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Effects of terlipressin on microcirculation of small bowel mesentery in rats with endotoxic shock

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ABSTRACT

Background: Septic shock is still related to unacceptably high morbidity and mortality. Microcirculatory alteration has been demonstrated to be one important reason associated with this evolution. Vasoactive drugs are often used to restore adequate arterial pressure and tissue perfusion in septic shock. To define the roles of different drugs, the effects of terlipressin (TP) on the microcirculation of small bowel mesentery in rats with endotoxic shock were evaluated and compared with those of norepinephrine (NE).

Methods: Twenty-five adult male Wistar rats were randomized to the control ($n = 5$), TP ($n = 10$), and NE ($n = 10$) groups. After endotoxic shock was induced by intravenous lipopolysaccharide administration for 30 min, rats in the NE and TP groups were infused with saline 5 mL/kg/h and simultaneously given NE 4 μg/kg/min or TP 8 μg/kg/h. The mean arterial pressure, heart rate, blood gas analysis, and microvascular blood flow images of small bowel mesentery were recorded.

Results: After fluid resuscitation and vasopressor infusion, the mean arterial pressure was restored to the baseline values in the NE and TP groups. In the TP group, the heart rate was significantly lower compared with the NE group ($P = 0.013$). The proportion of perfused vessels and the microvascular flow index (MFI) were significantly increased; furthermore, the heterogeneity index of small vessels was markedly decreased in both the interventional groups with respect to the control group. Compared with the NE group, the MFI was significantly higher ($P < 0.05$) and the heterogeneity index was significantly lower ($P < 0.05$) in the TP group.

Conclusions: Both TP and NE improved hemodynamic and microcirculatory alterations in rats with endotoxic shock. Compared with NE, TP was more effective in promoting MFI and improving the heterogeneity of small bowel mesentery in rats.

1. Introduction

Despite the significant progress that has been made in intensive care medicine, septic shock remains associated with high morbidity and mortality [1]. During septic shock, the pronounced low blood pressure and hypoperfusion are usually because of a decreased systemic vascular resistance. Early goal-directed therapy, based on aggressive volume resuscitation and the use of vasoactive drugs, aims to restore adequate arterial pressure and tissue perfusion. These measures have been shown to improve outcome in patients with septic shock [2]. The first choice of vasoactive drugs in

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critically septic shocked patients is catecholamines, including norepinephrine (NE) and epinephrine [3]. Although vasopressor therapy is an important part of hemodynamic support in patients with septic shock, the development of adrenergic hyposensitivity and the following high doses can lead to side effects or the failure of these drugs [4]. Based on this logic, the microcirculatory alteration may play an important role that could provide an explanation for these phenomena. Yasser Sakr *et al.* [5] showed that persistent microcirculatory alteration promotes organ failure and death in patients with septic shock. Therefore, agents that are able to reduce or solve this problem would be ideal drug candidates for septic shock.

Terlipressin (TP), a neurohypophyseal hormone, is an analog of vasopressin that has different actions and is mediated by tissue-specific receptors. In shock states, TP acts by binding receptors in vascular smooth muscle (G protein–coupled V1 receptors), leading to vasoconstriction and blood pressure maintenance. A previous study has demonstrated that TP effectively reduced the NE requirements without worsening the microcirculatory blood flow in fluid-resuscitated patients with septic shock [6]. A recent meta-analysis also found that the use of TP and vasopressin, compared with NE, may decrease mortality in patients with septic shock [7]. However, whether TP can improve microcirculation and ameliorate the prognosis of septic shock is still uncertain. Based on the initial clinical results obtained using TP in the treatment of hypotension associated with septic shock, we hypothesize that TP may maintain or improve microcirculatory perfusion.

Few studies have investigated the effect of TP on the microcirculation of the digestive system, which is among the first and the most commonly injured systems that are affected by microcirculation failure during shock. The aim of this study was to evaluate whether TP can improve the small bowel mesentery microcirculation in comparison with NE in rats with endotoxic shock.

2. Materials and methods

Adult male Wistar rats were obtained from the Center for Comparative Medicine (Yangzhou University, China) and kept in a pathogen-free environment on a 12-h light–dark cycle with free access to food and water. The rats were fasted by food only and overnight before the experiment.

All animals received humane care in accordance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and with the "Guide for the Care and Use of Laboratory Animals" by the Chinese Academy of Sciences. The study protocol was approved by the Ethical Committee of Southeast University Medical School, Nanjing, China.

2.1. Animal preparation

Each rat was anesthetized by the intraperitoneal injection of 2% pentobarbital (Sigma–Aldrich, St. Louis, MO) with a dose of 45 mg/kg. A venous catheter was inserted *via* the caudal vein for fluid and drug administration, and a tracheotomy was performed. Mechanical ventilation was ensured using a small animal ventilator (Inspira ASV55-7058; Harvard Apparatus, Cambridge, MA) in the assist or control mode with a tidal volume of 6 mL/kg, a respiratory rate of 60–80 breaths/min, a fraction of inspiratory oxygen of 30%, a positive end expiratory pressure of 5 cm H_2O, and an inspiratory or expiratory ratio of 1:1. An artery catheter (24 G; B. Braun Melsungen AG, Germany) was indwelled into the left femoral artery for blood pressure monitoring and blood sampling for gas analysis. The arterial catheter was connected to a pressure transducer (DTX pressure transducer; Ohmeda KG, Erlangen, Germany) and a physiological recorder (Hellige Servomed; Hellige, Freiburg, Germany).

Then, the abdominal cavity was opened along the Hunter's line and a small bowel segment of the ileum was exposured to record microcirculation imaging. The incision was covered with a wet sterile gauze to prevent heat and fluid loss. The body temperature of animals was continuously monitored and kept at 36°C–39°C during the experimental period using a desk lamp. Arterial blood gas samples were analyzed using an ABL 625 (Radiometer, Copenhagen, Denmark) and were corrected for body temperature.

2.2. Experimental protocol

After the animal preparation, the rats were randomly allocated to one of the following three groups: control ($n = 5$), TP ($n = 10$), and NE ($n = 10$). Rats in the control group received 0.5 mL of normal saline injection, whereas rats in the NE and TP groups were induced with endotoxic shock by a slow intravenous injection over 3 min of lipopolysaccharide (LPS, 5 mg/kg, serotype O111:B4; Sigma–Aldrich). When the mean arterial pressure (MAP) dropped by >40 mm Hg over the course of 30 min [8,9], the rats allocated into the NE and TP groups received fluid resuscitation with the simultaneous administration of normal saline (5 mL/kg/h) and either NE (4 μg/kg/min) or TP (8 μg/kg/h), respectively. Then, the NE and TP infusions were titrated to restore the baseline MAP. For the control group, no fluids or drugs were administered.

2.3. Data collection

The MAP, heart rate (HR), blood gas analysis, and microvascular blood flow images of the small bowel mesentery were recorded for all animals. In the control group, these parameters were collected at the baseline after 2 and 4 h, whereas in the interventional groups (NE and TP), parameters were collected at the baseline after 30 min from the onset of the shock and 30 min after resuscitation.

Microvascular blood flow was visualized using a sidestream dark field imaging device (MicroScan; MicroVision Medical, Amsterdam, The Netherlands) with a ×5 magnification lens through an optical probe applied to the small bowel segment.

Three good quality, 20 s sequences of microvascular blood flow images were recorded and analyzed off-line by a single blinded investigator using the proper software (Automated Vascular Analysis 3.0, MicroVision Medical, Amsterdam, Netherlands) [10]. For the microcirculation analysis, we considered only small vessels that had a diameter <20 μm, as previously described [10]. The total vessel density (TVD), perfused vessel density (PVD), proportion of perfused vessels