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**Endotoxin removal by
polymyxin B-immobilized
fiber column hemoperfusion
in septic shock**

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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. PMX treatment is based on the binding property of polymyxin B to lipid A of endotoxin. 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 systemic 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 treatment adsorbed endotoxin, monocytes and anandamide and reduced the blood concentrations of inflammatory cytokines (TNF- α , IL-6, IL-8) and other mediators such as plasminogen activator inhibitor (PAI)-1 and adhesion molecules. PMX increased blood pressure and reduced the dosage requirements for vasopressor/inotropic agents. Lastly, PMX improved pulmonary oxygenation and decreased the Sequential Organ Failure Assessment (SOFA) score and mortality.

The findings of this review suggest that PMX treatment has beneficial effects on the hemodynamics, pulmonary oxygenation, and mortality in patients with septic shock. These beneficial effects may be attributable to the direct adsorption of endotoxin, monocytes and anandamide, as well as indirect decrease in inflammatory cytokines and other mediators. Further studies will be needed to confirm the efficacy and mechanism of PMX treatment in septic shock.

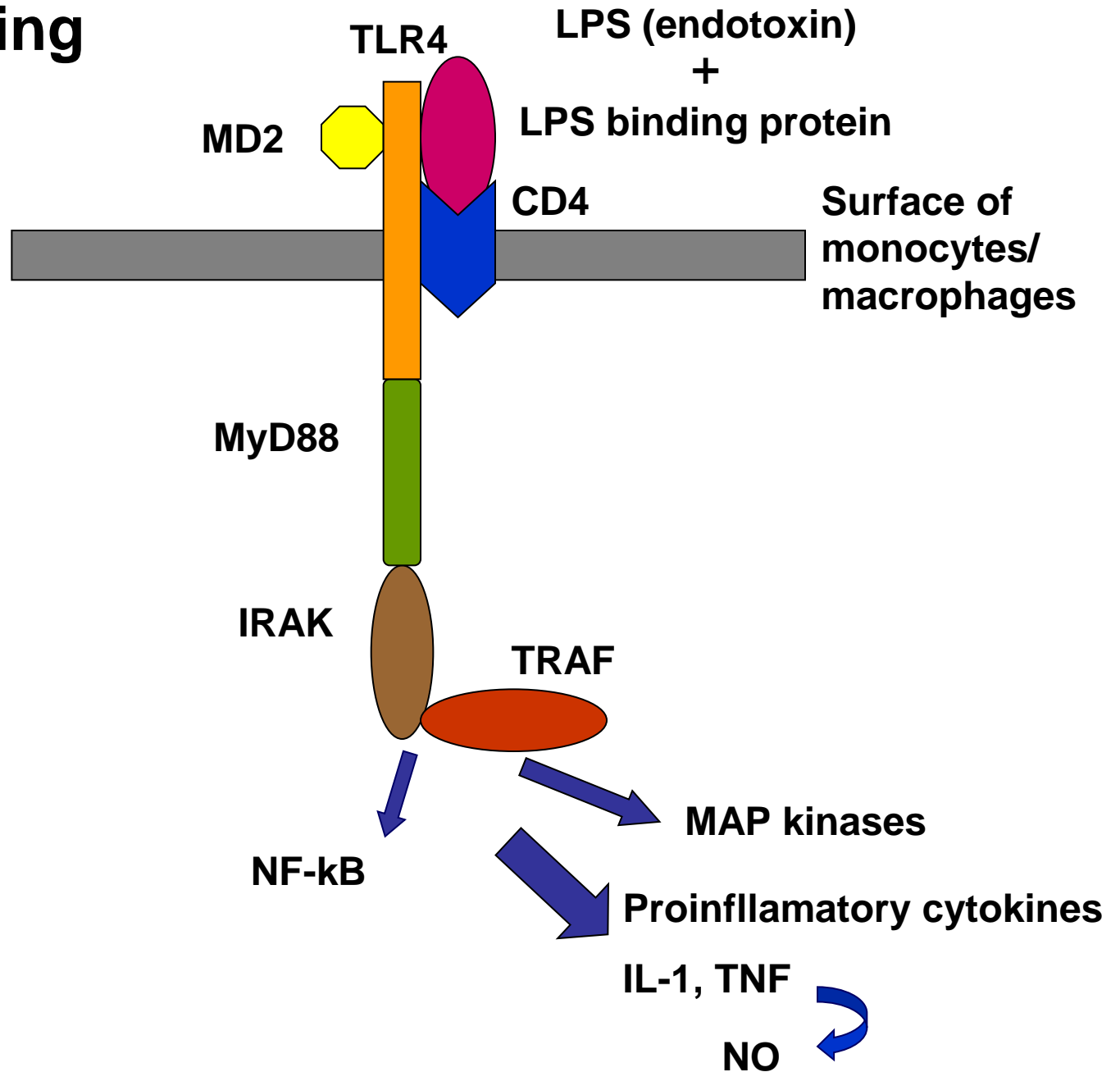
Key words: Endotoxin, polymyxin B (PMX), septic shock, TNF- α , IL-6, IL-8.



Introduction

- **Endotoxin, an outer membrane component of Gram-negative bacteria, plays an important role in the pathogenesis of septic shock.**

LPS signaling



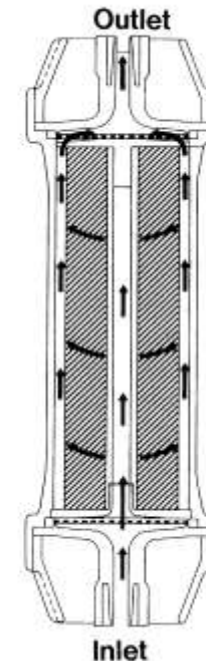


Endotoxin adsorption therapy

- 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.**

PMX (Toramyxin 20-R)

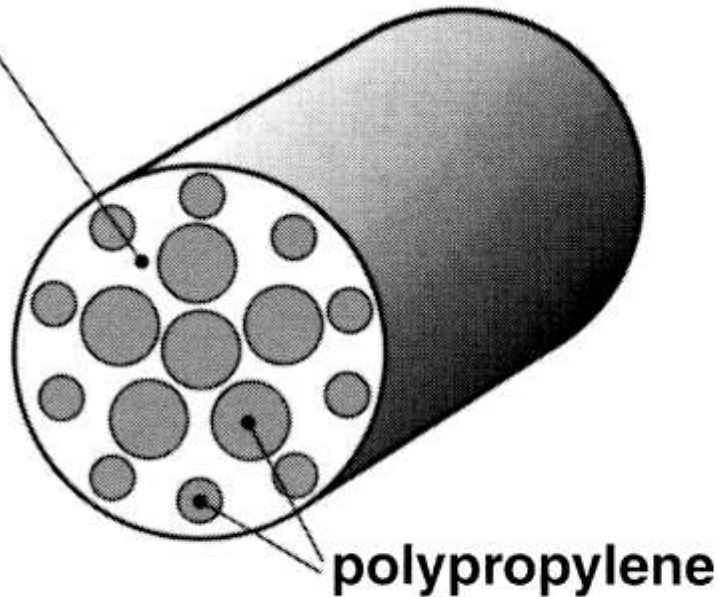
- PMX treatment is based on the binding property of polymyxin B to lipid A of endotoxin.
- The covalent binding of polymyxin B onto the surface of the polystyrene-based carrier fiber in PMX inactivates the endotoxin in the blood without exerting toxicity.



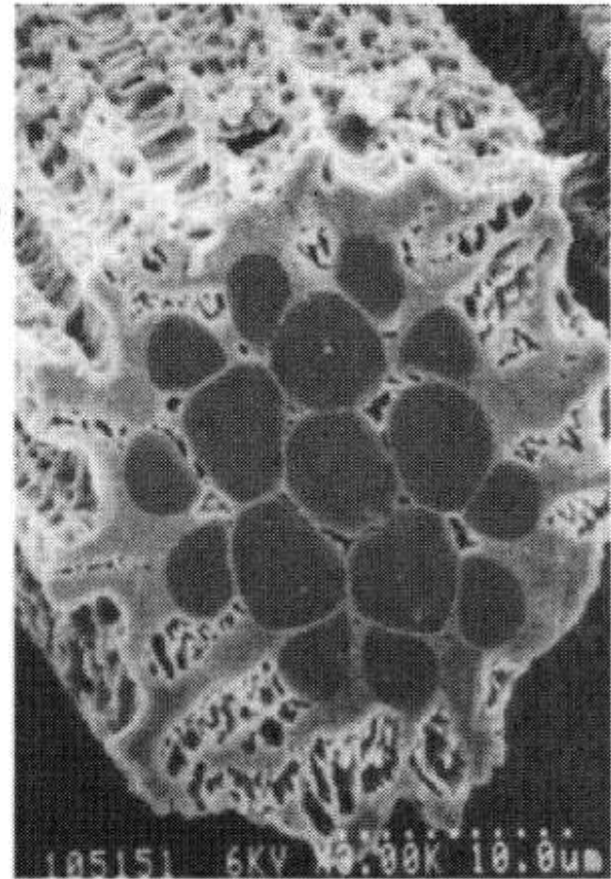
Schematic diagram of the blood flow within a Toramyxin cartridge (Ther Apher Dial 2003;7:1-8-114)

Conjugated fiber

polystyrene/polypropylene(9:1)

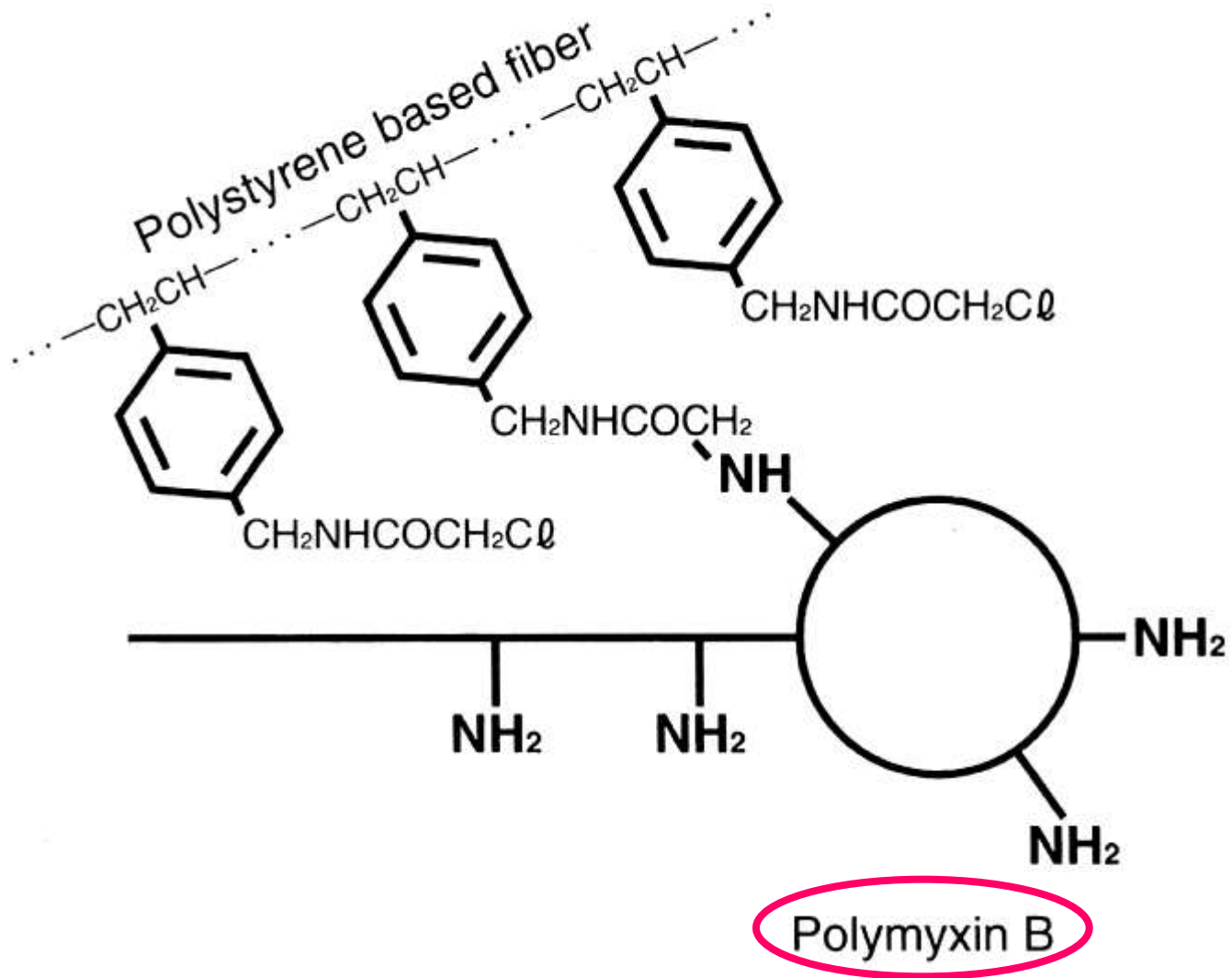


a



b

(a) Schematic diagram of the cross section of an island-sea type conjugated fiber filament. The island component is made up of a polypropylene polymer and the sea component is made of a mixed polymer of polystyrene and polypropylene (9:1). (b) Electronmicrograph of the cross section of fiber filament. (Ther Apher Dial 2003;7:108-114)



Schematic diagram of polymyxin B covalently bonded to the surface of the polystyrene-based carrier fiber. (Ther Apher Dial 2003;7:108-114)



Objective

- **This study was performed as a systemic review to evaluate the efficacy and mechanism of PMX treatment in patients with septic shock.**



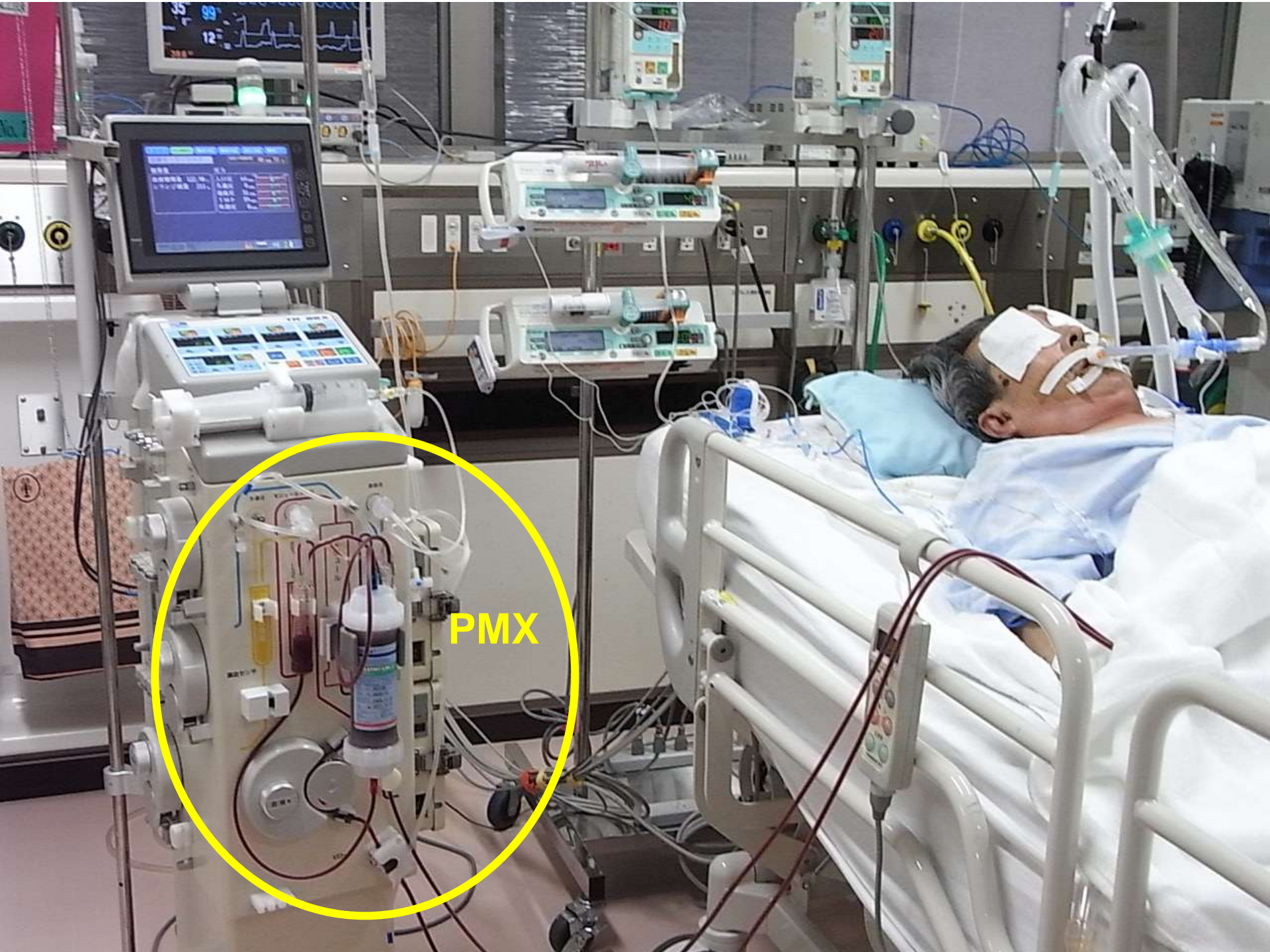
Methods

- **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.**



Interventions (PMX treatment)

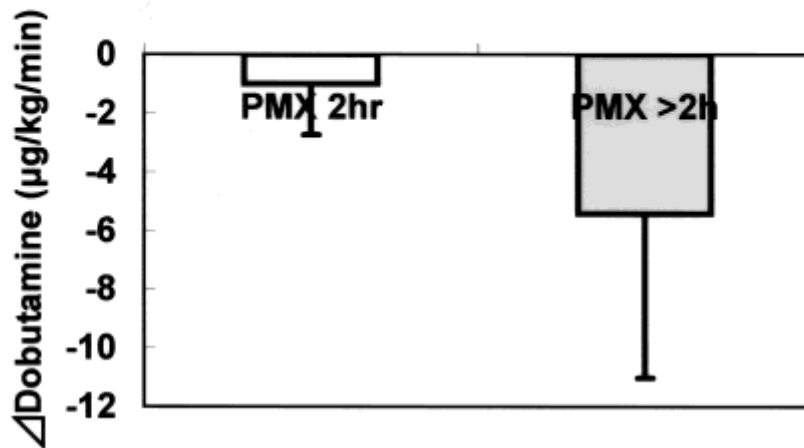
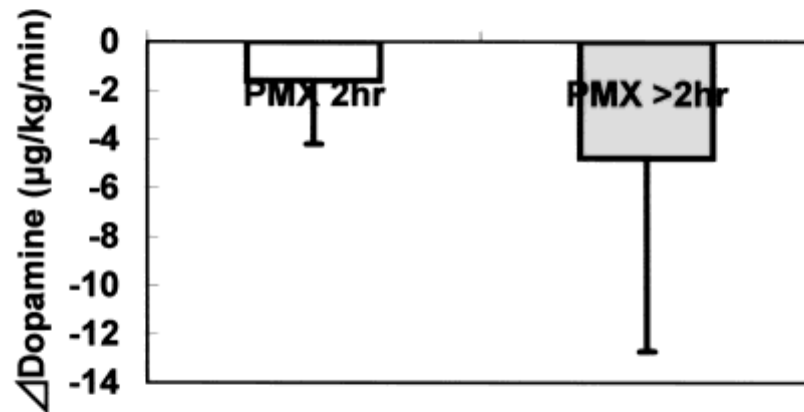
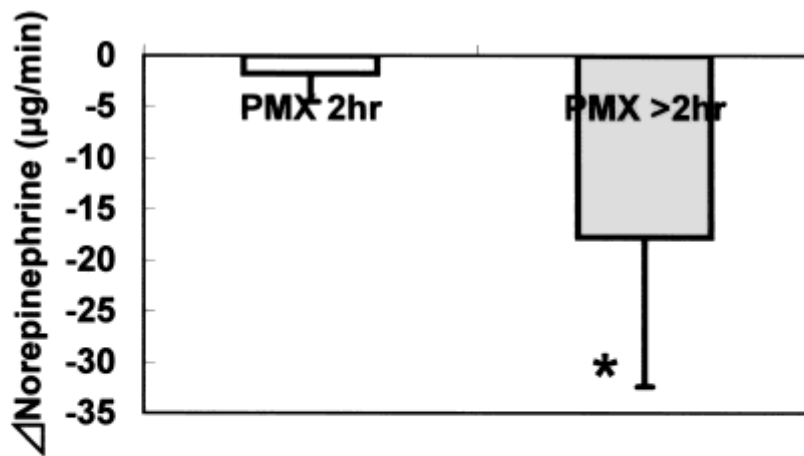
- A Toraymyxin 20-R is washed by perfusion with 4L of physiological saline.**
- After inserting a double lumen catheter into a central vein of patients, blood is drawn from the proximal port, perfused through Toraymyxin 20-R, and returned to the vein.**
- The blood is perfused at a rate of 80 to 100 ml/min using an anticoagulant.**
- A 2-to 24-hr PMX treatment is administered either once or in some patients up to two or three times, depending on the clinical response.**



PMX

Hemodynamics and dose of vasopressive/inotoropic agents

- **PMX treatment increased blood pressure in almost all studies.**
- **In a pilot study in Europe (Vincent JL, et al), PMX was found to significantly increase the CI, LVSWI, and DO_2I compared with control.**
- **The doses of dopamine, dobutamine, and norepinephrine were consistently reported to decrease after PMX treatment.**

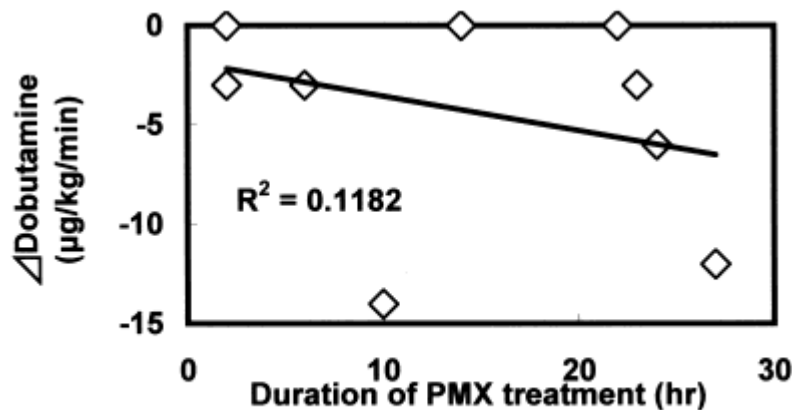
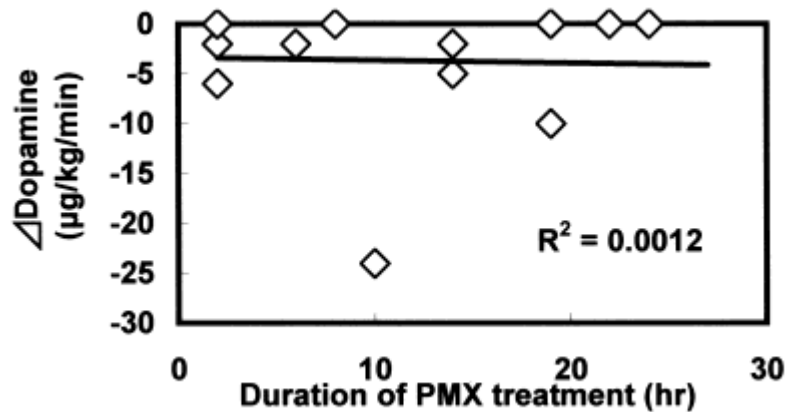
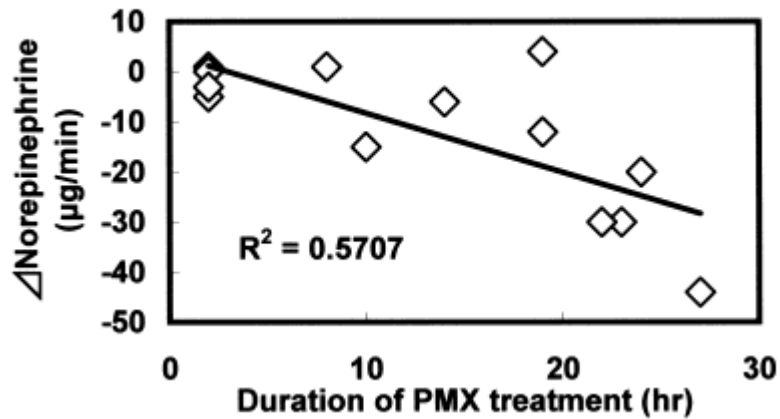


Changes in dose of norepinephrine, dopamine, and dobutamine before and after the PMX treatment in patients with septic shock

Each column with bar represents mean and SD.

*P<0.05 vs. the PMX 2-hr group

(Mitaka C, et al. Shock 2009;32:478-483)



Relationships between duration of PMX treatment and changes in doses of norepinephrine, dopamine, and dobutamine before and after the PMX treatment in patients with septic shock

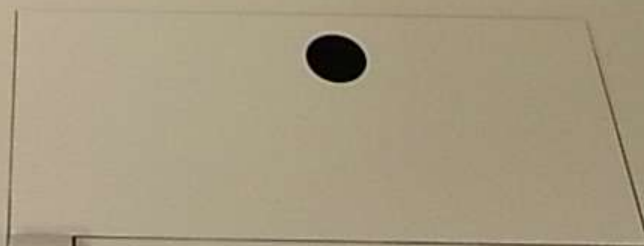
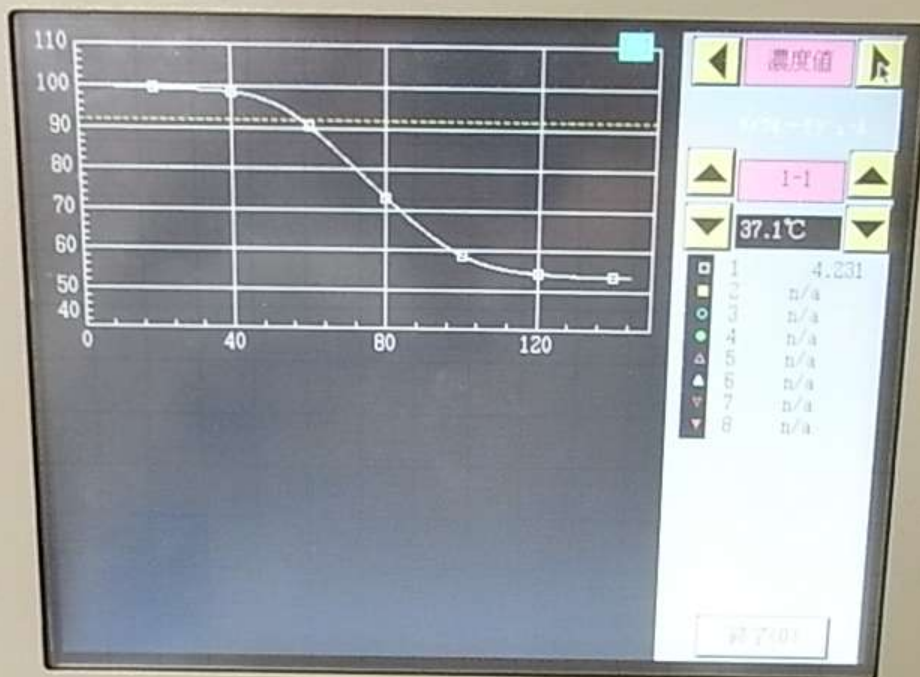
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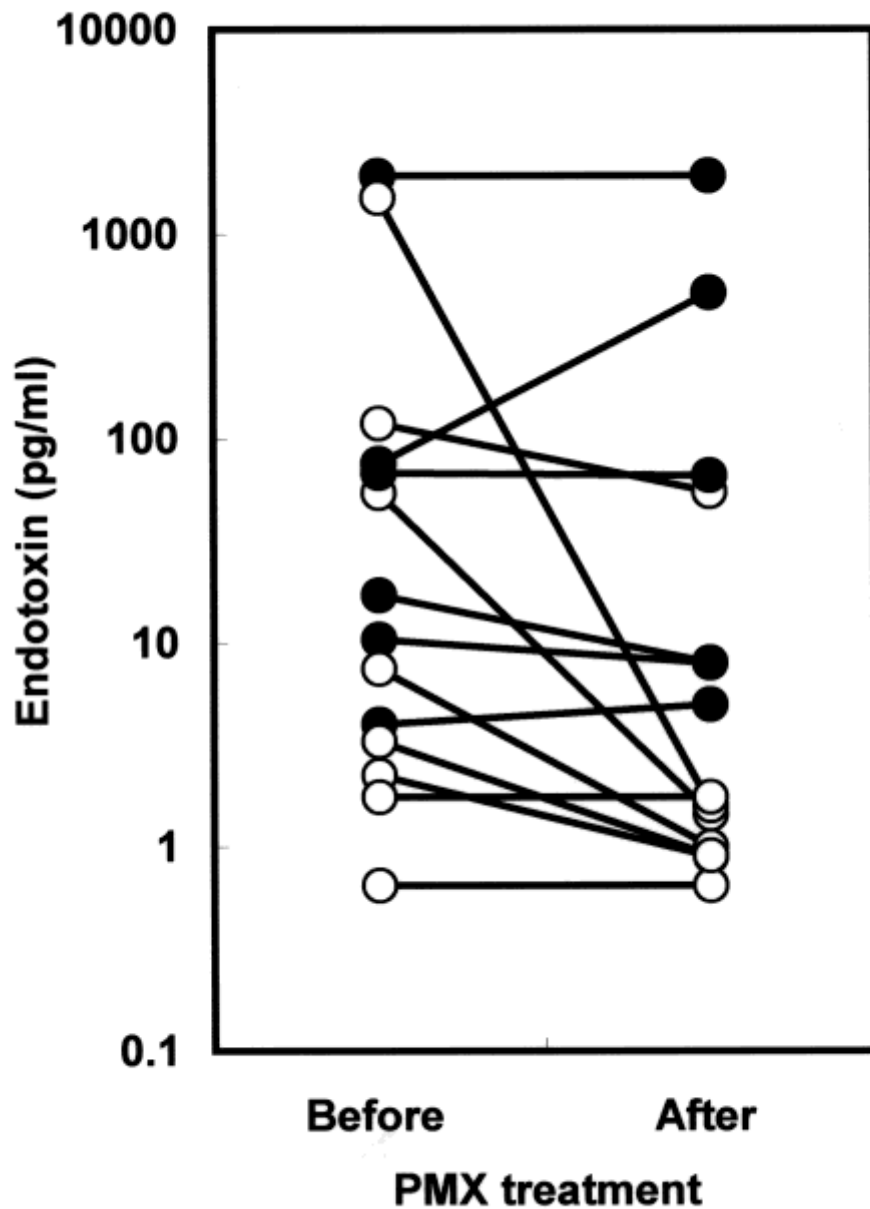


Endotoxin concentrations

- **Measurement of endotoxin concentration**
 - 1) **Endotoxin-specific assay (endospecky)**
 - 2) **Modified limulus amoebocyte lysate assay kit (COATEST)**
 - 3) **Kinetic turbidimetric limulus assay using a Toxinometer. This limulus assay test is specific to endotoxin and has no cross-reaction to β -glucan.**
- **Endotoxin concentrations decreased after PMX treatment in most of the studies, but did not significantly change in the European RCT study (Vincent JL, et al.).**

Kinetic turbidimetric limulus assay using a Toxinometer





Changes in plasma endotoxin concentrations before and after the PMX treatment in patients with septic shock

Open circles represent survivors, and closed circles represent nonsurvivors.

(Mitaka C, et al. Shock 2009;32:478-483)



Mediators

- **PMX decreased blood concentrations of IL-6, IL-8, IL-10, TNF- α , plasminogen activator inhibitor-1 (PAI-1), neutrophil elastase, platelet factor-4, β -thromboglobulin, soluble P selectin, soluble ELAM-1 and soluble ICAM-1, erythropoietin, and metalloproteinase-9.**
- **PMX decreased urinary NOx (Nakamura T, et al. Intensive Care Med 2004;30:1838-1841).**

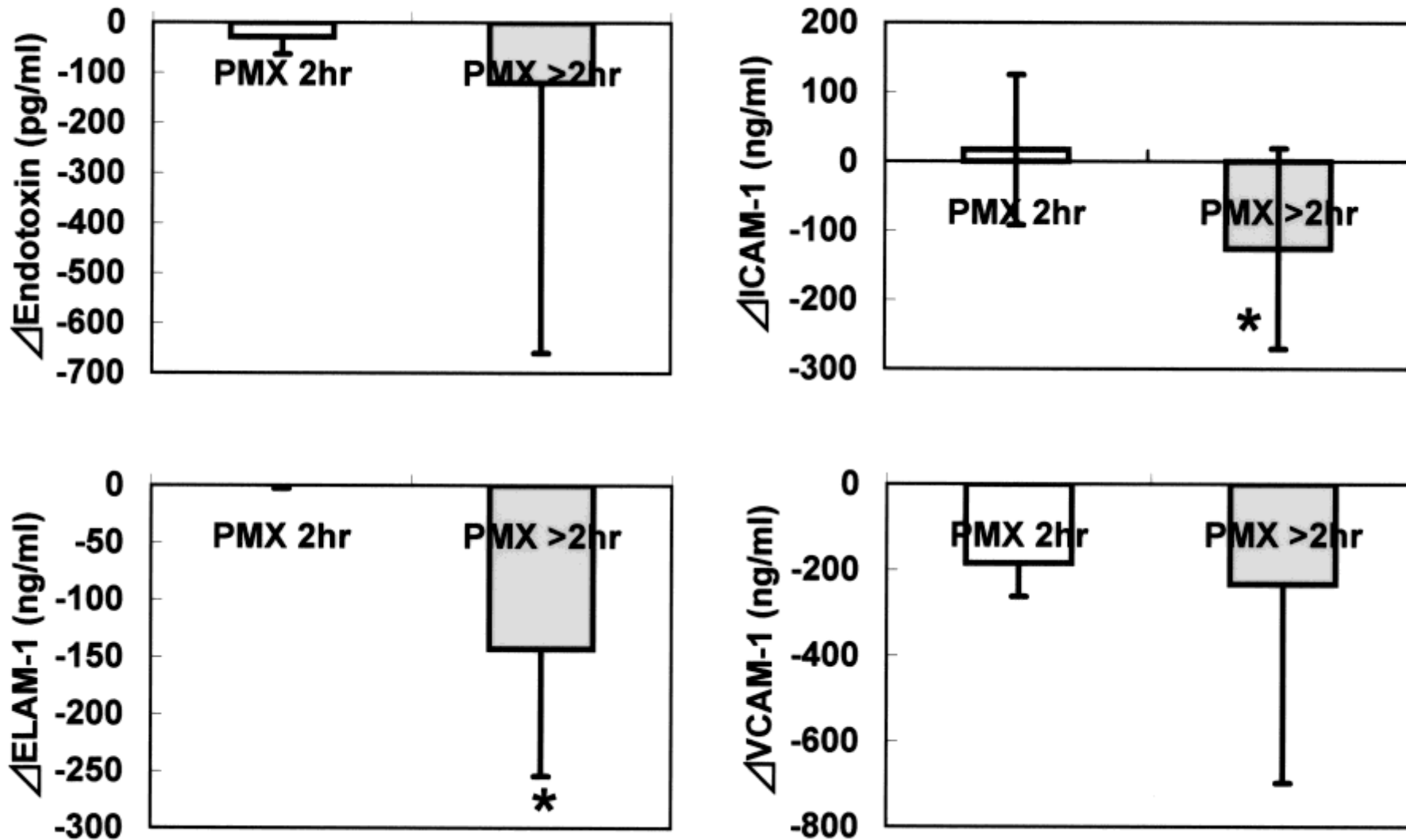


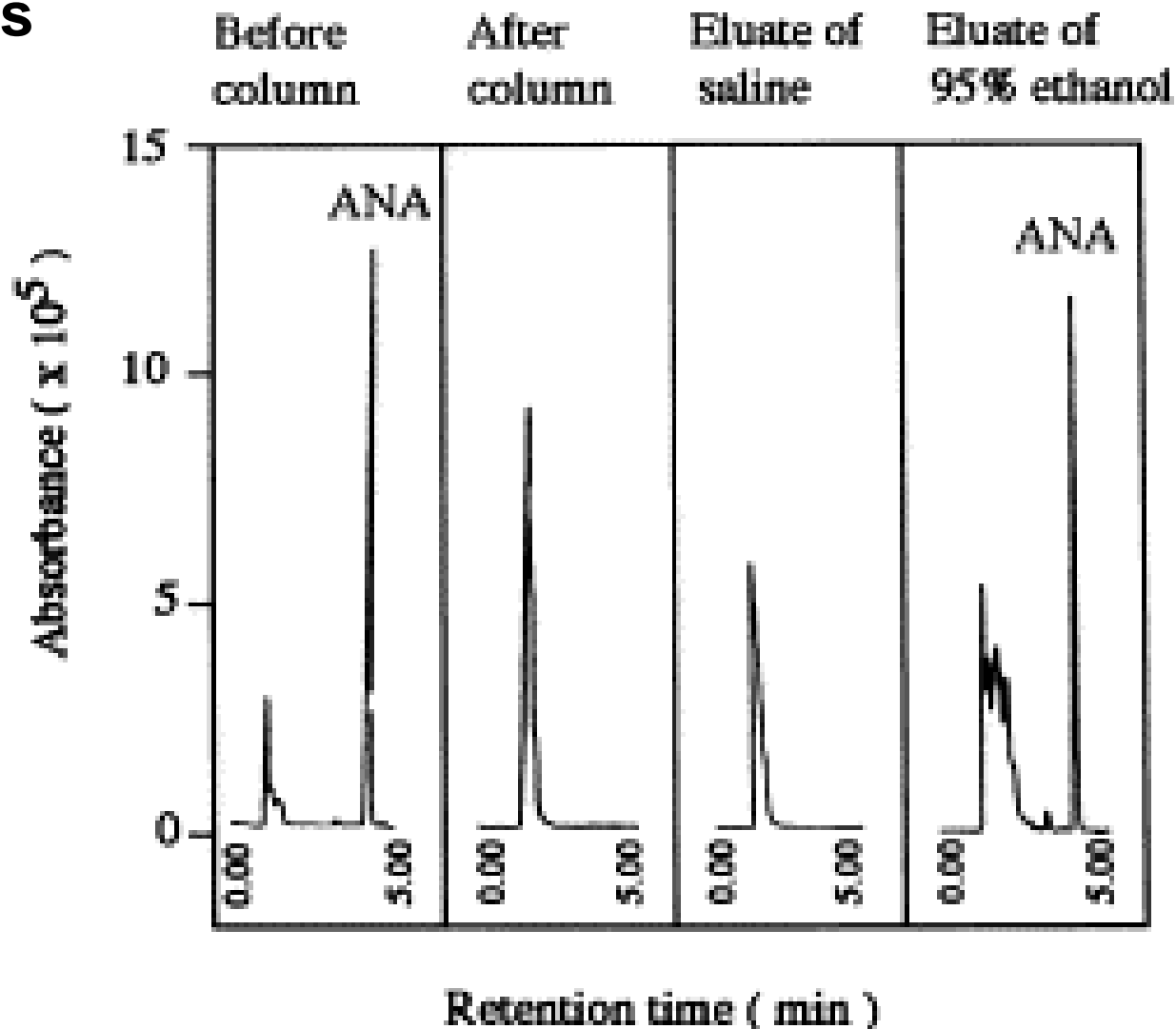
Fig. 4. Changes in plasma endotoxin, soluble ELAM-1, soluble ICAM-1, and soluble VCAM-1 before and after the PMX treatment in patients with septic shock. Each column with bar represents mean and SD. *P<0.05 vs. the PMX 2-hr group. (Mitaka C, et al. Shock 2009;32:478-483)



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 (Wang Y, et al. FEBS Lett 2000;470:151-155)**

RP-HPLC profiles showing the adsorption of anandamide (ANA) by polymyxin B-immobilized beads



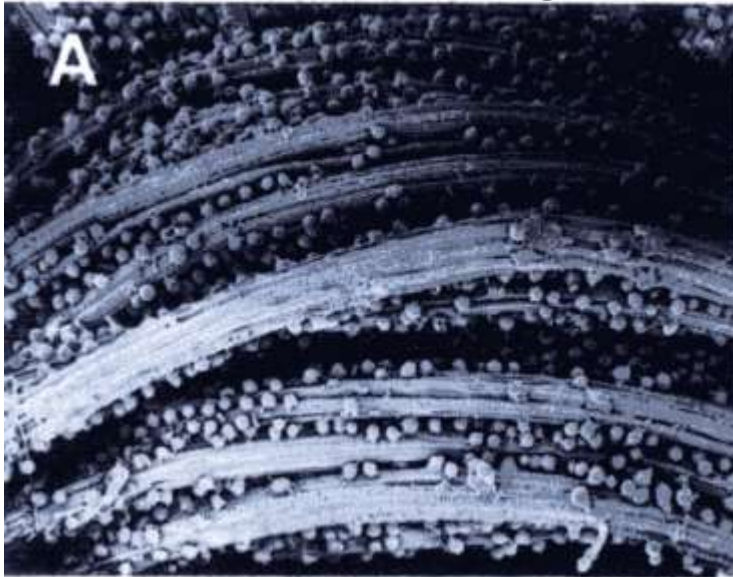


Removal of monocytes by PMX

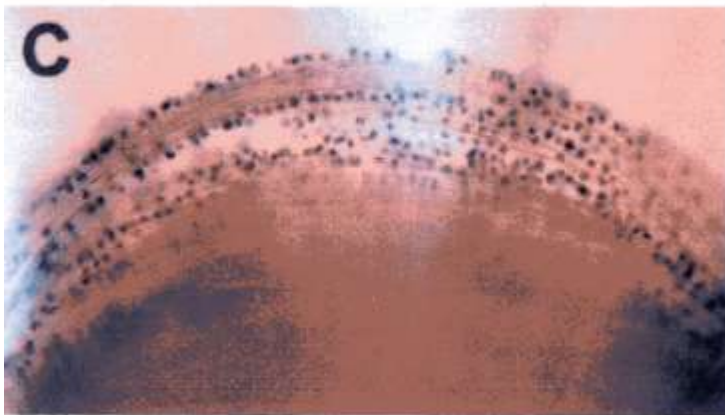
- In recent experiments using immunocytochemical and electron microscopic techniques to analyze the cells bound by the PMX column, the column was confirmed to bind monocytes from the peripheral blood leukocytes of septic patients (Nishibori M, et al. Acta Med Okayama 2009;63:65-69).**

Numerous leukocytes were trapped in the PMX columns

Before washing



After washing



Scanning electron micrographs of the filter (A,B)

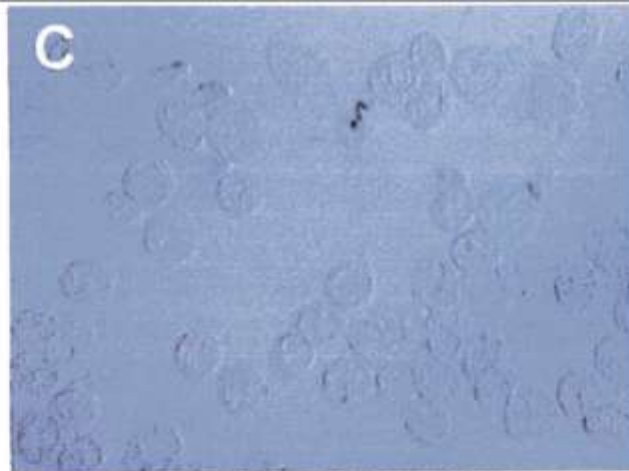
Light micrographs of the hematoxylin-stained filter (C,D)

**Leukocytes on
PMX filter**



A

**Smeared samples
(control)**

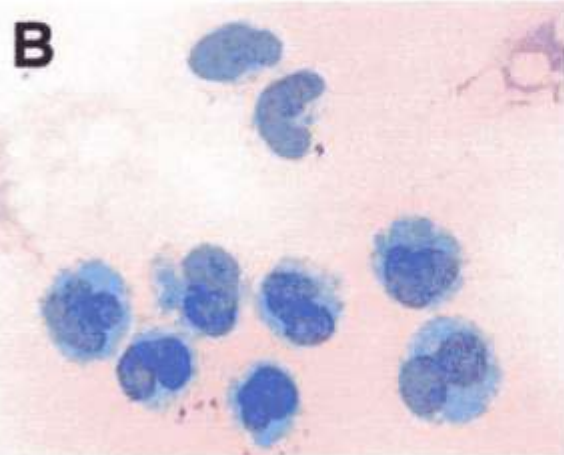


C

**Immunostained with FITC-
conjugated anti-CD68**

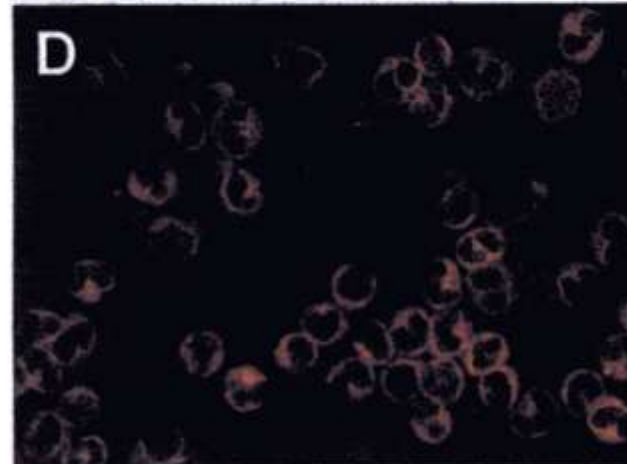


E



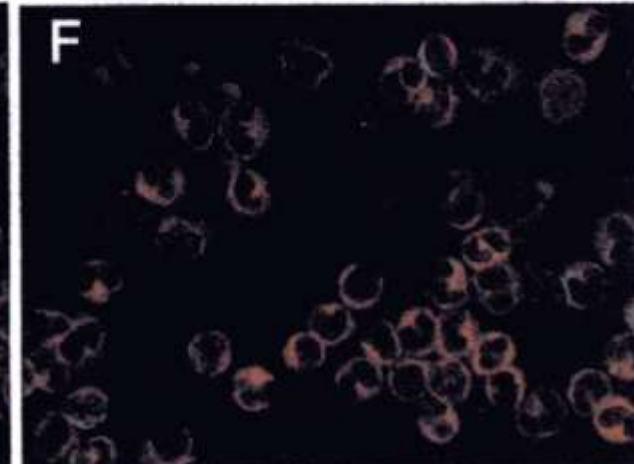
B

Monocytes



D

**Immunostained with PE-
conjugated anti-CD4**



F

Merged view



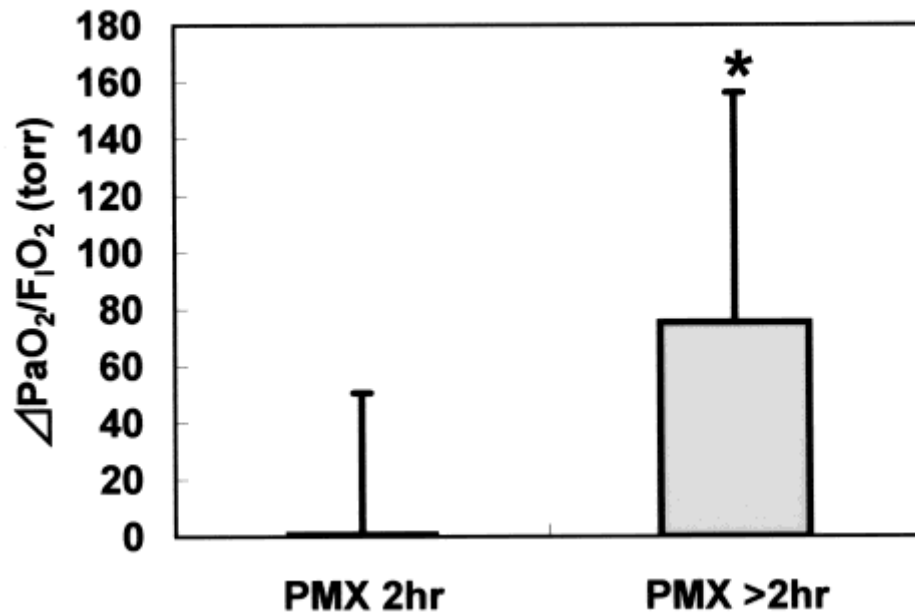
Pulmonary oxygenation

- The $\text{PaO}_2/\text{F}_1\text{O}_2$ ratio increased after PMX treatment in most of the studies.**



Organ dysfunction and Mortality

- **PMX treatment decreased SOFA score and seemed to significantly reduce mortality compared with conventional therapy.**

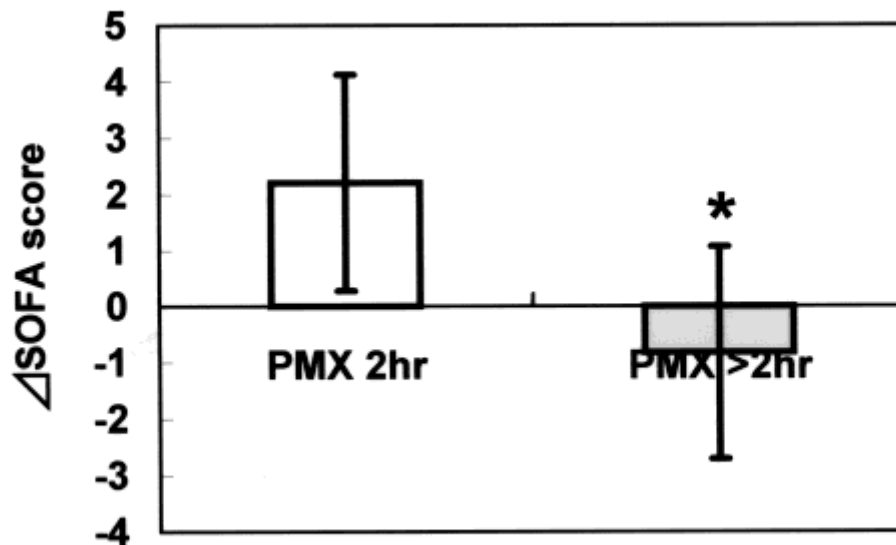


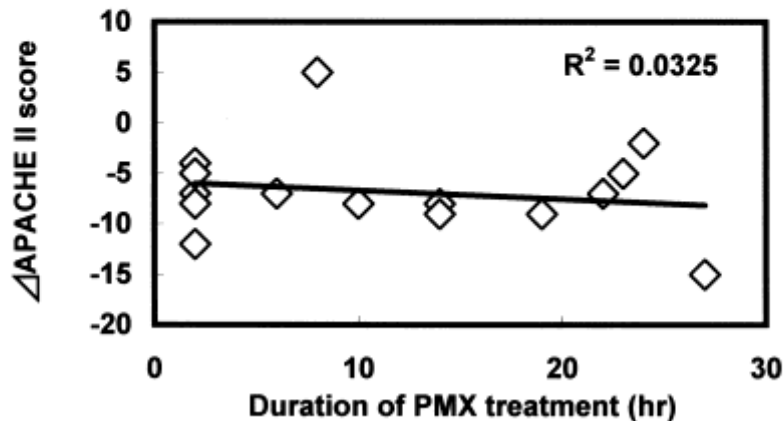
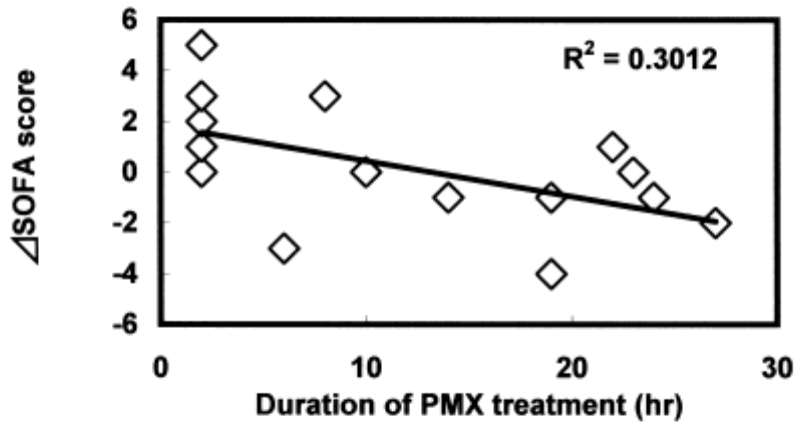
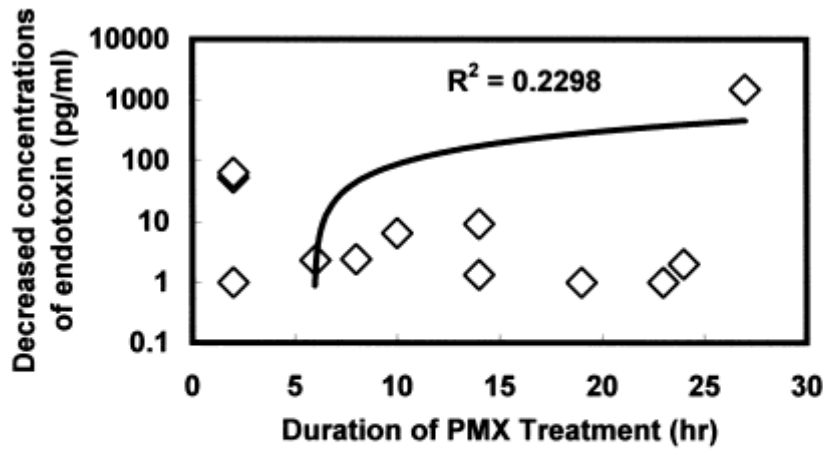
Changes in PaO₂/F_iO₂ ratio and SOFA score before and after the PMX treatment in patients with septic shock.

Each column with bar represents mean and SD.

*P<0.05 vs. the PMX 2-hr group

(Mitaka C, et al. Shock 2009;32:478-483)





Relationships between duration of PMX treatment and decreased concentrations of endotoxin, changes in SOFA score, and APACHE II score before and after the PMX treatment in patients with septic shock.

(Mitaka C, et al. Shock 2009;32:478-483)



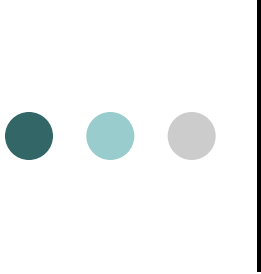
Summary of Results

- **PMX adsorbed endotoxin, anandamide and monocytes and reduced the blood concentrations of inflammatory cytokines (TNF- α , IL-6, IL-8) and other mediators such as plasminogen activator inhibitor (PAI)-1 and adhesion molecules.**
- **PMX increased blood pressure and reduced the dosage requirements for vasopressive/inotropic agents.**
- **PMX improved pulmonary oxygenation and decreased the SOFA score and mortality.**

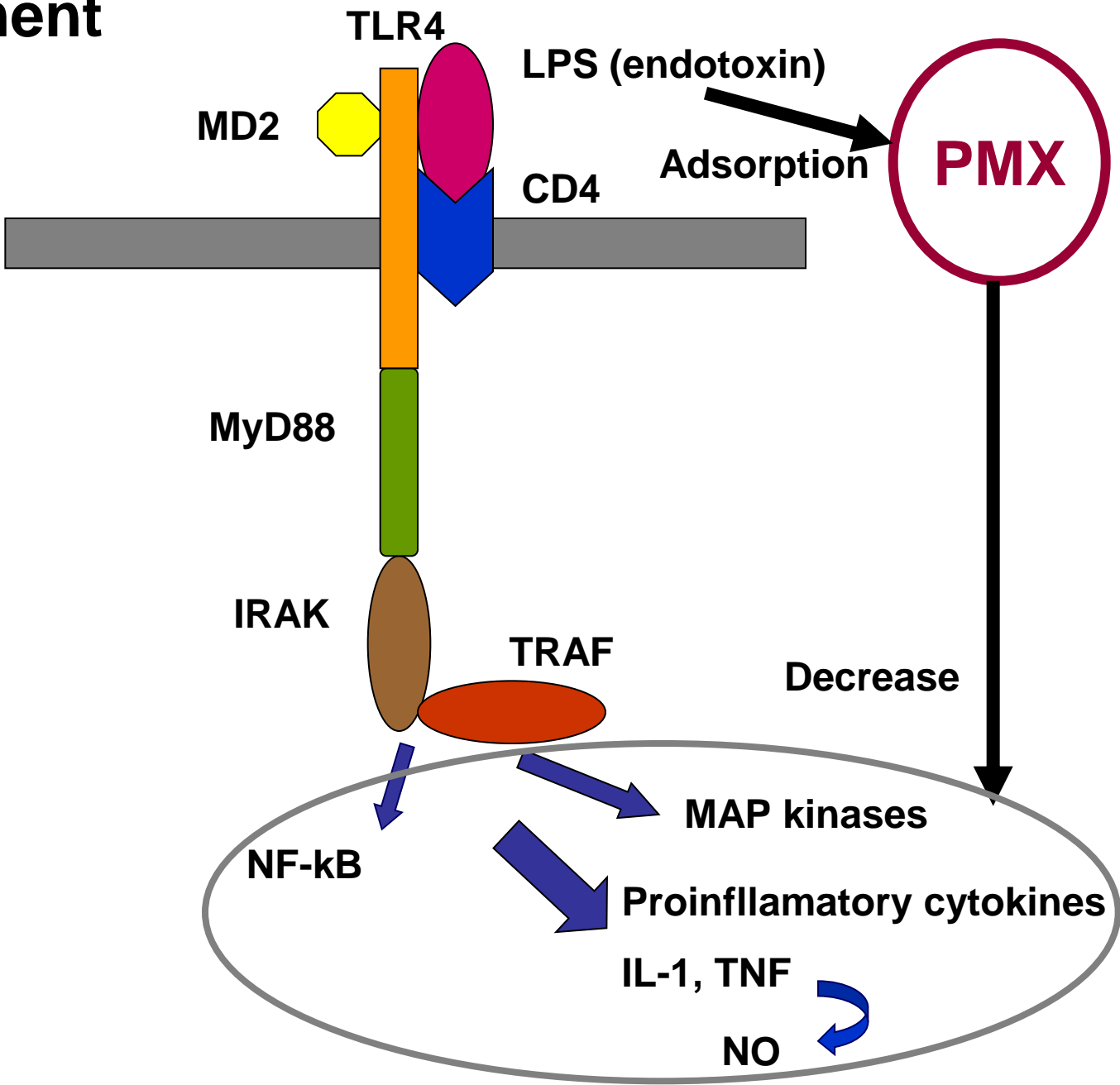


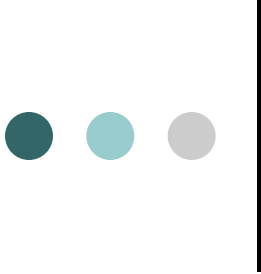
Discussion

- **The beneficial effects of PMX may be attributable to decrease in the concentrations of endotoxin and/or inflammatory cytokines.**
- **PMX might indirectly inhibit the release of inflammatory cytokines such as IL-6, IL-8, IL-10, and TNF- α .**
- **PMX treatment decreases NO breakdown products (NO_x) in urine. As such, the inhibition of NO production by PMX could prevent vasodilation and increase blood pressure.**

- 
- **PMX binds to anandamide and neutralizes bioactivity effects such as vasodilation and cytotoxicity**
 - **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.**

PMX treatment

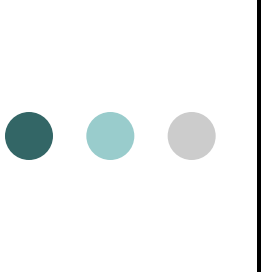


- 
- **The improved $\text{PaO}_2/\text{F}_i\text{O}_2$ ratio conferred by PMX treatment may be attributable to decreases in adhesion molecules, IL-8, neutrophil elastase, metalloproteinase-9 or the tissue inhibitor of metalloproteinase-1.**



Conclusion

- **The findings of this review suggest that PMX treatment has beneficial effects on the hemodynamics, pulmonary oxygenation, and mortality in patients with septic shock.**
- **These beneficial effects may be attributable to the direct adsorption of endotoxin, monocytes and anandamide, as well as indirect decrease in inflammatory cytokines and other mediators.**
- **Further studies will be needed to confirm the efficacy and mechanism of PMX treatment in septic shock.**



“ენდოტოქსინის გამოვლენა სექტიური შოკის დროს
პოლიმიქსინ B - იმობილიზირებული ბოჭკოების
მეშვეობით ჰემოპერფუზიულ სვეტებში”
ჩ.მიტაკა (ტოკიო, იაპონია)

ენდოტოქსინი, გრამ-ნეგატიური ბაქტერიის გარე მემბრანის კომპონენტი მნიშვნელოვან როლს თამაშობს სექტიური შოკის პათოგენეზში. ენდოტელეალიური უჯრედების გამოვლენა ენდოტოქსინში ააქტიურებს ენდოტელეალურ უჯრედებს და ზრდის ზედაპირის მოლეკულურ მიზიდულობას, ორგანულ დისფუნქციაში ენდოტელეალური დაზიანების მარკერებს. 1994 წლიდან ენდოტოქსინის აღსორბციის თერაპია პოლიმიქსინ B-ს საშუალებით - შეჩერებული ბოჭკოვანი მწკრივის ჰემოპერფუზი, იაპონიაში გამოიყენება სექტიური შოკის ავადმყოფთა სამკურნალოდ. ამ მოსაზრების შესამოწმებლად ჩვენ გავზომეთ ენდოტოქსინის და ხსნადი გადაბმული მოლეკულების პლაზმის კონცენტრაცია ავადმყოფებში, რომლებსაც მანამდე გადატანილი ჰქონდათ სექტიური შოკი და PMX-ს ჩატარების შემდეგ დავაკვირდით გამოყენების ფაქტიურ ხანგრძლივობასა და სხვადასხვა შედეგების ურთიერთდამოკიდებულებას. გამოკვლევულ იქნა თექვსმეტი პაციენტი სექტიური შოკით. ჩაწერილ იქნა დემოგრაფიული მონაცემები, ყოველდღიური ბიოქიმია, მიკრობიოლოგიური მონაცემები, ინფექციის სწარო, მწვავე ფიზიოლოგიური და ქრონიკული ჯანმრთელობის შეფასება II ქულა, თანმიმდევრული ორგანული უკმარისობის შეფასების ქულა, და 28-ე დღეზე სიკვდილიანობა. აღმოჩენები ადასტურებს, რომ PMX –ის მეტ ხანგრძლივობას შეუძლია გააუმჯობესოს პულმონარული ჟანგბადის მიწოდება, რომელიც ასოცირდება სექტიური შოკის დროს შემცირებულ გადაბმულ მოლეკულებთან.

Thank you !

