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Effects of pyruvate-enriched peritoneal dialysis solution on intestinal barrier in peritoneal resuscitation from hemorrhagic shock in rats

Xiao-Guang Lu, MD,[a,1,*] *Xin Kang, MM,*[b,1] *Fang-Qiang Zhou, MD,*[c,d,**] *Xiao-Zhou Wang, MM,*[a] *Shuai Guo, MM,*[a] *Zhi-Wei Fan, MM,*[a] *Zheng-Kai Liang, MM,*[e] *and Jie Yu, MM*[a]

[a] *Department of Emergency Medicine, Zhongshan Hospital, Dalian University, Dalian, China*
[b] *College (Institute) of Integrative Medicine, Dalian Medical University, Dalian, China*
[c] *Fresenius Medical Care, Dialysis Centers at Chicago, Rolling Meadows, Illinois*
[d] *Shanghai Sandai Pharmaceutical R & D Co., Pudong, Shanghai, China*
[e] *Graduate School of Zunyi Medical University, Zunyi, China*

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ABSTRACT

Background: To investigate protective effects of pyruvate-enriched peritoneal dialysis solution (P-PDS), compared with lactate-PDS (L-PDS), on the intestinal mucosal barrier in peritoneal resuscitation (PR) from severe hemorrhagic shock (HS) in rats.

Materials and methods: Fifty male SD rats were randomly divided into five groups ($n = 10$): group sham, group control (HS without fluid resuscitation), group intravenous resuscitation (IVR) (HS with IVR only), group L-PDS (HS with IV infusion plus PR with L-PDS), and group P-PDS (HS with IV infusion plus PR with P-PDS). HS was induced by hemorrhage with mean arterial pressure 40 mm Hg for 60 min. In three groups with fluid rehydration, IVR included shed blood and dl-lactate Ringer solution equal to two times the volume of shed blood during 60 min; in two groups with PR, 20 mL of L-PDS, or P-PDS were infused when IV infusion started after HS into the peritoneal cavity in 20 min, respectively. Blood samples were taken for determinations of pH, base excess, $PaCO_2$, PaO_2, and D-LA 60 min post fluid resuscitation. After rats were sacrificed, a segment of intestine was harvested for the detection of expressions of intestinal barrier proteins: zonula occludens-1 (ZO-1) and phosphorylated vasodilator-stimulated phosphoprotein (p-VASP) by Western blot and immunohistochemistry. Intestinal morphologic alterations were also observed.

Results: Blood pH, base excess, and PaO_2 were higher, whereas $PaCO_2$ and D-LA were lower in group P-PDS than in other three HS groups ($P < 0.05$ and $P < 0.01$, respectively). Severe acidosis was nearly corrected in group P-PDS. Intestinal barrier proteins ZO-1 and p-VASP were significantly preserved in group P-PDS than in group L-PDS ($P < 0.05$) although they were improved in group L-PDS in comparison with other two HS groups ($P < 0.05$ or

* *Corresponding author*. Department of Emergency Medicine, Zhongshan Hospital, Dalian University, Dalian 116001, China. Tel.: +86 81 411 62893126; fax: +86 81 411 62893555.
** *Corresponding author*. Fresenius Medical Care, Dialysis Centers at Chicago, 4180 Winnetka Avenue, Rolling Meadows, IL, 60008. Tel.: +17 08 785 3568; fax: +18 47 394 4621.
E-mail addresses: dllxg@126.com (X.-G. Lu), fqzh60130@yahoo.com (F.-Q. Zhou).
[1] These authors contributed equally to this work.

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$P < 0.01$). Expressions of barrier proteins by Western blotting in group P-PDS were reversed to normal. The score of intestinal epithelial damage index was reduced in group L-PDS, compared with other two HS groups ($P < 0.05$), however, it was significantly lower in group P-PDS than in group L-PDS ($P < 0.05$).

Conclusions: Pyruvate was superior to lactate in PDS in the correction of severe acidosis with PR. P-PDS was more preservative of expressions of intestinal ZO-1 and p-VASP and mucosal barrier function, compared with L-PDS in PR from severe HS in rats.

1. Introduction

Hemorrhagic shock (HS) is one of the most common critical illnesses in clinical settings, its main pathophysiologic alteration is the systemic reduction of effective circulating blood volume and tissue hypoperfusion. Currently, the major intervention of resuscitation from HS is intravenous rehydration. However, progressive visceral vasoconstriction and splanchnic hypoperfusion, including the reduction of intestinal blood flow, still remain despite aggressive intravenous fluids administration, which may lead to intestinal barrier dysfunction and endotoxemia, massive release of inflammatory mediators, and multiorgan failure [1–3].

Intraperitoneal resuscitation (PR) is a novel experimental protocol for resuscitation from HS first proposed in 2003, which used a commercial lactate-based 2.5% PD-2 peritoneal dialysis solution (L-PDS) infused into the peritoneal cavity when intravenous fluid resuscitation was started or completed [4,5]. Studies have shown that PR profoundly preserved intestinal mucosal barrier function, reversed visceral hypoperfusion, and improved survival in rats with severe HS [6–8]. PR may be a promising approach in clinical resuscitation from severe shock in the near future.

Although L-PDS has been safely applied for over half a century in clinical peritoneal dialysis and is available for PR in the potential clinical setting, it has been discovered that pyruvate is advantageous over lactate in PDS [9]. Pyruvate is a key metabolite of glycolysis and the metabolic hub of carbohydrates, proteins, and lipids. It has been demonstrated that pyruvate is multiorgan protective and enables to correct disturbances of glucose and acid-base metabolisms in hypoxic, even anoxic conditions [10–12]. Recent findings showed that pyruvate in PR or enteral resuscitation from severe HS not only greatly restored visceral blood perfusion, including liver, kidney, and intestine but also preferably preserved the integrity of intestinal mucosal barrier, including the expression of zonula occludens-1 (ZO-1) protein and Na^+–K^+–ATPase activity [13,14]. In addition, it has been discovered that vasodilator-stimulated phosphoprotein (VASP) is an important protein involved in intestinal tight junction regulatory processes and phosphorylated VASP (p-VASP) plays a pivotal role in determining its function [15,16]. Therefore, this study further focused on the protective effect of PR on intestinal barrier function, including ZO-1 and p-VASP expressions, with equimolar pyruvate-enriched peritoneal dialysis solution (P-PDS), compared with commercial L-PDS, in rats subjected to severe HS.

2. Materials and methods

2.1. Materials

Male Sprague–Dawley rats (50, aging 10–12 wk, weighing 280 ± 15.5 g) were purchased from Dalian Medical University Experimental Animal Center. Animals were housed in a colony room under a 12 h light–dark cycle at a constant temperature (23 ± 1°C) and humidity (50 ± 5%). Experimental animals were fasted over night, but allowed free access to water until 4 h before surgery. All procedures involved in animals and their care were conducted in conformity with National Institute of Health Guide for the Care and Use of Laboratory Animals and animal experiments were approved by the Dalian Medical University Animal Research Ethics Committee, Dalian, China.

2.2. Experimental methods

2.2.1. Reagents

L-PDS (2.5% Dianeal PD-2, pH 5.2 with the osmolality of 396 mOsmol/L) was purchased from Baxter Healthcare (Baxter, Guangzhou, China). P-PDS was prepared in the Pharmaceutical Department, Zhongshan Hospital, Dalian University with equimolar pyruvate to replace lactate in L-PDS with the following composition: sodium pyruvate 4.40 g, calcium chloride ($2H_2O$) 0.257 g, magnesium chloride ($6H_2O$) 0.05 g, sodium chloride 5.38 g, and anhydrous glucose 25 g in 1000 mL. The solution was adjusted to pH 5.2 with diluted HCl, disinfected with filtration, and restored at 4°C. Its calculated osmolality was 395 mOsmol/L. Both solutions were warmed to room temperature before the experiments. Pyruvate was purchased from Sigma (Sigma, St. Louis, MO). Sodium pentobarbital were obtained from Sinopharm Chemical Reagents (Beijing, China).

2.2.2. Preparation of an animal model with severe HS

Sodium pentobarbital (3%, 40 mg/kg, intraperitoneally) was used for anesthesia of rats during the experiment. Animals without tracheal intubations were allowed to breathe room air spontaneously. With aseptic technique, poly-ethylene (PE50) catheters were placed in the right carotid artery for continuous artery blood pressure monitoring with a multifunctional physiological recorder (BIOPAC, Goleta, CA), in the left femoral artery for blood withdrawal, and in the right femoral vein for fluid infusion. All animals were acclimated for about 10 min after surgery. Rectal temperature was maintained at 37°C with a heating pad and a heating lamp.