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Original Research

Fish oil–supplemented parenteral nutrition could alleviate acute lung injury, modulate immunity, and reduce inflammation in rats with abdominal sepsis

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ABSTRACT

The objectives were to confirm that intravenous fish oil (FO) emulsions could alleviate acute lung injury, modulate immunity, and reduce inflammation in rats with abdominal sepsis and to explore the mechanisms of these effects. Thirty-six adult male Sprague-Dawley rats were divided into 4 groups randomly. Two days after central venous catheterization, rats were subjected to cecal ligation and puncture to produce abdominal sepsis. Rats were assigned to receive normal saline or total parenteral nutrition (TPN) containing standard soybean oil emulsions or FO-supplemented TPN at the onset of sepsis for 5 days. A sham operation and control treatment were performed in control group rats. Acute lung injury scores, peripheral blood lymphocyte subsets, plasma cytokines, and Foxp3 expression in the spleen were determined. Compared with the normal saline and TPN without FO, FO-supplemented TPN beneficially altered the distributions of the T-lymphocyte subsets and downregulated the acute lung injury scores, plasma cytokines, and expression of Foxp3 due to sepsis. Fish oil–supplemented TPN can decrease acute lung injury scores, alleviate histopathology, reduce the bacterial load in the peritoneal lavage fluid, modulate the lymphocyte subpopulation in the peripheral blood, downregulate Foxp3 expression in the spleen, and reduce plasma cytokines, which means that FO-supplemented TPN can alleviate acute lung injury, modulate immunity, and reduce inflammation in rats with abdominal sepsis.

1. Introduction

Sepsis is one of the most common causes of death among admissions to medical intensive care units across the world. With the high mortality and poor prognosis of sepsis, focus has turned to treatment strategies. People are forced to reconsider the pathophysiological changes and pathogenesis of sepsis, and the following has been gradually recognized: normal immune

Abbreviations: ALI, acute lung injury; CLP, cecal ligation and puncture; FO, fish oil; IgA, immunoglobulin A; IL, interleukin; mAb, monoclonal antibody; NS, normal saline; PCR, quantitative real-time polymerase chain reaction; PE, phycoerythrin; PLF, peritoneal lavage fluid; PUFA, polyunsaturated fatty acid; SO, soybean oil; TPN, total parenteral nutrition.

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balance is disturbed because of excessive systemic inflammatory response syndrome and the accompanying compensatory anti-inflammatory response syndrome during the initial phase of sepsis; simultaneously, the body's reaction to these pathophysiological responses can be interrupted as a result of a severe immunodeficiency. [1] Thus, a new perspective is provided by modulation of the immune response for the treatment of sepsis.

Research indicates that sepsis-induced immunosuppression or immunoparalysis (including innate and adaptive immunity) plays a central role in the impaired clearance of microorganisms, resulting in patients' inability to fight primary bacterial infection and making the host more susceptible to intractable infection or to a new secondary infection [2]. The main function of Tregs ($CD4^+CD25^+Foxp3^+$ T cells) is to inhibit T-cell proliferation after trauma or sepsis and to inhibit the production of protective Th1-type cytokines. The goal of the immune response to sepsis is to maintain the balance between proinflammatory responses and anti-inflammatory responses. Tregs certainly play an important role in maintaining this balance; but if the negative immunoregulation of Tregs is abnormally dominant, the balance will be broken, resulting in incurable immunosuppression. Uncontrolled immunosuppression in the late period of sepsis is the main cause of poor prognosis and even death. Hence, perhaps the abnormal activities of Tregs contribute to severe sepsis and septic shock. In addition, it was reported that Tregs played a suppressive role by inhibiting T-cell proliferation and disrupting the production of protective Th1-type cytokines, such as interleukin (IL)-10 [3]. Therefore, the core of treatment strategies in sepsis depends on the timely restoration of the balance of proinflammatory and anti-inflammatory response [4], such as changing the relative number of Tregs.

Increasing evidence illustrates that diets supplemented with n-3 fatty acids exert anti-inflammatory effects [5]. Eicosanoids derived from n-6 polyunsaturated fatty acids (PUFAs) possess proinflammatory and immunoactive properties, whereas eicosanoids derived from n-3 PUFAs (eg, eicosapentenoic acid and docosahexenoic acid) have anti-inflammatory functions, traditionally by inhibiting the formation of n-6 PUFA–derived eicosanoids [5]. Possibly because n-3 PUFAs have a similar structure to n-6 PUFAs, n-3 PUFAs suppress the metabolism of n-6 PUFAs and the production of inflammatory mediators. However, it still remains controversial whether fish oil (FO) could modulate immune responses. Some studies have declared that FO possesses immunomodulation properties [6]. However, a recent randomized crossover study found that the short-term infusion of an FO-based lipid emulsion did not improve the immune function in healthy volunteers [7]. Therefore, we need more evidence to draw a definitive conclusion on this controversial issue.

In this study, we treated rats with total parenteral nutrition (TPN) with or without FO after inducing sepsis. The study was performed to confirm that FO emulsions could alleviate acute lung injury (ALI), modulate immunity, and reduce inflammation in rats with abdominal sepsis by measuring ALI scores, peripheral blood lymphocyte subsets, plasma cytokines, and Foxp3 expression in the spleen.

2. Methods and materials

2.1. Animals

This research was approved by the Animal Care and Use Committee of the Medical School of Qingdao University.

Adult male Sprague-Dawley rats (200-220 g) (Qingdao, China) were used for this study and were housed in a temperature- and light-controlled room (21°C-25°C; 12-hour light-dark cycle) with standard chow and water ad libitum for 1 week before the experiment.

2.2. Groups

Thirty-six rats were randomly divided into 4 groups, with 9 rats per group: (1) the control group (sham group): rats fed with standard chow and water ad libitum after the sham operation; (2) the normal saline (NS) group: rats underwent a cecal ligation and puncture (CLP) operation and received NS intravenously with standard chow and water ad libitum; (3) the FO group: rats received TPN containing a mixture of soybean oil (SO) and FO with water ad libitum after sepsis was induced; and (4) the SO group: rats received water ad libitum and TPN without FO after CLP operation.

2.3. Anesthesia

The animals were anesthetized with an intraperitoneal injection of chloral hydrate (0.3 mL/100 g body weight).

2.4. Central venous catheterization

After overnight fasting, the animals were anesthetized by the methods stated above and underwent central venous catheterization as previously described [8]. The rats were allowed to recover for 2 days with standard chow and water provided ad libitum, and NS was constantly infused (0.8 mL/h) using an infusion pump (Lifepum, Beijing, China) in the first 6 hours. Identical surgical procedures were performed on the control animals, but the catheter was placed subcutaneously.

2.5. Cecal ligation and puncture

Two days after tube insertion, sepsis was produced by CLP as described elsewhere [9]. In brief, the rats were anesthetized as described above after fasting for 12 hours. The cecum was exposed after a 2-cm midline laparotomy was performed, tightly ligated just distally to the ileocecal valve to avoid intestinal obstruction, punctured in a single pass through the anterior and posterior wall with a 20-gauge needle, and returned to the abdominal cavity; and the incision was closed. Standard fluid resuscitation was carried out in the first 6 hours of sepsis inducement. Rats in the NS group, the SO group, and the FO group were infused continuously with NS at 2 mL/h with free access to a standard chow diet, TPN containing a standard SO emulsion, or FO-supplemented TPN for 5 days, respectively. In the sham group, the cecum was identified after a laparotomy, lifted out of the peritoneal cavity, squeezed gently, and returned; and then the abdominal cavity was closed.