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Basic nutritional investigation

Parenteral glutamine supplementation in combination with enteral nutrition improves intestinal immunity in septic rats

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ABSTRACT

Objectives: The gut-associated lymphoid tissue is continuously exposed to antigens in the gut lumen and becomes the first line of defense against enteric bacteria and associated toxin. The aim of this study was to investigate the effects of parenteral glutamine (GLN) supplementation in combination with enteral nutrition (EN) on intestinal mucosal immunity in septic rats by cecal ligation and puncture (CLP).

Methods: Male Sprague-Dawley rats were randomly assigned into four groups: A sham CLP + EN + saline group (n = 10), a sham CLP + EN + GLN group (n = 10), a CLP + EN + saline group (n = 10), and a CLP + EN + GLN group (n = 10). At 2 h after CLP or sham CLP, all rats in each of the four groups received an identical enteral nutrition solution as their base formula. Then, the rats in the sham CLP + EN + GLN group and CLP + EN + GLN group were given 0.35 g GLN/kg body weight daily for 7 d, all at the same time, via a tail vein injection; whereas those in the sham CLP + EN + saline group and CLP + EN + saline group were daily administered isovolumic sterile 0.9% saline for comparison. All rats in each of the four groups were given 290 kcal/kg body wt/d for 7 d. At the end of the seventh day after the nutritional program was finished, all rats were euthanized and the entire intestine was collected. Total Peyer's patches (PP) cell yield was counted by a hemocytometer. The percentage of PP lymphocyte subsets was analyzed by flow cytometry. The number of intestinal lamina propria IgA plasma cells was determined by the immunohistochemistry technique. The intestinal immunoglobulin A (IgA) levels were assessed by ELISA. PP apoptosis was evaluated by terminal deoxyuridine nick-end labeling.

Results: The results revealed total PP cell yield, the numbers of PP lymphocyte subsets, intestinal lamina propria IgA plasma cells, and intestinal IgA levels in the CLP + EN + GLN group were significantly increased when compared with the CLP + EN + saline group ($P < 0.05$). On the other hand, the number of TUNEL-stained cells within PPs in the CLP + EN + GLN group was markedly decreased as compared with the CLP + EN + saline group ($P < 0.05$).

Conclusion: The results of this study show that parenteral glutamine supplementation in combination with enteral nutrition may attenuate PP apoptosis, increase PP cell yield and intestinal lamina propria IgA plasma cells, and subsequently improve intestinal mucosal immunity. Clinically, these results suggest therapeutic efforts at improving intestinal immunity may contribute to the prevention and treatment of sepsis.

Introduction

Severe sepsis is still one of the leading causes of death in critically ill patients [1,2]. The failure of the intestinal barrier appears to play a crucial role in the initiation and the development of this detrimental state, because the intestine is not only a nutritional organ to digest and absorb nutrients, but also a reservoir of bacteria and endotoxin, which might translocate into systemic organs and systemic circulation under the condition of the stress and injury [3]. Therefore, amending the impaired intestinal immune barrier function, reversing immune

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dysfunction, and modulating uncontrolled inflammatory response play an important role in the prevention and treatment of sepsis.

Enteral nutrition (EN) has exhibited clinical benefits such as supporting the immune and metabolic responses as well as preserving gut barrier integrity [4]. In critically ill septic patients, a calorie and protein delivery closer to recommended amounts by EN in the early phase of intensive care unit stay was associated with a more favorable outcome [5]. It appears, however, that unless there is severe protein-calorie malnutrition, the provision of the traditional nutritional support has little effect on host immune function. With the development of the nutritional therapeutic theory and technique, it has been recognized that nutritional support is not just simply the provision of calories and nitrogen for critically ill patients to prevent and treat malnutrition, which is of particular importance, but it also attains the aim of disease treatment and metabolic modulation through the pharmacologic role of some specific nutrients [6–8]. One of the more heavily studied nutrients for this purpose is the amino acid glutamine (GLN).

GLN, traditionally considered to be a nonessential amino acid, is now regarded as 'conditionally essential' during inflammatory response and the hypermetabolic state. Numerous published data have demonstrated that GLN can improve gut barrier function, modulate inflammatory response and stimulate immune function [9–21]. GLN has been widely applied in the critically ill patients. However, recently a meta-analysis of randomized clinical trials and a randomized controlled trial proclaimed GLN supplementation in critically ill patients had no significant effects on infections, length of stay and mortality [22,23]. Also, Briassouli et al. found although apparently safe in animal models, premature infants, and critically ill children, glutamine supplementation did not reduce mortality or late onset sepsis [24]. Further, Heyland et al. even reported GLN supplementation was associated with an increase in mortality among critically ill patients with multiple organ failure [25,26]; whereas Wischmeyer et al. strongly supported that parenteral GLN supplementation in critically ill patients could improve survival rate and decrease infectious complications, costs and hospital length of stay [27–32]. These conflicting and confusing results may bring about the controversy over GLN supplementation in critically ill patients. Although current data are contradictory, the potential benefit of parenteral GLN supplementation has been one of the most heavily investigated nutritional interventions in critical care medicine and the further exploration of the mechanisms of the GLN pharmacologic role appears warranted and necessary.

The gut-associated lymphoid tissue (GALT), being one of the largest lymphoid organs in the body and containing up to 70% of the body's immune cells [33], is continuously exposed to antigens in the gut lumen and becomes the first line of defense against enteric bacteria and associated toxin [34]. However, major thermal injury, trauma-hemorrhagic shock and sepsis frequently contribute to the impairment of the GALT function, subsequently leading to the suppression of intestinal mucosal immunity, which is closely associated with increased gut-origin bacterial translocation [18,19,34–37]. The aim of this study was to investigate whether parenteral glutamine supplementation in combination with enteral nutrition could reverse GALT dysfunction and improve intestinal mucosal immunity in septic rats by cecal ligation and puncture (CLP).

Materials and methods

Animals

Male Sprague-Dawley (SD) rats weighing 200–250 g were used in this experiment. All rats were housed in stainless steel cages maintained in a temperature- and humidity-controlled room. Rats were allowed free access to a rodent chow and water for a 7-d acclimatization period. All procedures of this study were in accordance with the guide for the care and the use of laboratory animals published by the Ministry of Science and Technology of China and were approved by the Ethics Committee of our institute.

Surgical procedure and grouping

Rats were randomly assigned into 4 groups: A sham CLP + EN + saline group (n = 10), a sham CLP + EN + GLN group (n = 10), a CLP + EN + saline group (n = 10), and a CLP + EN + GLN group (n = 10). Sepsis was induced by CLP. Briefly, the rats in the CLP + EN + saline group and the CLP + EN + GLN group were weighted and anesthetized by intraperitoneal (i.p.) injection of 50 mg/kg body weight pentobarbital sodium, were fixed in supine position, the entire ventral surface was shaved, and the abdomen was sterilely opened through a 2-cm middle incision. The cecum was exposed. The distal 1 cm of the cecum was ligated with a 4–0 silk suture and perforated twice through-and-through with a 21-gauge needle. A small amount of feces was extruded to ensure the wound was patent. The cecum was placed back into the abdominal cavity, and the abdomen was closed with 4–0 silk suture in two layers. Immediately the CLP rats received a 50 mL/kg body weight i.p. injection of sterile 0.9% saline for fluid resuscitation. The rats in the sham CLP + EN + saline group and the sham CLP + EN + GLN group were treated in the same procedure as previously mentioned, except the cecum was exposed but not ligated or punctured, and then replaced.

Nutrition program and sample collection

Enteral feeding was administered by oral gavage at 2 h after CLP or sham CLP and continued for 7 d. All rats in each of the four groups received an identical enteral nutrition solution as their base formula. As described in Table 1, convention enteral nutrition solution provided, per L, 6300 KJ, 60 g protein, 185 g carbohydrate, and 58.4 g fat. The supplied energy ratio of the enteral nutrition solution was 49% carbohydrate, 35% fat, and 16% protein and the proportion of non protein calorie to nitrogen was 133:1. Then, GLN was administered as alanyl-glutamine dipeptide (Dipeptiven, Fresenius-Kabi, Homburg, Germany). The rats in the sham CLP + EN + GLN group and the CLP + EN + GLN group were given 0.35 g GLN/kg body weight daily for 7 d, all at the same time, via a tail vein injection; whereas those in the sham CLP + EN + saline group and the CLP + EN + saline group were administered isovolumic sterile 0.9% saline daily for comparison. All rats in each of the four groups were given 290 kcal/kg body wt/d by oral gavage. A quarter of the daily requirement of energy was supplied within the first 24 h, and a half in the second 24 h. After the second day, the full energy requirement was given. All rats were allowed to drink water freely. At the end of the seventh day after the nutritional program was finished, all rats were sacrificed, and a middle abdominal incision was made, and the entire intestine was carefully removed for further analysis.

Cell isolation

The number of Peyer's patches (PPs) from each small intestine removed was counted and lymphocytes were isolated from PPs. Briefly, PPs were excised from the serosal side of the intestine and placed in Petri dishes containing 5 mL of cold Hank's balanced salt solution (HBSS). The tissues were gently glass ground, and the cell suspension was passed through a 150 mesh stainless-steel screen into a

Table 1
Formulas of enteral nutrition solution

Component (units)	Enteral nutrition solution
Calorie (KJ)	6300
Protein (g/L)	60
Nitrogen (g/L)	9.4
Non protein calorie: Nitrogen (kcal:g)	133:1
Fat (g/L)	58.4
Carbohydrate (g/L)	185
Composition of total Calorie	
Protein	16%
Fat	35%
Carbohydrate	49%