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ORIGINAL ARTICLE

Serum citrulline as a diagnostic marker of sepsis-induced intestinal dysfunction

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Summary

Objective: To investigate the use of citrulline as an indicator for diagnosing septic acute intestinal dysfunction (SAID) in a rat model.

Methods: SD rats were divided into three groups: a normal group (A), a model group (B), and a glutamine group (C). Group B was divided into a 36-h group (B1) and a 72-h feeding group (B2). The concentrations of serum citrulline, intestinal fatty acid-binding protein (I-FABP) and intestinal glutamine and histopathological changes were measured.

Results: The lengths of the villus and thicknesses of the mucosal layer in groups B1, B2 and C were significantly different from those in group A. Citrulline concentrations in groups B1, B2 and C were lower than in group A; the serum concentrations in group C were significantly greater than in groups B1 and B2. The I-FABP levels of groups B1, B2 and C were higher than group A; I-FABP levels of groups B1 and B2 were higher than group C. Intestinal glutamine levels of groups B1 and B2 were lower than groups A and C. The serum citrulline of group C was negatively correlated with I-FABP and Chiu's score.

Conclusions: Serum citrulline could be used as the diagnostic indicator of SAID.

Introduction

Critically ill patients exhibit various degrees of functional damage to the intestinal epithelium because of reduced splanchnic blood flow, increased mucosal permeability and bacterial translocation [1]. Reduced splanchnic blood flow is also a major concern during the development of multiple organ dysfunction syndrome (MODS) [1]. Currently, there is no simple, accurate and objective method for assessing the intestinal functions of critically ill patients. The most frequently used procedures for measuring enterocyte function in critically ill patients are sugar absorption tests, bomb calorimetry, and tonometry. However, the above methods

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have some drawbacks and limitations, and there is no gold standard for determining gastrointestinal function [2].

Citrulline is a non-protein amino acid, produced almost entirely by enterocytes from glutamine [3] that could serve as an index reflecting the function and mass of small intestinal epithelial cells [4]. Previous studies on citrulline have focused only on diseases such as short bowel syndrome [5], viral enteritis [4], intestinal transplants [6], intestinal toxicity due to chemotherapy [7], and Crohn's disease [8]. More recently, there have been a few studies that have focused on critically ill patients [9,10]. Crenn et al. showed that plasma citrulline levels decreased, and were lower when digestive bacterial translocation occurred at the onset of septic shock. The decreased citrulline levels could be a reflection of early acute intestinal dysfunction [11]. There has also been a report showing that glutamine concentrations decrease in the muscles and plasma of critically ill patients [12]. A citrulline generation test (CGT) [13] was developed based on the assumption that functional competent enterocytes are required to convert glutamine to citrulline. The aim of the current study was to investigate the use of citrulline levels as an indicator of septic acute intestinal dysfunction using a rat model.

Materials and methods

Experimental animals and environment

The Shanghai Super B&K Laboratory Animal Co., Ltd provided 30 male Sprague-Dawley rats (6 months old and weighing 140—160 g). The feeding and experiments were all performed in the laboratory of Nanjing Keygen Technology Development Co., Ltd. For experiments, rats were put into in cages individually, at 20—25 °C, with a humidity of 40—70%, and 12 h light-dark cycles. The experimental animals had free access to food and water. The experiments had been approved by the Animal Care and Use Committee of the Institute of Nanjing University of Chinese Medicine.

Drugs and preparation

Lipopolysaccharide (LPS), L-glutamine, and *Escherichia coli* 055 (B5, No. 2880) were obtained from SIGMA, (St. Louis, MO, USA). PEPTI-2000 variant powder, consisting of short peptides of whey protein, medium-chain triglycerides and maltodextrin, was produced by Nutricia (Zoetermeer, Dutch).

LPS was dissolved in saline at a concentration of 0.45 mg/mL. Peptisorb (125 g) was dissolved in 500 mL water. L-glutamine was dissolved in double distilled water at a concentration of 0.375 mg/mL.

An I-FABP ELISA kit was obtained from Shanghai Hufeng Chemical Co., Ltd. Freeze-drying agent (Christ, Germany), phenylisothioovyanate (PITC) (Sigma, St. Louis, MO, USA), triethylamine, sodium acetate, ethanol, disodium hydrogen phosphate, phosphoric acid and acetic acid (all analytical grade) were obtained from Sinopharm, (Sinopharm, Shanghai, China). HPLC-grade methanol and acetonitrile were obtained from Merck (Darmstadt, Germany). Citrulline and Glu standards were obtained from Sigma.

Instrumentation

Transmission electron microscopy was performed using a JEM-1011 (Japan). Metabolite analyses were done on a 1525 HPLC (Waters, USA), and a 2489 UV detector (Waters, USA), using an XBridge C18 column (5 μm, 4.6 × 250 mm), driven by an Empower2 chromatography workstation.

Experimental methods

Experimental grouping

Each of the 30 rats was designated to a group (for a total of 10 rats per group): a normal group (A); a model group (B), which was subdivided into groups B1 and group B2 with five rats in each subgroup; and a glutamine group (C).

Preparation of animal models

LPS can cause intestinal mucosal edema, cell necrosis, increased intestinal permeability, and intestinal mucosal barrier damage, eventually leading to mucosal damage [14]. A sepsis model [15] was prepared using lipopolysaccharide (LPS) at a dose of 4.5 mg/kg as described previously by Mercer et al. [16].

Group A rats were injected intraperitoneally with saline, and were fed standard laboratory feed (343.5 kcal/kg) at a rate of 80 kcal/d (Xietong Organism CO., Ltd.) for 72 h. Group B rats were injected intraperitoneally with 0.45 mg/mL LPS solution at a dose of 1 mL/100 g. Three intraperitoneal injections were completed within 10 minutes. Beginning 12 h later, 4 mL of 25.2% peptisorb solution (80 kcal) was orally administrated by gavage five times per day for 36 h (a mean of 7 doses in 36 h) in group B1 or 72 h (a mean of 15 doses in 72 h) in group B2. Group C rats were injected intraperitoneally with LPS as for group A, and gavaged with peptisorb and glutamine (3.75 g/kg/d) for 72 h.

Specimen collection

Twelve hours after the final feedings, blood was collected from the orbital venous plexus, and rats were sacrificed for pathological examination of the ileum. Blood was centrifuged at 13,000 rpm for 10 minutes; serum was stored at −80 °C until further testing.

Analyses

Pathological examination

Small intestines were fixed in 10% formalin followed by dehydration, paraffin embedding, and sectioning. The sections were stained with HE [17] and examined microscopically for edema of the villi, degeneration and necrosis of epithelial cells, congestion, edema and inflammatory cell infiltration of intestinal layers. Samples were scored according to the six-point Chiu score [18]: the degree of pathology ranged from mild to serious based on a scale from 1 to 5; no obvious lesions received 0 points. For EM analysis, software was used to measure the length of the villi and mucosal thickness (DX45, Olympus, Japan, DP2-BSW image analysis system). We calculated the ratio of the average length of villi to mucosal thickness of each animal, and the average