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Effects of endotoxin absorber hemoperfusion on microcirculation in septic pigs

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ABSTRACT

Background: Endotoxins contribute to systemic inflammatory response and microcirculatory dysfunctions under conditions of sepsis. Polymyxin B hemoperfusion (PMX-HP) is used to remove circulating endotoxins and improve clinical outcomes. This study aims to investigate the effect of PMX-HP on microcirculation in septic pigs.

Materials and methods: By using a septic pig model, we tested the hypothesis that PMX-HP can correct intestinal microcirculation, tissue oxygenation saturation, and histopathologic alterations. A total of 18 male pigs were divided into three groups: (1) sham; (2) sepsis (fecal peritonitis); and (3) sepsis + PMX-HP groups. A sidestream dark field video microscope was used to record microcirculation throughout the terminal ileal mucosa, colon mucosa, kidney surface, and sublingual area. A superficial tissue oxygenation monitor employing the light reflectance spectroscopy technique was used to measure the tissue oxygen saturation. Hematoxylin and eosin staining was used for histologic examination.

Results: The perfused small vessel density and tissue oxygen saturation of the ileal mucosa at 6 h were higher in the sepsis + PMX-HP group than those in the sepsis group. The fluid amount and norepinephrine infusion rate between the sepsis group and sepsis + PMX-HP groups did not differ significantly. The histologic score for the ileal mucosa was lower in the sepsis + PMX-HP group than that in the sepsis group. Finally, the urine output was higher in the sepsis + PMX-HP group than it was in the sepsis group.

Conclusions: This study demonstrates that PMX-HP attenuates microcirculatory dysfunction, tissue desaturation, and histopathologic alterations in the ileal mucosa in septic pigs.

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Introduction

Endotoxins (lipopolysaccharide) contribute to systemic inflammation responses and result in microcirculatory dysfunction under conditions of sepsis. Sepsis-related microcirculatory dysfunction includes decreased perfused vessel density,[1] microthrombosis,[2] shunting,[3] endothelial dysfunction, and capillary hyperpermeability.[4] Frey *et al.* reported that splanchnic perfusion decreases early under conditions of sepsis.[5] Intestinal mucosal ischemia causes epithelial death and barrier disruption, leading to subsequent bacterial translocation to aseptic viscera.[6-8] Severe microcirculatory dysfunction and subsequent multiple organ failure can result in associated morbidity or death.

Polymyxin B, which can bind and neutralize endotoxins, has been immobilized to a polystyrene-derived fiber in a hemoperfusion cartridge to remove circulating endotoxins.[9] A European multicenter pilot trial conducted by Vincent *et al.* revealed that polymyxin B hemoperfusion (PMX-HP) improved mean arterial pressure (MAP) and reduced the requirement for renal replacement therapy in patients with severe sepsis.[10] Kushi *et al.* reported that early PMX-HP eliminates humoral mediators and improves pulmonary oxygenation in sepsis patients with acute respiratory distress syndrome.[11] We hypothesized that reducing circulating endotoxins level by PMX-HP may improve microcirculation. Iba *et al.* reported that mesenteric microcirculation was more effectively maintained using PMX-HP treatment in septic rats.[12] To our knowledge, there was no published information about the effect of PMX-HP on the microcirculation in intestinal mucosa, kidney, and sublingual area. Therefore, we tested this hypothesis through a septic pig model by examining microcirculation with a sidestream dark field (SDF) video microscope and a superficial tissue oxygenation monitor.

Materials and methods

A total of 18 male Lanyu pigs (Taitung Animal Propagation Station, Livestock Research Institute, Council of Agriculture, Executive Yuan, Taiwan) weighing 25 ± 5 kg were used in this study; use of these animals was approved by the Animal Care and Use Committee of the Laboratory Animal Center, College of Medicine, National Taiwan University, Taiwan (No. 20130367). This study adhered to the animal welfare guidelines, and the pigs were maintained on a 12-h light-dark cycle and had ad libitum access to water and food.

Animal preparation and grouping

Each pig was anesthetized using an intramuscular injection of zolazepam and tiletamine (4 mg/kg, Virbac Laboratories, France) and atropine (0.04 mg/kg, Taiwan Biotech Co, Ltd, Taiwan). After anesthesia, each pig was placed on an animal surgical table, and a water-heated blanket was used to maintain normal body temperature. An intravenous catheter was inserted into the ear vein. The lower abdominal wall was disinfected with chlorhexidine solution, and a 6-cm incision was made to open the abdomen. After localization of the cecum, a 1-cm incision was made in the cecum, and 35 mL of autologous feces was aspirated. The wound of the cecum was closed. The pigs were divided into three groups: (1) sham, (2) sepsis (fecal peritonitis), and (3) sepsis + PMX-HP groups (fecal peritonitis and polymyxin B hemoperfusion). The pigs in the sham group received intraperitoneally 200 mL of warm 5% dextrose. The pigs in the sepsis and sepsis + PMX-HP groups received intraperitoneally 1 g/kg of autologous feces dissolved in 200 mL of warm 5% dextrose.[13,14] The wound of the abdominal wall was closed. Intravenous ketorolac (1 mg/kg) was administered for postoperative analgesia. The pigs were sent back to their cage and had ad libitum access to water.

Experimental protocol

Fifteen hours after the establishment of fecal peritonitis or the sham procedure, the pigs were anesthetized with intramuscular injection of zolazepam and tiletamine (4 mg/kg for pigs in the sham group and 2 mg/kg for pigs in the sepsis and sepsis + PMX-HP groups; Virbac Laboratories) and atropine (0.04 mg/kg for all groups, Taiwan Biotech Co, Ltd). After anesthesia, each pig was placed on an animal surgical table, and a water-heated blanket was used to maintain normal body temperature. The rectal temperature was continuously monitored. Each pig was intubated with a size 5.5 or 6.0 endotracheal tube and ventilated with an anesthesia machine with a tidal volume of 8 mL/kg and a respiratory rate of 15 breaths/min. Subsequent anesthesia was maintained using isoflurane (0.6%-1.2% in 100% oxygen; Attane, Johnson Veterinary Supply Co Ltd, Taiwan). A pulse oximeter probe was placed on the tongue to monitor oxygen saturation. A three-lumen central catheter venous catheter (16-18-18F, Abbott Laboratories, USA) was inserted into the left carotid vein for blood sampling, fluid supplement, and drug administration. A total of 500 mL of lactated Ringer's solution (Haforman; Nang Kuang Pharmaceutical Co Ltd, Taiwan) was infused as an initial fluid supplement for anesthesia and sepsis-related hypotension. A PiCCO catheter (Pulsion Medical System, Germany) was inserted into the right femoral artery for hemodynamic monitoring with the PiCCO system (Pulsion Medical System). Cardiac index and MAP were used to guide the subsequent resuscitation with fluid supplement and norepinephrine administration to maintain a MAP ≥65 mmHg. A double-lumen central catheter (14-14F, Abbott Laboratories) was inserted into the right carotid vein in the pigs of the sepsis + PMX-HP group for subsequent hemoperfusion. The abdominal wall was disinfected with chlorhexidine solution and opened using a 30-cm incision. The autologous feces and dirty ascites were carefully removed and aspirated. Two 3-cm incisions were made at the antimesenteric site of the terminal ileum at approximately 15-20 and 35-40 cm proximal to the ileocecal junction, and the exposed mucosa was used for microcirculation examination and tissue oxygen saturation measurement. Because two segments of ileal mucosa were used for microcirculation examination, the sample size is 12 for ileal mucosa. A 3-cm incision was made at the antimesenteric site of the colon at approximately 30-40 cm distal to the ileocecal junction, and the exposed mucosa was used for microcirculation examination and tissue oxygen measurement. The left kidney was exposed from the retroperitoneal cavity, and a 2-cm square capsule was carefully removed to expose the