Tri-iodothyronine supplement protects gut barrier in septic rats

Zhi-Li Yang, Lian-Yue Yang, Geng-Wen Huang, He-Li Liu

INTRODUCTION

In recent years, laboratory and clinical researches have strongly shown that the gastrointestinal tract plays a pivotal role in the occurrence of sepsis and multiple organ dysfunction syndrome (MODS)[1-4]. It is believed that gut barrier failure is a “trigger point” in sepsis and MODS, and some substances including intestinal trefoil factor[5], glucagon-like peptide 2[6-8] and glutamine[9,10] may protect gut barrier, but we are still facing either poor efficacy in clinical application or the challenge of translating the findings from the bench to the bedside. Therefore, it has become a breakthrough that may prevent MODS occurrence, improve septic prognosis and further seek for protective substances of gut barrier.

The “euthyroid sick syndrome, ESS” is defined as a decreased concentration of plasma tri-iodothyronine (T3) with normal or low thyroxine (T4), but serum thyroid-stimulating hormone concentration is normal[11,12]. This syndrome is seen in states in which there are significant insults to the host including surgery, starvation, myocardial infarction, hypothermia and sepsis. In the study of ESS, it was reported that T3 supplement may protect some organ functions[13], such as pulmonary function during sepsis[14], and donor myocardial function after transplantation[15]. Recently, in sepsis, T3 augmentation was shown by Chapital et al to increase the circulating antithrombin III levels, which is a critical material to prevent disseminated intravascular coagulation (DIC)[16]. An earlier study revealed that hypothroid hormone resulted in atrophy of intestinal epithelial cells, decreased mucosal DNA and protein contents, shortened the villi height, decreased the cryt depth and rates of utilization of glucose and glutamine, and consequently impaired the gut barrier[17]. Based on these studies, we hypothesized that T3 supplement may protect gut barrier in sepsis. This study was to investigate the relationship between T3 and gut barrier in septic rats.

METHODS

Twenty-two rats were randomized into three groups: sham group (n=6), sepsis group (n=8), and sepsis plus tri-iodothyronine (T3) group (n=8). Septic rat model was established through cecal ligation and puncture (CLP). After 5 h, sham and sepsis groups received saline, and the remaining group received T3 intraperitoneally. Twenty-one hrs After CLP, intestinal permeability and serum free T3 and T4 were measured with fluorescence spectrophotometer and by radioimmunoassay, respectively. Intestinal ultrastructure and histologic morphology were observed under transmission electron microscopy (TEM) and light microscopy, respectively.

RESULTS

After 21 h, septic symptoms and signs in sepsis plus T3 group were milder than those in sepsis group. Serum FT3 or FT4 concentration in sepsis group was lower than that in sham group (1.59±0.20, 3.41±2.14 pmol/L vs 3.44±1.40, 9.53±3.39 pmol/L, P<0.05), and FT3 concentration in sepsis plus T3 group (3.40±1.65 pmol/L, P<0.05) was corrected. Portal concentration of fluorescein isothiocyanate-dextran (FITC-D) in sepsis group (2.51±0.56 mg/L) was higher than that in sham group (1.22±0.21 mg/L, P<0.01), and in sepsis plus T3 group (1.68±0.38 mg/L) it was decreased significantly (P<0.01). TEM and light microscopy showed that T3 supplement preserved well ultrastructure and morphology of intestinal mucosa in septic rats.

CONCLUSION

Tri-iodothyronine supplement protects gut barrier in septic rats.

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MATERIALS AND METHODS

Animals

Twenty-two adult male specific pathogen free (SPF) Sprague-Dawley rats weighing from 250 to 350 g were utilized in this investigation. The animals were purchased from Department of Animal Laboratory of Xiangya Medical College. Rats used in the present study were cared in accordance with the directory of Central South University Animal Care Unit, and the guidelines of the National Institutes of Health on welfare of laboratory animals.

Animal model

Rats were randomly divided into three groups: sham group (n=6), sepsis group (n=8), and sepsis plus T3 group (n=8). Sepsis was induced by cecal ligation and puncture (CLP) as described by Wichterman et al[18]. Under 3 % pentobarbital natrium anesthesia, a laparotomy was performed (the size of the incision was 2.0 cm), and the cecum was ligated just distally to the ileocecal valve to avoid any intestinal obstruction and was punctured across the intestine once with an 18 gauge needle. Punctured holes were placed 1-0 silk thread in case they were blocked up. The cecum was then returned to the peritoneal cavity and the abdomen was closed in two layers. Laparotomy in sham group was performed and the cecum was manipulated, but neither ligated nor punctured. All animals were resuscitated subcutaneously with 50 ml/kg body weight of normal saline at the completion of surgery. After 5 h, sepsis plus T3 group were injected intraperitoneally with 1.5 ml/kg body weight of T3 (0.01 g/L, Sigma), and sham group and sepsis group received 1.5ml/kg body weight of normal saline. All animals were anesthetized 2hrs post-CLP once more with

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were frozen at -20°C were evaluated under light microscopy. After 21 hrs, 3 ml blood samples were collected from inferior vena cava, centrifuged at 1500×g for 15 min. The supernatants haematoxylin and eosin. Intestinal morphologic characteristics in paraffin wax. Tissue sections (5 µm) were processed by conventional methods. The tissue was embedded in citrate, and examined under a Hitachi H-600 electron microscope. A longitudinal 1 cm segment of intestine was removed and rinsed in normal saline, fixed in 10% neutral formalin, and counter-stained with uranyl acetate and lead on copper grids. Transmission electron microscopy (TEM) was then dehydrated in graded series of ethanol and propylene for at least 24 hours, and counter-fixed in 2% osmium tetroxide containing 150 mM chloride natrium. The diluted plasma was immediately diluted with 1.9 ml of 50 Mm Tris(pH10.3) buffer, pH7.4 for one hour. They were then dehydrated in graded series of ethanol and propylene oxide and embedded in Epon 812. Sections were cut, collected on copper grids, counter stained with uranyl acetate and lead citrate, and examined under a Hitachi H-600 electron microscope.

Histological assessment
A longitudinal 1 cm segment of intestine was removed and rinsed in normal saline, fixed in 10% neutral formalin, and processed by conventional methods. The tissue was embedded in paraffin wax. Tissue sections (5 µm) were stained with haematoxylin and eosin. Intestinal morphologic characteristics were evaluated under light microscopy.

Assay of serum free T3 and T4 (FT3, FT4)
After 21 hrs, 3 ml blood samples were collected from inferior vena cava, centrifuged at 15000 g for 15 min. The supernatants were frozen at -20°C for later FT3 and FT4 assay by radioimmunoassay (RIA).

Statistical analysis
Data were expressed as ±s and compared using one-way analysis of variance (ANOVA). The statistical analyses were made using the Statistical Package for the Social Science (SPSS10.0) software. Differences were considered as significant when the probability was less than 0.05.

RESULTS
All rats of sepsis group exhibited symptoms and signs of sepsis, including lethargy, piloerrection, decreased grooming, and diarrhea. Above symptoms and signs of sepsis plus T3 group were milder, and sham group were normal.

Serum FT3 levels in sepsis group were decreased significantly compared with tri-iodothyronine treated rats at 21 hrs, sepsis plus T3 rats had normal or slightly decreased FT3 levels after CLP 21 hrs compared with sham operated rats, and FT3 levels in sepsis group were also much lower than those in sham operated rats (Table 1).

| Groups            | n  | FT3 (pmol/L) | FT4 (pmol/L) |
|-------------------|----|--------------|--------------|
| Sham              | 6  | 3.44±1.40    | 9.53±3.39    |
| Sepsis            | 8  | 1.59±0.20    | 3.41±2.14    |
| Sepsis plus T3    | 8  | 3.40±1.65    | 6.37±4.45    |

p <0.05 vs sepsis group.

Rats receiving normal saline after CLP showed a significant increase in intestinal permeability in comparison with sham group (P<0.01), rats with administered T3 after CLP showed a significant decrease in intestinal permeability in comparison with the sepsis plus normal saline group (P<0.01) (Figure 1).

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In sepsis group, the ultrastructure of intestinal epithelial cells showed that the microvilli were sharply reduced and deformed, and loss was patchy. The edema of the villi cells was more pronounced with the mitochondria dropsy and vacuolar change, gaps of enterocytes were sharply widened, junctional complex among enterocytes were shortened and widened (Figure 2 A). In contrast, ultrastructure of sepsis plus T₃ group showed that the microvilli were dense and regular with a jagged and interlocking pattern among enterocytes and the mitochondria were clear (Figure 2 B).

Under photomicrography, septic rats showed severe edema and sloughing of the villous tips compared with sham animals (Figure 3A and B). Rats with administered T₃ after CLP showed relatively normal villous tips without sloughing (Figure 3 C).

**Figure 3** In sham rats, intestinal mucosal architecture is well preserved (A). Sepsis induced significantly edema of villous tips and sloughing of villous enterocytes (B). The tri-iodothyronine prevented the intestinal damages induced by sepsis (C). HE, ×100.

**DISCUSSION**

For more than 3 decades, it has been known that the euthyroid sick syndrome exists probably in any severe illness, including starvation[20], pulmonary tuberculosis[21], sepsis[22,23], surgery[24,25], myocardial infarction[26,27], bypass[28] and bone marrow transplantation[29]. The syndrome is also called low T₃ syndrome or nonthyroidal illness, characterized by low serum T₃ levels, and serum free T₃ levels are commonly below normal, but may be normal or above normal. It is likely that mechanism of thyroid hormone suppression in these illness is multifactorial and may differ in different groups of patients, as for low serum T₃ levels in this syndrome, one important cause is a decreased generation of T₃ by type I iodothyronine deiodinase in the liver and a reduced degradation of r T₃[30]. Subsequently, Nagaya et al demonstrated that in severe illness activated NF-κB could inhibit T₃-dependent induction of type I 5' - deiodinase mRNA and enzyme activity[31]. In addition, the degree of low T₃ in circulation has been shown to correlate with the severity of the underlying disorders and the prognosis[32]. Some authors believe that this is an abnormal state with decreased production rather than increased degradation, others content that this may be the body’s adaptation to stress protecting the body against exaggerated catabolism. Hitherto, some T₃ supplementing studies suggested that the former might be more reasonable than the latter[24,26,35].

In this study, we used cecum ligation and puncture (CLP) to establish classic animal model of sepsis. During the whole investigation, our septic models were coincident with the previous studies[33,34]. An acute decrease in circulating levels of free T₃ and T₄ was seen after 21hrs CLP. T₃ supplement prevented the decrease in serum free T₃ concentration with sepsis. ESS was seen in our septic model. Moley et al showed in their study that absence of thyroid hormone abolished the hyperdynamic phase of sepsis, increased susceptibility to sepsis, and significantly increased the mortality in sepsis, and thyroxine replacement following thyroidectomy prevented the increased mortality from sepsis[35]. In our research, T₃ supplement showed that septic symptoms and signs of the rats were abated to a certain extent. Thus, our data has confirmed that T₃ replacement in septic rats with ESS may be beneficial to the general condition of the patients.

The progression from sepsis to severe sepsis (sepsis with dysfunction of one organ) to multiple organ dysfunction syndrome and then to septic death requires escalation of treatment[36]. During the course of the progression, gut barrier disruption is believed to be the “motor” of “irreversible” shock and multiple system organ failure[37]. The critical cause is that translocation of bacteria and endotoxins contribute to the infection and injury of the body. Increased intestinal mucosal permeability is considered to be a quantitative index of injury or dysfunction of the intestinal mucosa barrier. The molecular probe FITC-D used in this study is considered to penetrate through a paracellular route toward portal vein via the tight junctions according to its size. Gut permeability indicated by FITC-D was coincident with the pathologic changes of injured gut in inflammation[38]. Assay of FITC-D does not depend on systemic circulation and renal function compared with other probe molecule, and so application of FITC-D assay can indicate more exactly the gut permeability in this experimental model. Our data showed that T₃ administration significantly decreased gut permeability in septic rats. Photomicrography and TEM showed that T₃ supplement well preserved the ultrastructure and morphology of intestinal mucosa. Therefore, these data indicate that T₃ administration significantly decreased gut permeability in septic rats. The proximate molecular mechanism by which T₃ regulates gut barrier in sepsis is not known. It is possible that T₃ administration is associated with protective substances synthesis of intestinal epithelial cells. Smith et al reported that administration of T₃ could induce expression of heme oxygenase-1 (HO-1) and stimulate activity of HO-1 in liver of thyroidectomized rats[39]. HO-1 is a stress-associated protein whose expression is stimulated by hypoxia, and increases adaptive response of cells to hypoxia. Hypoxia inducible factor-1 (HIF-1) mediates transcriptional activation of HO-1 gene in
response to hypoxia[40]. Our study showed that T3 supplement increased expression of HIF-1α in intestinal epithelial cells of septic rats (unpublished data). Thus, promoting adaptive response of cells to hypoxia may be one of approaches to improve gut barrier in sepsis by T3.

In conclusion, thyroid hormone is one of the critical hormones in mammals and plays an indispensable role in development as well as in lipid, protein, and carbohydrate metabolism and energy generation. Our results demonstrate that tri-iodothyronine, active form of thyroid hormone, can protect gut barrier in septic rats. Obviously, biologic functions of thyroid hormone are expanded, and moreover, it may supply a novel method to protect from the injury of gut barrier in critic illness. It is of important theoretical significance and practical value to further investigate its protective mechanism.

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