Anandamide absorption by direct hemoperfusion with polymixin B-immobilized fiber improves the prognosis and organ failure assessment score in patients with sepsis

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Abstract

Purpose. Direct hemoperfusion (DHP) with polymixin B-immobilized fiber (PMX) has been reported to be effective for patients with septic shock. The aim of this study was to clarify the mechanism of PMX-DHP effect on septic shock.

Methods. The following parameters were measured in septic shock patients who were treated with PMX-DHP: survival rate, sepsis-related organ failure assessment (SOFA) score, acute physiology and chronic health evaluation II (APACHE-II) score, and plasma concentrations of cannabinoids [anandamide (ANA) and 2-arachidonyl glyceride (2-AG)], cytokines [interleukin (IL)-6, IL-8, IL-10], transforming growth factor β (TGF-β), and calcitonin gene-related peptide (CGRP)]. The primary end point was mortality from all causes at day 28 after intensive care unit (ICU) admission or discharge.

Results. The survival rate of all patients at 28 days after ICU admission was 37.5% (9/24). The survival group showed significantly lower SOFA and APACHE-II scores than the nonsurvival group after PMX-DHP treatment (P = 0.008 and 0.028, respectively). The improved SOFA score group showed a better survival rate than the nonimproved SOFA score group (71.4% versus 23.5%, P = 0.028). Plasma ANA level significantly decreased after PMX-DHP treatment both in the improved SOFA score group and in the survival group. The level of 2-AG, however, showed no significant change in either group.

Conclusion. ANA, an intrinsic cannabinoid that induces hypotension in septic shock, is inferred to be the main mechanism of the PMX-DHP effect. Removal of ANA by PMX-DHP could be key to successful septic shock treatment.

Key words Direct hemoperfusion · Polymixin B-immobilized fiber · Septic shock · Sepsis organ failure assessment · Anandamide

Introduction

In the management of sepsis, previous research focused on the importance of Gram-negative bacteria and endotoxins. Polymixin B-immobilized fiber (PMX) was first developed as a biomaterial for selective adsorption of endotoxin in patients with Gram-negative bacterial infection [1–3]. Endotoxin adsorption using PMX provided some successful results for Gram-negative bacteria-induced sepsis [4].

On the other hand, antilipopolysaccharide (LPS) monoclonal antibodies (HA-IA [5], E5 [6]) have been developed against endotoxin, but clinical trials have not provided reproducible survival benefits in LPS shock patients [7]. Direct hemoperfusion (DHP) with PMX (PMX-DHP) has been reported to be effective for patients with septic shock who are infected not only by Gram-negative bacteria but also by Gram-positive bacteria without endotoxin release [8]. From these facts, it is supposed that the therapeutic effect of PMX-DHP on septic shock would depend on the other mediators, except for endotoxin removal. The aim of this study was therefore to clarify the mechanism of PMX-DHP effect on septic shock.

Cytokines are regarded as important mediators in the pathophysiology of sepsis and septic shock. Several studies have demonstrated an increase in serum levels of inflammatory cytokines in critically ill patients [9]. However, it has also been reported that cytokines are not removed by PMX-DHP [10], and pharmacological antagonism of cytokine effects failed to provide protection from the hypotension of septic shock [11]. It is supposed that cytokines are not a major mechanism of the PMX-DHP effect for septic patients. Both Gram-negative and Gram-positive bacteria have been suggested to release endogenous cannabinoids in animal studies. Recent evidence indicates that during certain shock conditions, platelets and macrophages produce at least two different endogenous cannab-
inoids, anandamide (ANA) and 2-arachidonyl glyceride (2-AG), which may be paracrine mediators of hypotension during shock, acting via CB1, a cannabinoid receptor subtype localized in the peripheral vasculature [12–16]. Several studies have demonstrated that ANA and 2-AG can elicit CB1 receptor-mediated hypotension in rats [12,17,18]. ANA induces cell death, and pretreatment with polymixin B neutralizes its cytotoxicity [14]. Based on these studies, we hypothesized that the mechanism of improvement for septic shock patients by PMX-DHP treatment might be the reduction of blood levels of cannabinoids.

A prospective clinical study was performed, and the several parameters described above were measured in septic shock patients who were treated with PMX-DHP, as well as other parameters for critically ill patients: survival rate, sepsis-related organ failure assessment (SOFA) score, acute physiology and chronic health evaluation II (APACHE-II) score, and catecholamine pressure index (CAPI).

**Patients and methods**

**Study design**

This study was approved by the review board of our institution, and informed consent was obtained from the family members of patients. This prospective consecutive study was conducted at Sapporo Medical University and three community hospitals in Japan from the beginning of January 2000 to December 2002. Standard treatments including regular antibiotic therapy were started before the induction of PMX-DHP treatment.

**Patients**

Twenty-four patients with a clinical diagnosis of septic shock as defined by the American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference [19] were included in this study. Antibiotic therapy was judged to be adequate when the patient received an antibiotic to which each isolated organism was sensitive. Standard treatments for septic shock were continued during and after PMX-DHP. The primary end point was mortality from all causes at day 28 after intensive care unit (ICU) admission or discharge, if patients were discharged from the hospital or transferred to another hospital within the 28 days.

**PMX-DHP treatment**

The PMX was produced by immobilizing polymyxin B on polystyrene fiber using covalent bonding without its release. The column for DHP contained 53 g of PMX, supplied by Toray Industries (Tokyo, Japan) [20]. During PMX-DHP, the blood flow volume was about 80 to 100 ml·min⁻¹ by the venovenous method with a double lumen catheter. The PMX-DHP treatment lasted for 2 h and 20 mg of nafamostat mesilate (Torii Pharmaceutical, Tokyo, Japan), an anticoagulant was administered concurrently.

**Clinical and laboratory evaluation**

SOFA and APACHE-II scores were calculated before and after DHP-PMX treatment. SOFA is a scoring system that was devised in 1994 to describe the degree of organ dysfunction in sepsis [21]. The system scores the function of six different organ systems: respiratory, cardiovascular, central nervous system, coagulation, hepatic, and renal systems, and each system is scored (1–4) according to the level of physiological derangement. APACHE-II uses a point score based upon initial values of 12 routine physiological measurements, age, and previous health status to provide a general measure of severity of disease [22]. CAPI was measured before/after and 1 day after the PMX treatment. CAPI was calculated by the following equation: \[ \frac{[\text{dopamine} \left( \mu g \cdot kg^{-1} \cdot min^{-1} \right) + \text{dobutamine} \left( \mu g \cdot kg^{-1} \cdot min^{-1} \right) + 100 \cdot \text{adrenaline} \left( \mu g \cdot kg^{-1} \cdot min^{-1} \right) + 100 \cdot \text{noradrenaline} \left( \mu g \cdot kg^{-1} \cdot min^{-1} \right)]}{\text{systolic blood pressure (mmHg)}}. \]

Blood samples were also collected, and serum concentrations of ANA, 2-AG, transforming growth factor β (TGF-β), interleukin (IL)-6, IL-8, IL-10, and calcitonin gene-related peptide (CGRP) were measured before, just after, and 24 h after DHP-PMX treatment. Collected blood samples were centrifuged at 1500 g for 10 min, and the plasma samples were stored at −70°C until measurements. All samples were transported to and analyzed at the Department of Laboratory Medicine, Kagoshima University School of Medicine, as previously reported [13].

**Statistics**

Data are expressed as numbers or means ± SD. All parameters were compared in two sets of two groups, using an unpaired t test or a \( \chi^2 \) test, between survival and nonsurvival groups, and between improved and nonimproved SOFA score groups. The changes in parameters obtained before, just after, and 24 h after PMX-DHP treatment were analyzed by one-way analysis of variance (ANOVA) with Fisher’s post hoc test in each group. All \( P \) values < 0.05 were considered significant.

**Results**

Of the 24 patients diagnosed as having septic shock during the study period, 18 patients were men and 6