

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Tohru Tani, Tomoharu Shimizu, Masaji Tani, Hisataka Shoji, and Yoshihiro Endo

Abstract

Polymyxin B is an antibiotic that shows strong bactericidal activity against Gram-negative bacteria, by binding to and inactivating endotoxin. Systemic administration of polymyxin B in humans is restricted because of its nephrotoxicity and neurotoxicity, and this compound was therefore considered a strong candidate ligand for the extracorporeal selective adsorption of circulating endotoxin in the blood. Toraymyxin® is a direct hemoperfusion column that uses polymyxin B attached to an insoluble carrier to bind endotoxin in the blood. In 1994, the Japanese National Health Insurance system approved the use of Toraymyxin for the treatment of endotoxemia and septic shock.

In this chapter, we will review the development, clinical use, and efficacy of Toraymyxin, examine the structure of the Toraymyxin column, and comment on the current position of Toraymyxin in the treatment of severe sepsis and septic shock. We will also highlight some potential new applications of Toraymyxin for pulmonary diseases.

Keywords

Polymyxin · Lipopolysaccharide · Endotoxemia · Septic shock · Toraymyxin

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19.1 Introduction

Endotoxin is one of major constituents of the cell wall in Gram-negative bacteria. Endotoxin is recognized through toll-like receptor-4 as a pathogen-associated molecular component that activates macrophages and other leukocytes to produce various inflammatory mediators. Endotoxin plays a major role in the development of toxic symptoms in Gram-negative bacterial infections. A recent review showed that co-detection of Gram-negative bacteria and endotoxemia is strongly predictive of increased risk of mortality compared to detection of neither [1]. It is therefore a reasonable therapeutic approach to evaluate and remove circulating endotoxin in

the blood of patients with Gram-negative bacterial infections.

Polymyxin B is a polycationic antibiotic that binds to and inactivates endotoxin, thereby neutralizing endotoxin-associated toxicity in humans [2]. Systemic administration of polymyxin B in humans is restricted because of its nephrotoxicity and neurotoxicity, and this compound was considered a strong candidate ligand for the extracorporeal selective adsorption of circulating endotoxin in the blood.

The selective removal of endotoxin from blood has been discussed since the 1970s, when Nolan and Ali first demonstrated the adsorption of endotoxin via the ion-exchange resin cholestyramine [3]. Various non-selective adsorbents such as charcoal or ion-exchange resins have since been considered, but a new selective adsorbent was needed for the specific and efficient removal of circulating endotoxin from blood. Sepharose 4B beads to which polymyxin B was

covalently immobilized were shown to be effective adsorbents for the removal of endotoxin in a rat model and in human hemodialysis systems [4, 5]. Polyethylenimine [6] and albumin [7], rather than polymyxin B, have also been used as ligands for the selective adsorption of endotoxin.

Initial research into polymyxin B-immobilized fibers began in 1981 at the Department of Surgery, Shiga University of Medical Science, Japan, as a collaboration between our research group and Toray Medical Co., Ltd. (Chiba, Japan) (Fig. 19.1). In 1982, the preliminary results of this research were published, demonstrating that polymyxin B-immobilized fibers reduce endotoxin activity in saline solution, as measured using a semi-quantitative limulus amoebocyte lysate (LAL) assay.

In 1983, we began *in vitro* and *in vivo* experimental studies. A pre-clinical study showed that selective removal of endotoxin from the blood by extracorporeal direct hemoperfusion with

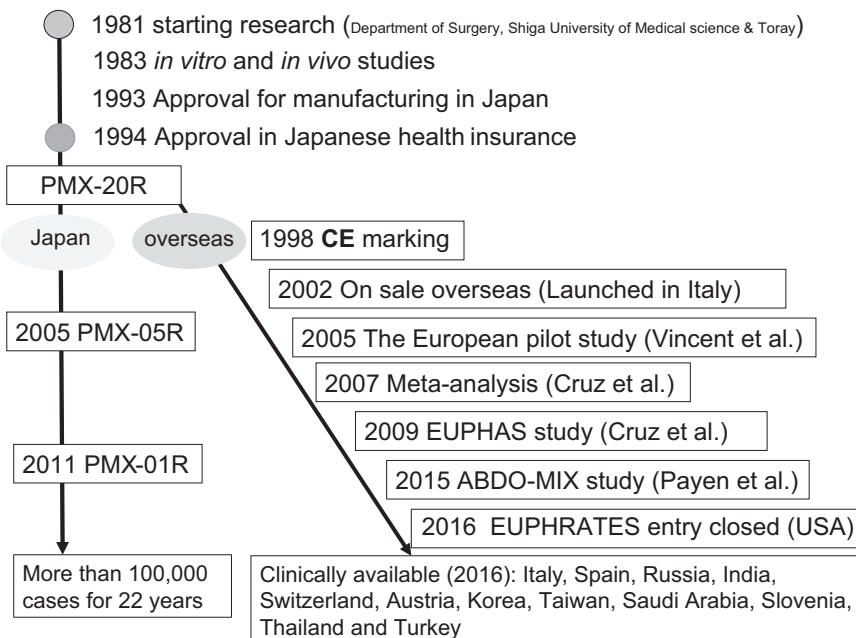


Fig. 19.1 History of the development of Toraymyxin
 EUPHAS: Early use of polymyxin B hemoperfusion in abdominal sepsis
 ABDO-MIX trial: Effects of hemoperfusion with a polymyxin B membrane in peritonitis with septic shock

EUPHRATES trial: Evaluating the use of polymyxin B hemoperfusion in a randomized controlled trial of adults treated for endotoxemia and septic shock

polymyxin B-immobilized fibers resulted in improved survival in an endotoxemia canine model [8]. Survival also improved in a Gram-negative bacterium (*Escherichia coli*) intravenous administration canine model after extracorporeal hemoperfusion with polymyxin B-immobilized fibers, indicating the potential for use thereof in humans [9].

The polymyxin B-immobilized fibers blood purification column, Toraymyxin[®], was developed as a medical device to be used in conjunction with direct hemoperfusion to remove circulating endotoxin from the blood in humans. A phase I clinical study was initiated in 1989, and in 1994 the first clinical report of 16 patients with septic multiple organ failure treated with direct hemoperfusion with Toraymyxin (PMX) was published [10]. PMX for 2 h significantly decreased the level of circulating endotoxin from 76 to 21 pg/mL. Furthermore, the patients' hyperdynamic state (in terms of cardiac index), which is characteristic of endotoxic shock, returned to normal after treatment [10]. Regarding the technical term for Toraymyxin, we have suggested defining "PMX" as "direct hemoperfusion with the polymyxin B-immobilized fiber column (Toraymyxin)".

In 1994, Toraymyxin was adopted by the Japanese National Health Insurance system for the treatment of endotoxemia and septic shock [11]. Since then, Toraymyxin has been used safely in more than 100,000 cases in emergency and intensive care units in Japan. Three different columns are currently available for clinical use in Japan: PMX-20R for use in adults, PMX-05R for use in children, and PMX-01R for use in babies (Fig. 19.1).

In 1998, Toraymyxin received CE mark approval in Europe. In 2005, the results of the first randomized controlled trial (RCT) in Europe were published, showing that treatment with Toraymyxin is safe, and improves cardiac and renal dysfunction due to sepsis or septic shock [12]. In 2007, the findings of a meta-analysis demonstrated the favorable effects of PMX [13]. In 2009, the results of the second RCT in Europe, the EUPHAS study (Early Use of Polymyxin B Hemoperfusion in Abdominal Sepsis), which was conducted in Italy, were pub-

lished, showing that Toraymyxin treatment results in a significant reduction in sepsis-associated mortality [14]. A large RCT, the ABDO-MIX trial in France, has failed to show survival benefit and improvement in organ failure with Toraymyxin treatment compared to conventional treatment of peritonitis-induced septic shock [15]. Recruitment into another large RCT, the EUPHRATES trial in the USA, is now closed and the results showed that polymyxin B hemoperfusion plus conventional medical therapy did not reduce mortality at 28 days in patients with septic shock and high endotoxin activity (Fig. 19.1, ClinicalTrials.gov Identifier: NCT01046669). Toraymyxin is currently available for use in clinical settings in Italy, Spain, Russia, India, Switzerland, Austria, Korea, Taiwan, Saudi Arabia, Slovenia, Thailand, and Turkey.

In this chapter, we will review the development, clinical use, and efficacy of Toraymyxin, examine the structure of the Toraymyxin column, and comment on the current position of Toraymyxin in the treatment of severe sepsis and septic shock. We will also highlight some potential new applications for Toraymyxin.

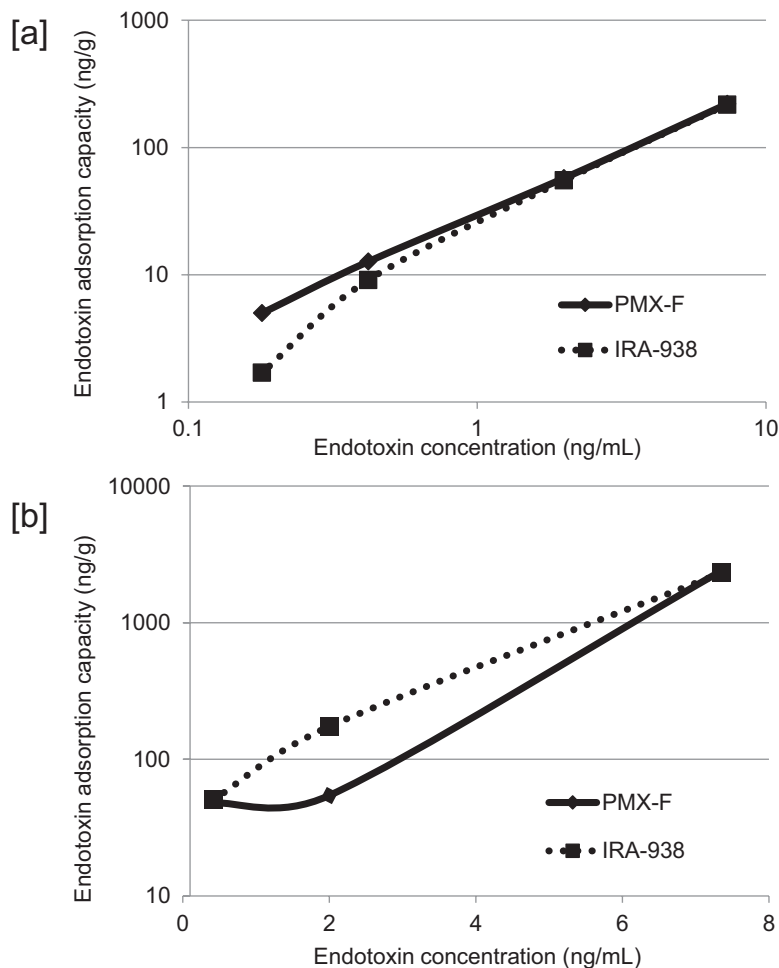
19.2 Development of Polymyxin B-immobilized Fibers

19.2.1 Polymyxin B and Endotoxin

Polymyxin B has strong bactericidal activity against Gram-negative bacteria. The bactericidal properties of polymyxin B are discussed in more detail in other chapters. Polymyxin B also neutralizes the lethal toxicity, limulus gelation activity, and hemodynamic effects of endotoxin, a major component of the outer membrane of Gram-negative bacteria, by binding to the lipid A domain, which is the active center of the endotoxin molecule [16]. The protective effects of polymyxin B against endotoxemia and septicemia have been demonstrated in various animal models [17–19].

Electron microscopy imaging has revealed that when endotoxin is exposed to polymyxin B, its usual ribbon-like structure partially or fully disaggregates [20]. The detergent-like activity of polymyxin B arises from its ability to prompt the

Fig. 19.3 Endotoxin adsorption capacity of polymyxin B-immobilized fiber or IRA-938 data from Shoji et al. [24]. Two grams of polymyxin B-immobilized fibers (polymyxin B-immobilized fibers, solid line) or ion-exchange resin (IRA-938, dotted line) were incubated with 30 mL of various concentrations of endotoxin in (a) aqueous solution or (b) bovine serum solution



(Fig. 19.4a) [24]. In an *in vivo* canine experiment, the survival rate of endotoxin-challenged dogs was higher when the immobilized polymyxin B had a large number of residual primary amino groups (Fig. 19.4b) [24]. These results suggested that polymyxin B-immobilized fibers had a maximal endotoxin detoxification capacity when three primary amino groups were left unbound from the carrier fiber. Furthermore, concentrations of immobilized polymyxin B above 3.5 $\mu\text{g}/\text{mg}$ of fiber were suggested to be optimal. Because the primary amino groups are positively charged, it is likely that they play a major role in the ionic binding of polymyxin B to endotoxin [24].

In addition to detoxifying the limulus activity of endotoxin, polymyxin B-immobilized fibers have also been shown to neutralize the pyrogenic activity of endotoxin solution in a rabbit pyrogen

test. Therefore, polymyxin B-immobilized fibers are also effective in removing pyrogenic agents from serum (Fig. 19.5) [24].

19.2.3 Endotoxin Adsorption Capacity and Bactericidal Activity of Polymyxin B-immobilized Fibers *In Vitro*

When polymyxin B-immobilized fibers, or carrier fibers alone, were incubated with synthetic lipid A *in vitro*, polymyxin B-immobilized fibers effectively adsorbed synthetic lipid A, whereas the carrier fibers alone did not, as assessed using an LAL assay, showing that the capability of polymyxin B to bind to lipid A is retained even when polymyxin B is immobilized (Fig. 19.6a)

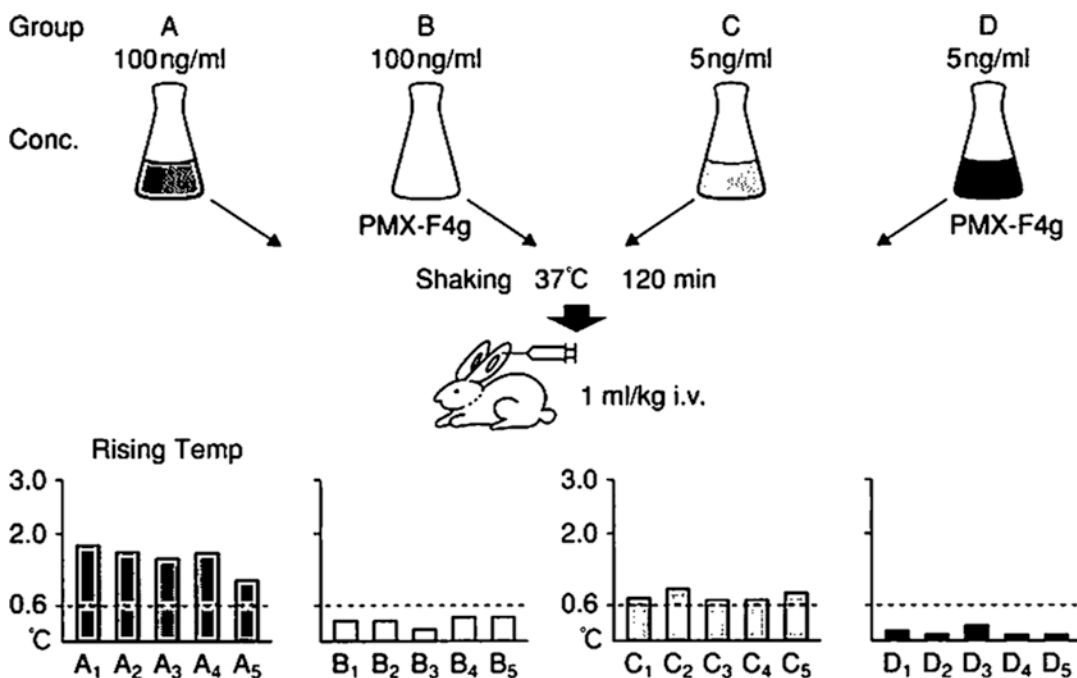


Fig. 19.5 Endotoxin removal by polymyxin B-immobilized fibers examined in a rabbit pyrogen test. Polymyxin B-immobilized fibers (PMX-F) were incubated with various concentrations of endotoxin in an

aqueous solution at 37 °C for 120 min, and then intravenously administered to rabbits. Treatment with polymyxin B-immobilized fibers suppressed the elevation of body temperature

toxin binding and bactericidal properties of free polymyxin B [24].

19.2.4 Endotoxin Adsorption Capacity of Polymyxin B-immobilized Fibers *In Vivo*

Direct hemoperfusion under heparin infusion for 2 h with an animal-use polymyxin B-immobilized fiber column improved mortality and hemodynamic parameters in endotoxemic dogs receiving intravenous endotoxin administration, compared with carrier fiber alone, charcoal, and ion-exchange resin (Fig. 19.7) [25]. Direct hemoperfusion with an animal-use polymyxin B-immobilized fiber column has also been shown to improve hemodynamic parameters in dogs with sepsis induced by an intravenous infusion of *E. coli*. Blood lactate levels were improved in dogs treated with polymyxin B-immobilized fibers compared with non-treated dogs; however,

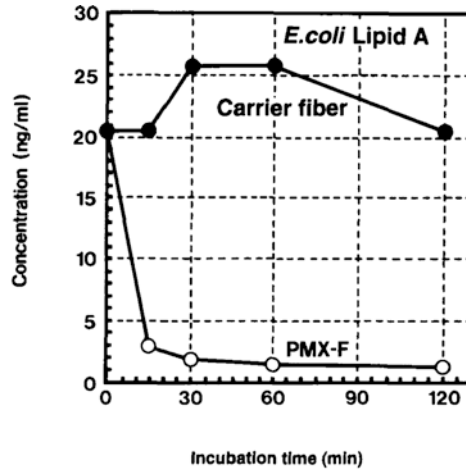
platelet counts were significantly lower, which is an adverse effect of direct hemoperfusion with a polymyxin B-immobilized fiber column for animal use (Fig. 19.8) [9].

19.3 Toraymyxin, a Polymyxin B-immobilized Fiber Column for Human Use

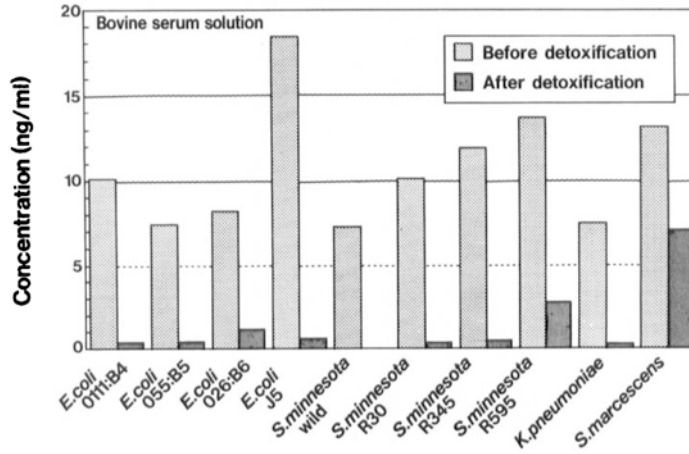
19.3.1 Physical Structure of Toraymyxin Cartridge

The Toraymyxin cartridge comprises a plastic case containing a knitted roll of polymyxin B-immobilized fiber fabric for human use. The Toraymyxin cartridge is sterilized via autoclave and filled with physiological saline. The use of a thin, fibrous carrier produces a hemoadsorption cartridge with a large surface area that does not cause a large pressure drop in the blood flow compartment (Fig. 19.2). Flow through the

[a]



[b]



[c]

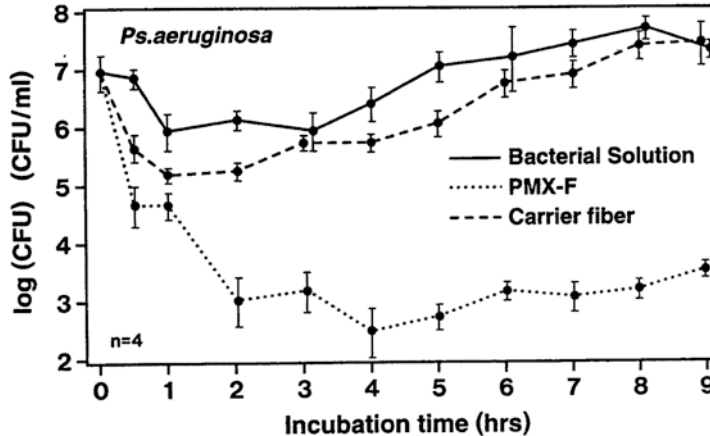


Fig. 19.6 The endotoxin adsorption ability and bactericidal activity of polymyxin B-immobilized fibers. (Reproduced from Shoji et al. [24])

(a) Alteration in lipid A concentration by polymyxin B-immobilized fibers

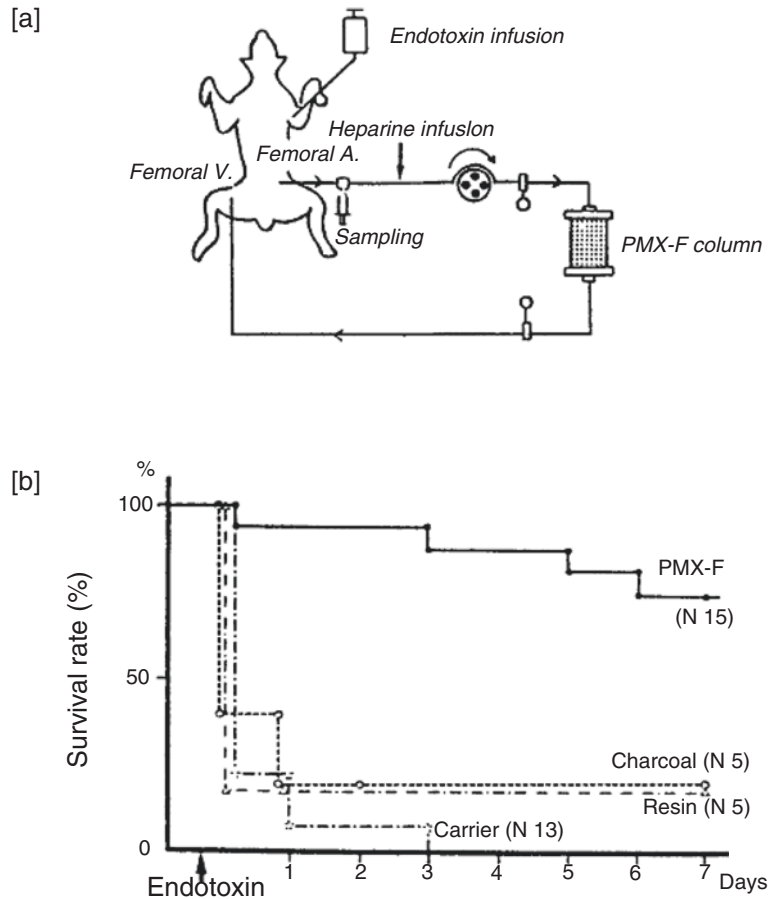
Polymyxin B-immobilized fibers (PMX-F) were incubated with a bovine serum solution containing 20 ng/mL of synthetic *Escherichia coli* lipid A with continuous stirring. Lipid A concentration was measured via limulus amoebocyte lysate assay

(b) Detoxification of endotoxin extracted from different species or strains of Gram-negative bacteria by use of polymyxin B-immobilized fibers

(c) Bactericidal activity of polymyxin B-immobilized fibers against *Pseudomonas aeruginosa*

Polymyxin B-immobilized fibers (PMX-F) or carrier fibers alone were incubated with a solution of *Pseudomonas aeruginosa* for 9 h and residual bacterial numbers were assessed

Fig. 19.7 Endotoxin adsorption by polymyxin B-immobilized fibers in an endotoxemia dog model. (Reproduced from Kodama et al. [25]) (a) Schematic of the experimental model: endotoxin was infused intravenously to dogs. Extracorporeal direct-hemoperfusion with a polymyxin B-immobilized fiber (PMX-F) column or control column was initiated (b) Survival rates after PMX-F treatment were compared with those after treatment with a PMX-F column, or a charcoal, resin, or carrier fiber column



Toraymyxin column is unidirectional and moves radially from the center to the outside of the roll of polymyxin B-immobilized fiber fabric, which improves adsorption capacity by ensuring a homogeneous distribution of blood within the column (Fig. 19.9a). Therefore, direct hemoperfusion can be performed in the clinical setting without the need for a complicated extracorporeal blood perfusion circuit.

The endotoxin adsorption capacity of the Toraymyxin column was evaluated in a bovine serum perfusion model. An endotoxin-containing bovine serum solution (10 ng/mL of endotoxin, total 1.5 L) was perfused through the Toraymyxin cartridge at a flow rate of 100 mL/min, and the change in endotoxin level was monitored using an LAL assay. Endotoxin concentration reached equilibrium after 2 h of perfusion (Fig. 19.9b) [11].

19.3.2 Toraymyxin as a Medical Device

There are three types of Toraymyxin columns currently available for clinical use in Japan: PMX-20R® (volume: 135 mL), PMX-05R® (40 mL), and PMX-01R® (8 mL) (Fig. 19.10a). The first Toraymyxin column released was PMX-20R, which was developed for the treatment of adults with septic shock [11]. The second column released was PMX-05R, which was developed for use in pediatric or elderly patients with smaller circulating blood volumes or a body weight less than 40 kg [26]. Several reports in Japanese literature have shown acceptable results by using PMX-05R in pediatric or elderly patients with sepsis. In 2011, PMX-01R was released for use in newborn or premature babies, and there are case reports describing the clinical use of PMX-

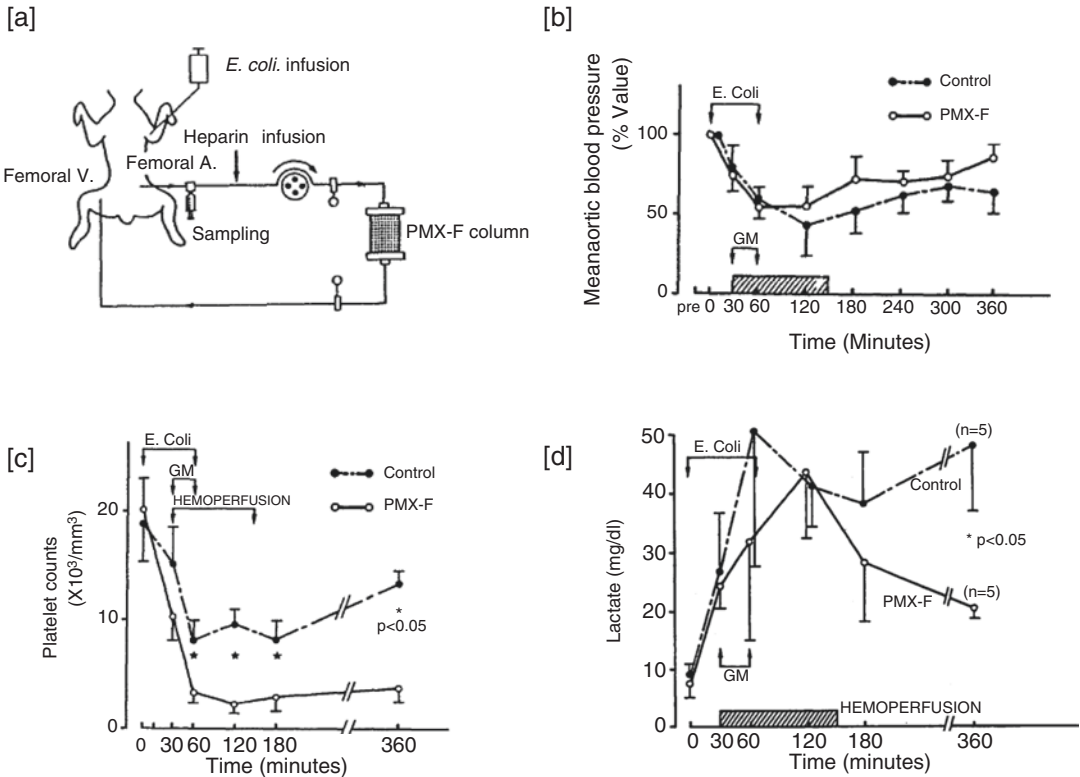


Fig. 19.8 Efficacy of polymyxin B-immobilized fibers in a dog bacterial infusion model. (Reproduced from Hanasawa et al. [9])

(a) Schematic of the experimental model: the solution containing *Escherichia coli* was infused intravenously to dogs. Extracorporeal direct-hemoperfusion with a polymyxin B-immobilized fiber (PMX-F) column or control column was initiated

(b) Alteration in mean aortic blood pressure: mean aortic blood pressure after PMX-F treatment increased compared to that after treatment with the control column

(c) Alteration in platelet counts: platelet count significantly decreased following PMX-F treatment compared that following treatment with the control column

(d) Alteration in blood lactate levels: blood lactate levels significantly decreased following PMX-F treatment compared to those following treatment with the control column

01R in patients with a body weight less than 1000 g with severe sepsis [27, 28].

19.3.3 Use of the Toraymyxin Column in Hemoperfusion

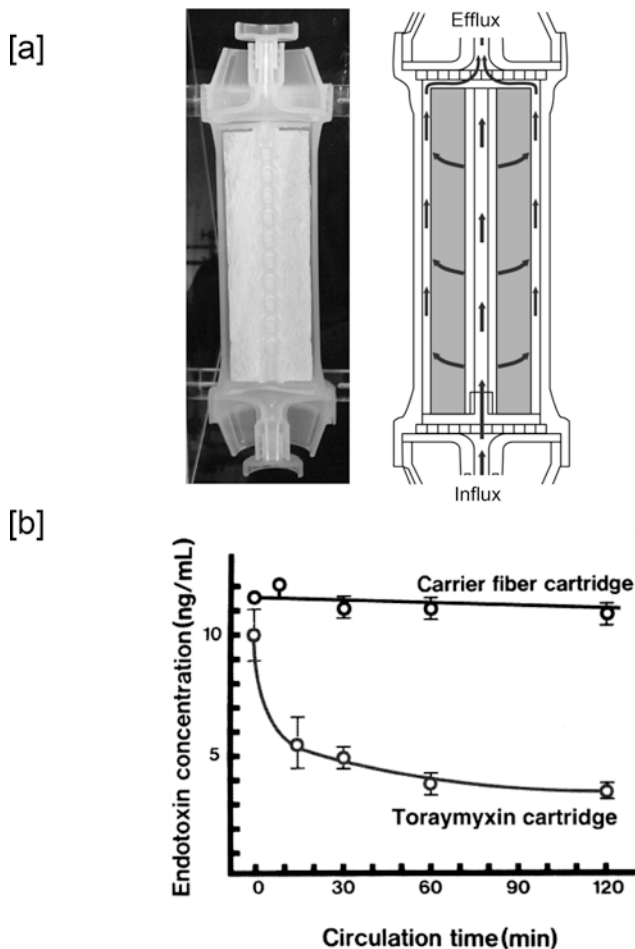
Prior to use, Toraymyxin columns must be rinsed with physiological saline and then primed. For example, a PMX-20R column must be washed out with 4 L of physiological saline and then primed with 1 L of physiological saline containing 20 mg of nafamostat mesilate or 1000 U of unfractionated heparin. For patients in an extremely critical condition or with an extremely low blood pressure, an albumin solution contain-

ing anticoagulation agents may be used as the final priming solution.

Vascular access for hemoperfusion is usually via a central vein (internal cervical vein, subclavian vein, or femoral vein). The standard blood flow rate through the Toraymyxin column depends on the size of the column: 80–120 mL/min for PMX-20R, 20–40 mL/min for PMX-05R, and 8–12 mL/min for PMX-01R. An anticoagulant (20–30 mg/h nafamostat mesilate or bolus 40–60 U/kg and 40–60 U/kg/h unfractionated heparin, depends on patients' coagulation condition) should also be administered to prevent coagulation within the blood circuit (Fig. 19.10b). Since coagulation dysfunction is common in patients with septic shock, the short half-lives of

Fig. 19.9 The inside structure and endotoxin adsorption capacity of the Toraymyxin cartridge

(a) Structure of a Toraymyxin cartridge and schematic of the blood flow inside the adsorption cartridge. (Provided by Toray Medical Co., Ltd.)
 (b) Endotoxin adsorption capacity of the Toraymyxin cartridge compared with the carrier fiber cartridge alone. Bovine serum solution containing 10 ng/mL of endotoxin was circulated through the Toraymyxin and carrier fiber cartridges. (Reproduced from Shoji et al. [11])



these regional anticoagulants in the blood mean that they are safe for use during hemoperfusion. Nafamostat mesilate is the most commonly used anticoagulant for this purpose in Japan.

Since 1994, Toraymyxin has been used in more than 100,000 cases in Japan. Despite adverse effects such as thrombocytopenia or hypotension being reported, their incidence is rare, and there have been no reports of any serious adverse effects [11].

19.4 Mechanism of Action of PMX Therapy

19.4.1 Removal of Endotoxin

The Toraymyxin column was originally designed to specifically adsorb endotoxin. Plasma endo-

toxin levels significantly decrease immediately after PMX treatment compared with pre-treatment levels [10]. By using data from a multicenter study, Tani reported that PMX treatment significantly reduced plasma endotoxin concentrations from 83.7 ± 26.7 pg/mL to 56.4 ± 27.9 pg/mL after 2 h of direct hemoperfusion with a Toraymyxin column [29]. Although almost all of the studies conducted so far have demonstrated a significant decrease in plasma endotoxin levels following PMX treatment, a recent RCT failed to show such an effect [12]. However, this could be due to the use of the LAL assay, which is prone to contamination and lacks precision and accuracy resulting in both false positive and false negative results [30]. Indeed, our previous study showed that sensitivity for the detection of endotoxin in the turbidimetric LAL assay was very low (26.9%; 14 of 52 patients) in patients with severe sepsis and sep-

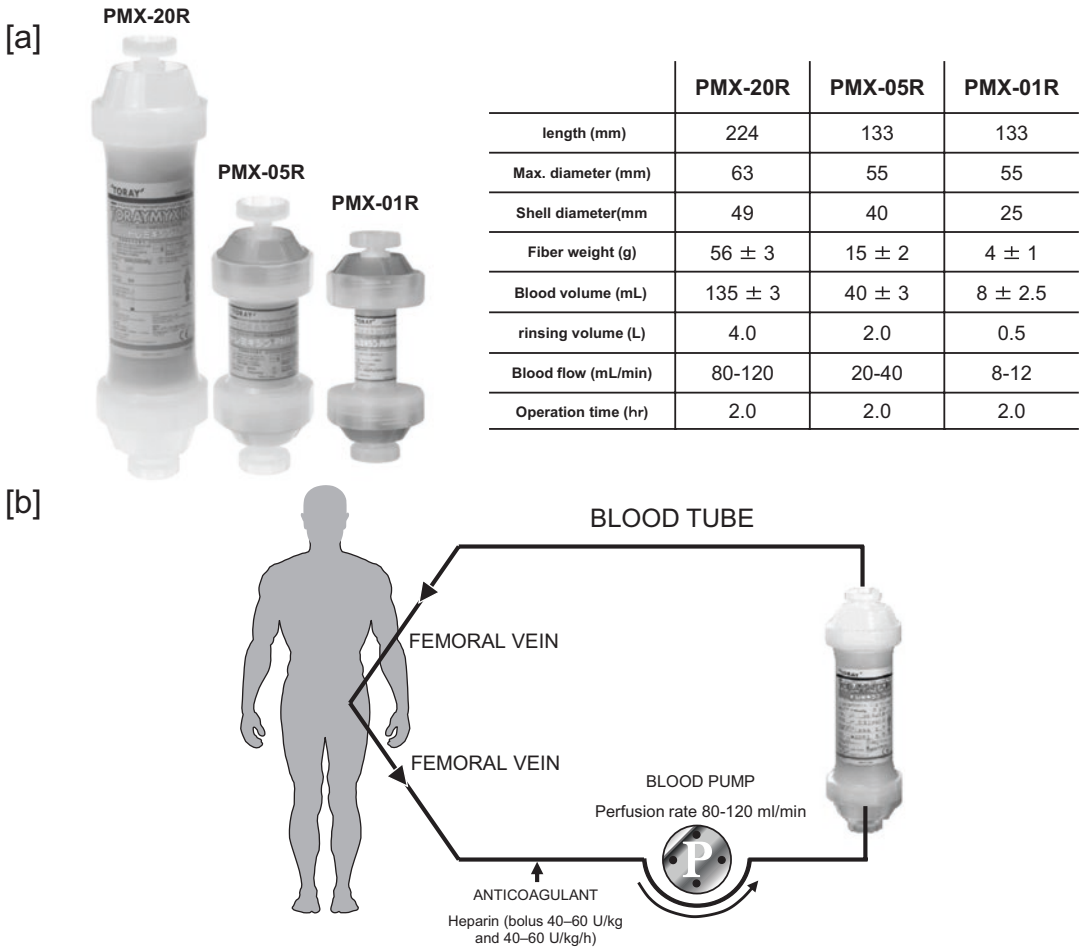


Fig. 19.10 The specifications of the Toraymyxin cartridge
 (a) Overview of the three Toraymyxin cartridges currently available for clinical use. (Provided by Toray Medical Co., Ltd.)

(b) Schematic of direct hemoperfusion with a Toraymyxin cartridge. (Provided by Toray Medical Co., Ltd.)

tic shock who required PMX treatment [31]. A new method for the detection and quantitation of endotoxin is therefore immediately needed.

Recently, Romaschin developed a rapid assay, called the endotoxin activity assay (EAA), that detects endotoxin in whole blood by using autologous neutrophil-dependent chemiluminescence [32]. The EEA has been approved by the United States Food and Drug Administration as a clinical method for the diagnosis of endotoxemia, and was used in the EUPHRATES trial (see Sect. 19.5.1) [33].

A rapid LAL assay for detecting endotoxin by using a laser light-scattering particle-counting method called endotoxin scattering photometry (ESP) has also been developed. Endotoxin binds to and activates factor C, a clotting enzyme, early in the LAL cascade, which ultimately results in the production of the insoluble protein coagulin. Coagulin spontaneously forms a polymer, and it is this polymer formation that is detected in the turbidimetric LAL assay. Obata et al. reported that by using ESP they were able to detect coagulin particles formed under agitation of the LAL cascade, and that the transferring period of the

LAL cascade after endotoxin stimulation depends on the concentration of endotoxin before gelation [34]. The ESP method allows for more rapid and sensitive detection of endotoxin compared with the turbidimetric LAL method.

Furthermore, ESP is able to discriminate between patients with sepsis and those with septic shock undergoing gastrointestinal emergency surgery compared with the standard turbidimetric LAL assay [35]. After undergoing PMX therapy for a longer-duration (> 2 h), the hemodynamic condition of patients with septic shock due to intra-abdominal infection improved, and the reduction in plasma endotoxin concentration could be detected via ESP but not through the standard turbidimetric method. We have demonstrated the ability of endotoxin adsorption in longer-duration PMX treatment [36]. Using the ESP method, we observed a reduction in endotoxin after passing through the Toraymyxin column even when PMX duration was greater than 2 h. Therefore, ESP appears to be sensitive enough to detect circulating endotoxin in the plasma of patients with septic shock who require PMX therapy.

19.4.2 Other Potential Mechanism of Action of PMX Therapy

Although Toraymyxin was originally developed for the adsorption of circulating endotoxin from the blood, recent studies have shown other potential applications. Jaber reported that it may be possible to use Toraymyxin to remove lipoteichoic acid (LTA) from peripheral blood [47]. In an *in vitro* study, polymyxin B significantly suppressed LTA-induced tumor necrosis factor (TNF)- α production by peripheral blood mononuclear cells, suggesting that LTA may also be removed during direct hemoperfusion with PMX and that this removal may be due to binding of LTA to polymyxin B.

Other recent studies have reported a possible relationship between immune cells and the reduction in inflammatory mediators. Ono demonstrated that the expression levels of HLA-DR, a major histocompatibility complex class II cell

surface receptor on monocytes, and CD16 on granulocytes, markedly decrease in patients with septic shock. However, PMX treatment had beneficial effects by increasing leukocyte expression of these surface antigens [37]. The number of CD16⁺CD14⁺ monocytes and the expression level of monocytic toll-like receptor 4 dramatically increase in patients with severe infection, and recent studies have shown that PMX treatment is effective in reducing both of these in patients with septic shock [38]. Moreover, Ono demonstrated that the removal of surplus circulating CD4⁺CD25⁺Foxp3⁺ regulatory T cells in patients with septic shock via hemoperfusion with Toraymyxin might represent a novel strategy for inducing recovery from sepsis-associated immunosuppression [39]. Tsuzuki have demonstrated immediate inhibition of NF- κ B binding activity and suppression of TNF- α secretion after endotoxin neutralization with polymyxin B regardless of whether the peripheral blood mononuclear cells were already producing TNF- α or not [40]. Reducing the number of circulating monocytes or modulating their function may contribute to improving pro-inflammatory responses following PMX treatment.

Anandamide and 2-arachidonyl glyceride are endogenous cannabinoids that are released by activated macrophages and platelets during endotoxic shock, and play a crucial role in the induction of shock-related hypotension [41]. Wang showed that anandamide was efficiently adsorbed by a polymyxin B-immobilized bead column *in vitro* [42]. Therefore, the adsorption of cannabinoids by polymyxin B may be an important mechanism for improving hemodynamic dysfunction following PMX treatment.

Macrophage migration inhibitory factor (MIF) is constitutively expressed by monocytes, macrophages, T cells, B cells, endocrine cells, and epithelial cells. Microbial toxins and cytokines are powerful inducers of MIF release by immune cells, and up-regulation of MIF expression during the course of inflammatory and infectious diseases plays an important role in the pathogenesis of sepsis and septic shock [43]. In a recent study, we demonstrated that Toraymyxin directly adsorbed MIF *in vitro* (unpublished data).

High-mobility group box 1 protein (HMGB1), which is a protein previously known only as a nuclear transcription factor, is now implicated as a mediator of delayed endotoxin lethality and systemic inflammation [44]. An experimental study demonstrated that serum levels of HMGB1 were lower in PMX-treated patients than in controls [45]. The reduction in circulating HMGB1 level may also contribute to the beneficial effects of PMX treatment in patients with sepsis [46–48].

Further studies are required to investigate the clinical efficacy of PMX treatment and elucidate the precise mechanism. Nevertheless, in Japan, PMX treatment is a widely accepted method to improve the condition of patients with septic shock, and this useful clinical device is gaining acceptance as its use becomes more widespread.

19.5 Clinical Use of PMX Therapy

19.5.1 PMX Therapy for the Treatment of Sepsis

The first clinical report from a phase I study of PMX therapy for the treatment of sepsis was published in 1994 [10]. Sixteen patients with septic multiple organ failure were treated with direct hemoperfusion with a Toraymyxin column over 2 h. PMX treatment significantly decreased the endotoxin level from 76 to 21 pg/mL (Fig. 19.11a). Furthermore, the patients' hyperdynamic state (in terms of cardiac index), which is characteristic of endotoxic shock, returned to normal after treatment (Fig. 19.11b) [10].

The results of the first prospective, multicenter, observational clinical trial of Toraymyxin for the treatment of patients with sepsis in Japan were published in 1998 [29]. The survival rate was significantly higher in the treatment (PMX) group (54.0%) compared with the control group who received standard therapies (36.4%). In the treatment group, the mean plasma endotoxin concentration was significantly lowered from 83.7 pg/mL before treatment to 56.4 pg/mL immediately after treatment. The day after treatment, the mean plasma endotoxin concentration

was found to be 28.5 pg/mL. Post treatment, the mean plasma endotoxin concentration was lower in those who survived (18.8 pg/mL) compared with those who died (88 pg/mL). Other cardiac function parameters also improved after treatment.

Nemoto demonstrated that the rate of overall survival in patients with sepsis significantly improved after PMX treatment compared with standard therapies without PMX (41% vs. 11%) in a RCT of 98 patients with sepsis in Japan [31]. PMX improved the rate of survival in patients with an APACHE II (Acute Physiology and Chronic Health Evaluation II) score <30, but not in those with scores >30, demonstrating that Toraymyxin treatment is most effective at improving patient outcome when applied during the early stages of sepsis [49].

In 1998, Toraymyxin received CE mark approval in Europe. In 2005, Vincent et al. published the results of a multicenter, open-label, pilot, randomized controlled study conducted in the intensive care units of six academic medical centers in Europe, which was the first RCT of Toraymyxin conducted outside of Japan [11]. Thirty-six postoperative patients with severe sepsis or septic shock secondary to intra-abdominal infection were randomized to either PMX treatment for 2 h ($n = 17$) or standard therapy ($n = 19$). PMX treatment was well tolerated and no significant adverse effects were observed. Patients treated with PMX showed significant increases in cardiac index ($P = 0.012$ and 0.032 at days 1 and 2, respectively), left ventricular stroke work index ($P = 0.015$ at day 2), and oxygen delivery index ($P = 0.007$ at day 2) compared with controls. Furthermore, the need for continuous renal replacement therapy after study entry was significantly reduced in the PMX group ($P = 0.043$). There were no statistically significant differences in endotoxin and interleukin six levels, and organ dysfunction as assessed by the Sequential Organ Failure Assessment (SOFA) score, or 28-day mortality. Note that sepsis in the patients enrolled in this study was likely too severe to find statistical significance in the mortality outcome. Together, these results show that PMX treatment

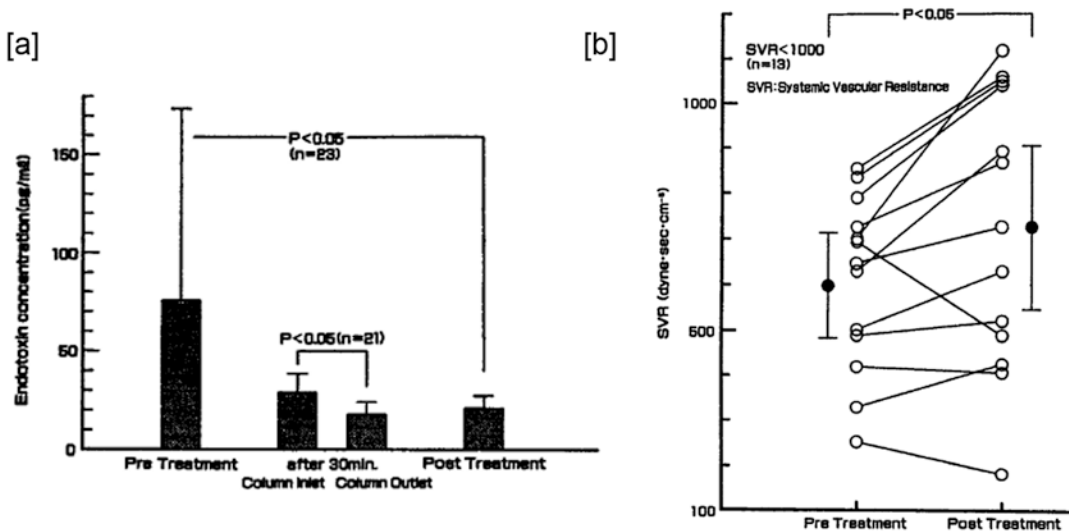


Fig. 19.11 Results from the first clinical trial of Toraymyxin in Japan

(a) Endotoxin concentrations before and after Toraymyxin treatment

(b) Systemic vascular resistance (SVR) before and after Toraymyxin treatment. Toraymyxin treatment decreased the concentration of endotoxin in the blood and improved hemodynamic status in patients with severe sepsis and septic shock. (Reproduced from Aoki et al. [10])

is safe and that it improves cardiac and renal dysfunction due to sepsis or septic shock.

In 2007, Cruz et al. published a systematic review of the effectiveness of Toraymyxin for the treatment of sepsis [12]. They included 28 publications that reported at least one of the specified outcome measures for PMX therapy. In this meta-analysis of 1425 patients (PMX therapy, 978 patients; conventional therapy, 447 patients), PMX therapy was associated with a significantly lower risk of mortality compared with conventional therapy (PMX, 33.5% vs. conventional treatment, 66.5%; risk ratio, 0.53; 95% CI, 0.43–0.65). A 33–80% reduction in plasma endotoxin levels compared to pre-treatment levels was also observed. The large decrease in mortality was associated with an improvement in hemodynamic condition: after PMX therapy, mean arterial pressure significantly increased by 19 mmHg (mean increase, 26%; range, 14–42%), and dopamine/dobutamine dose was decreased by 1.8 $\mu\text{g}/\text{kg}/\text{min}$. Improvement in pulmonary function was also demonstrated: the mean ratio of partial pressure arterial oxygen to fraction of inspired oxygen ($\text{PaO}_2/\text{FiO}_2$) increased by 32 units (95% CI, 23–41 units; $P < 0.001$) (Fig. 19.12).

Cruz et al. have also published the results of the EUPHAS (Early Use of Polymyxin B Hemoperfusion in Abdominal Sepsis) trial, which was a prospective, multicenter, RCT conducted at the intensive care units of ten Italian tertiary care hospitals in 2009 [14]. Sixty-four patients with severe sepsis or septic shock who had undergone emergency surgery for intra-abdominal infection were enrolled in this study. Patients were randomized within 6 h after open abdominal surgery to either conventional therapy ($n = 30$) or conventional therapy plus two sessions of 2 h PMX with an interval of 24 h between sessions ($n = 34$). Twenty-eight-day mortality was significantly improved in the PMX group (32%; 11/34 patients) compared with that in the conventional therapy group (53%; 16/30 patients). In the PMX group, mean arterial pressure significantly increased from 76 mmHg (before treatment) to 84 mmHg (72 h after treatment; $P = 0.001$) and vasopressor requirement (measured as inotropic score) significantly decreased from 29.9 (before treatment) to 6.8 (72 h after treatment; $P < 0.001$). In contrast, in the conventional therapy group, mean arterial pressure (before, 74 mmHg; 72 h after, 77 mmHg; $P = 0.37$) and inotropic score

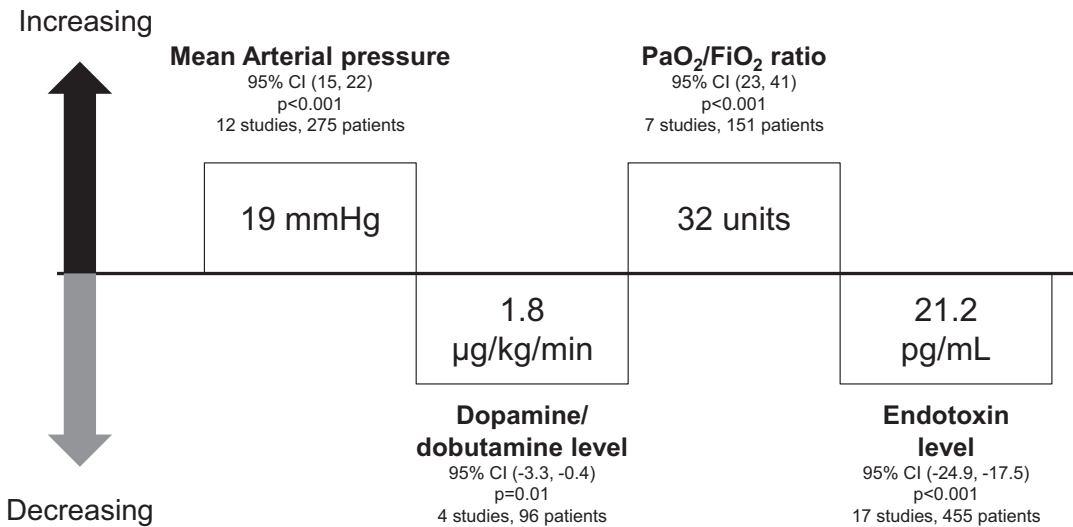


Fig. 19.12 Main results of a meta-analysis of Toraymyxin treatment. (Provided by Toray Medical Co., Ltd.) MAP mean arterial pressure, PaO₂/FIO₂ ratio of partial

pressure arterial oxygen and fraction of inspired oxygen, CI confidence interval

(before, 28.6; after, 22.4; $P = 0.37$) did not change significantly with treatment. Ratio of partial pressure arterial oxygen and fraction of inspired oxygen (PaO₂/FIO₂ ratio) significantly increased (before, 235; after, 264; $P = 0.049$) in the PMX group but not in the conventional therapy group (before, 217; after, 228; $P = 0.79$). Moreover, SOFA score, which is an indicator of the severity of organ dysfunction, improved in the PMX group compared with the conventional therapy group (change in SOFA score, -3.4 vs. -0.1; $P < 0.001$).

Although the goal was planned to enroll 120 patients in this study, the interim analysis revealed that the risk of mortality in the conventional treatment group was significantly higher than that in the PMX group; thus, the study was terminated midway. Despite the high mortality rate in the conventional treatment group and the lack of evaluation of circulating endotoxin levels, this was the first report showing an improved rate of survival in a RCT conducted outside of Japan.

A multicenter randomized controlled trial, The ABDOMIX trial (Effects of Hemoperfusion with a Polymyxin B Membrane in Peritonitis with Septic Shock), was conducted in France [15]. A total of 243 patients with peritonitis-

induced septic shock from abdominal infections were enrolled and the primary end point of the study was 28-day mortality. This multicenter randomized controlled study demonstrated a non-significant increase in mortality and no improvement in organ failure with PMX treatment compared to conventional treatment of peritonitis-induced septic shock. However, Antonelli et al. pointed out that major differences in mortality and completion rates of two scheduled sessions of PMX compared to the EUPHAS study may jeopardize their comparability, suggesting that any definitive conclusion be put on hold [50]. The 28-day mortality rate recorded in both groups was significantly lower than that reported in larger studies (between 32.7% and 53% for similar patient cohorts) [51–53]. Furthermore, only 81 of the 119 treated patients completed the two scheduled sessions of PMX. In the previously published EUPHAS study, all patients enrolled had completed the two planned sessions of PMX, and a significantly higher mortality rate was recorded in the control group [50].

The EUPHRATES trial (Evaluating the Use of Polymyxin B Hemoperfusion in a Randomized Controlled Trial of Adults Treated for Endotoxemia and Septic Shock), which is ongoing

ing in the USA and Canada [33], is a trial designed to address the criticisms of previous studies. Circulating endotoxin levels in patients with septic shock were evaluated by using a novel endotoxin detection method called the endotoxin activity assay (EAA). A total of 432 patients with septic shock and high EAA activity (> 0.6) were enrolled and randomized to either PMX or conventional treatment. Dellinger et al. reported that the primary endpoint for mortality rate (44.3% in placebo group and 43.75% in PMX group) was not met in the full intention-to-treat population. Their interim results showed that the mortality rate in the per protocol population (36.9% in placebo group and 31.9% in PMX group) was 5% in favor of PMX treatment ($P = 0.407$). Interestingly, the final results of the EUPHRATES trial did not show reduced mortality at 28 days in patients with PMX treatment (ClinicalTrials.gov Identifier: NCT01046669).

Direct hemoperfusion sessions with Toraymyxin column usually last 2 h; however, the obvious clinical efficacy of longer-duration PMX (> 6 h) in such cases has been reported [36, 54–56]. No adverse effects, such as thrombocytopenia, have been reported with longer duration Toraymyxin treatment [36, 54]. Further studies to clarify the suitability of longer-duration Toraymyxin treatment are expected.

The combination of PMX and continuous hemodiafiltration (CHDF) has been reported to be more beneficial for patients with septic renal dysfunction than CHDF alone. Combination treatment significantly decreases the concentration of circulating interleukin-6 and improves patient survival (Fig. 19.13a) [57]. Moreover, PMX followed by CHDF with a polymethyl methacrylate (PMMA) membrane significantly decreased the concentrations of plasminogen activator inhibitor-1, protein C, interleukin-6, and endogenous anandamide compared with CHDF with a polyacrylonitrile membrane in patients with septic shock [58]. Therefore, the combination of PMX with PMMA-CHDF is beneficial for patients with septic shock and septic renal dysfunction. In combination therapy, the Toraymyxin column is generally placed before or after CHDF on a single circuit. However, when

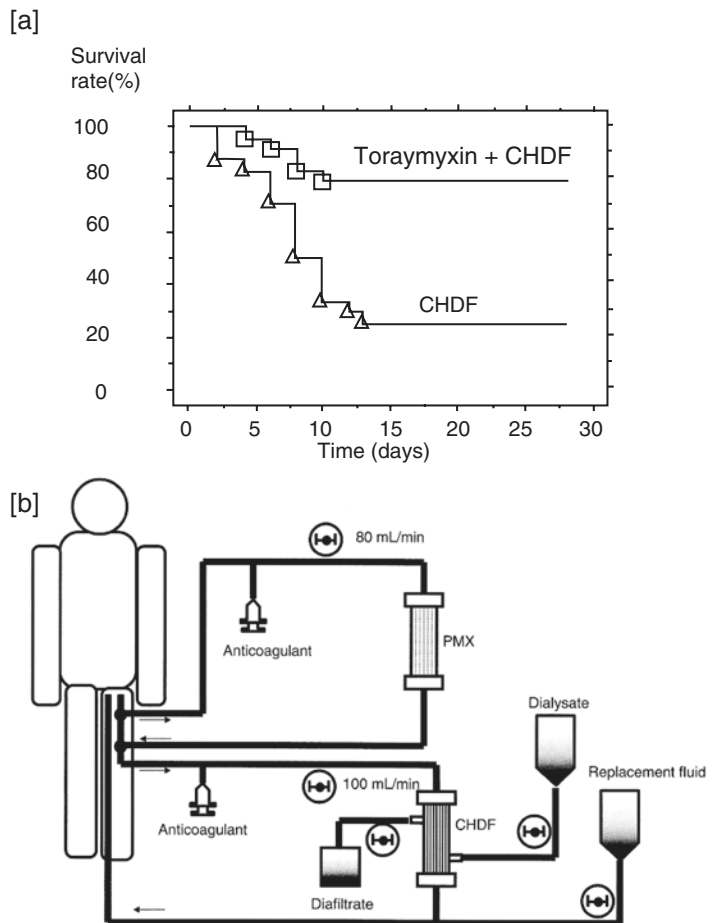
CHDF cannot be interrupted, for example in patients with renal failure, the Toraymyxin circuit can be connected in parallel or in series with the CHDF circuit (Fig. 19.13b) [59].

19.5.2 PMX Therapy for the Treatment of Acute Exacerbation of Idiopathic Pulmonary Fibrosis and Acute Respiratory Distress Syndrome

Idiopathic pulmonary fibrosis (IPF) is one of a heterogeneous group of diffuse parenchymal lung disorders of unknown etiology. Acute exacerbation of IPF is characterized by severe worsening dyspnea and high mortality. IPF is one of the most common presentations of idiopathic interstitial pneumonia. Previous reports have suggested that PMX treatment improves oxygenation in patients with acute lung injury or acute respiratory distress syndrome (ARDS) due to severe sepsis [60, 61]. The potential benefit of PMX therapy for the treatment of acute exacerbation of IPF has also been reported [62]. A multicenter retrospective analysis of 160 patients showed that PMX therapy improves oxygenation and survival in patients experiencing acute exacerbation of IPF [63]. A large-scale, prospective, RCT in patients experiencing acute exacerbation of IPF will be conducted in the near future.

The successful treatment of severe ARDS due to influenza virus infection with PMX has been reported recently. Yokoyama et al. reported a case of severe ARDS caused by novel swine-origin influenza virus (A/H1N1pdm) [64]. The patient underwent PMX, after which her hypoxemia improved and she survived. This is the first report of severe, life-threatening ARDS due to a novel influenza virus in which PMX was beneficial. Yatera et al. reported a case of ARDS due to influenza A infection that was successfully treated with PMX [65]. Kudo et al. has reported cases of severe pneumonia due to highly pathogenic avian influenza A (H5N1) in Vietnam successfully treated with CHDF coupled with Toraymyxin hemoperfusion, suggesting that it is

Fig. 19.13 Use of Toraymyxin in combination with continuous hemodiafiltration
(a) Survival of patients receiving Toraymyxin therapy in combination with continuous hemodiafiltration.
 (Reproduced from Suzuki et al. [57])
 The use of this combination significantly improved survival rate in patients with sepsis and acute renal failure
(b) Schematic of the combination of direct hemoperfusion with Toraymyxin and continuous hemodiafiltration in a series-parallel circuit



an effective candidate treatment for ARDS due to the H5N1 virus if applied early in the disease [66].

These data show that new indications for PMX treatment in the field of pulmonary medicine will likely be available in the near future.

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