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Sepsis leads to thyroid impairment and dysfunction in rat model

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ABSTRACT

Sepsis was a systemic response to a local infection. Apoptosis was observed in the experimental sepsis. In this study, cecal ligation and puncture (CLP)-induced sepsis was established in rats. We found that sepsis decreased thyroid hormone levels, including triiodothyronine (T3), thyroxine (T4), free T3 (fT3), and free T4 (fT4). Besides, we detected the increasing expression level of Caspase-3 and increasing ratio of TUNEL positive cells in the thyroid after sepsis. Furthermore, a series of pathological ultrastructural changes were observed in thyroid follicular epithelial cells by CLP-induced sepsis. This study established a sepsis animal model and provided the cellular and molecular basis for decoding the pathological mechanism in thyroid with the occurrence of sepsis.

1. Introduction

Sepsis is a condition of systemic inflammation initiated in response to an infection by microbes and endotoxins leading to organ dysfunction and high mortality (Ma et al., 2015; Rossaint and Zarbock, 2015). The incidence of sepsis is steadily increasing. The annual incidence of sepsis is in the range of 200–1000 cases per 100,000 inhabitants (Angus et al., 2001; Flaatten, 2004; Wilhelms et al., 2010). One major hallmark of sepsis is the development of multi-organ dysfunction syndrome (MODS). The vital organs, such as kidneys, lung, and liver are especially vulnerable to damage and functional impairment during sepsis (Ramirez, 2013). However, the exact pathological pathway leading to the development of MODS during sepsis is still not completely known to us. Until now, treatment of sepsis is not effective. The most promising strategy is the combination therapy, such as the use of antibiotics in combination with candidate drug molecule (Aydin et al., 2005; Bauhofer et al., 2004; Christ et al., 1995; Fei et al., 2011).

Thyroid gland is responsible for the secretion of thyroid hormones, including thyroxine (T4) and tri-iodothyronine (T3). Thyroid-stimulating hormone (TSH) is secreted by the pituitary gland. In health, thyroid hormone homeostasis is regulated by hypothalamic-hypophyseal-thyroid (HHT) axis (Willard, 1983). By contrast, in patients with critical illness such as sepsis, changes in thyroid hormone concentrations arise in the absence of abnormality in thyroid function, known as the syndrome of nonthyroidal illness (NTI) (Warner and Beckett, 2010). Production of inflammatory cytokines and reactive oxygen species are two critical factors contributing to NTI in patients with sepsis, suppressing TSH release (Wajner and Maia, 2012). Besides, Plasma T3, T4 and TSH concentrations decrease along with the progression of sepsis and septic shock (Mebis and van den Berghe, 2009; Peeters et al., 2006; Yildizdas et al., 2004). However, the study on morphological and functional alteration in the thyroid gland is limited.

In this study, we made use of the sepsis rat model to determine the changes after sepsis happened. The objective of this study was to assess thyroid function, apoptosis and pathological ultrastructural change with the occurrence of sepsis. We hypothesized that the thyroid hormone level change, apoptosis in thyroid epithelial cells and apparent morphological change in follicular epithelial cell would be associated with the high mortality.

2. Methods and materials

2.1. Animals

Male Sprague-Dawley rats (weight: 180–220 g) were purchased from Fujian Medical University Animal Center. All procedures of animal handling were carried out in accordance with the protocols approved by the Institutional Animal Care and Use Committee of the Fujian Medical University. The animals were randomly assigned

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Table 1
The comparison of thyroid hormone levels among control and surgery groups.

Group	T3	T4	fT3	fT4	TSH
Control	0.453 ± 0.239	39.848 ± 7.316	2.693 ± 0. 983	7.920 ± 1.769	0.506 ± 0.144
12h	0.364 ± 0.147	19.862 ± 7.881*	1.643 ± 0.766	5.386 ± 1.693	0. 422 ± 0.080
24h	0.271 ± 0.051	12.784 ± 4.394*	1.155 ± 0.417*	3.413 ± 1.115*	0. 458 ± 0.087
36h	0.217 ± 0.051*	10.880 ± 6.371*	1.185 ± 0.532*	3.991 ± 2.423*	0. 471 ± 0.140

Hormone levels were presented as $\bar{x} \pm s$. The difference was determined by one way ANOVA by comparison to control group, $^*p < 0.05$.

to four groups, including control group (C group), and three experimental groups (12 h, 24 h and 36 h after surgery).

2.2. Sepsis rat model

The cecal ligation and puncture (CLP)-induced sepsis model was established as previously described (Hu et al., 2015). In brief, after an overnight fast, rats were anesthetized using 10% chloral hydrate (3 ml/kg, intraperitoneal). A midline incision about 1.5 cm was made on the anterior abdomen. The cecum was carefully isolated and the distal 1 cm was ligated. Then the cecum was punctured once with a sterile 18-gauge needle, and was squeezed to extrude the cecal contents from the wounds. The cecum was repositioned and the abdomen was closed. Rats in the control group underwent the same surgery, and the cecum was manipulated, without being ligated or perforated. All rats were administered with saline (2 ml/100 g body weight) subcutaneously immediately after surgery for fluid resuscitation.

2.3. Thyroid function analysis

Blood was collected from right common carotid artery at 12 h, 24 h and 36 h after surgery. The samples were centrifuged and the supernatants (serum) were used to determine the level of T3, T4, fT3, fT4, and TSH. Thyroid function was analyzed by radioimmunoassay kit (Beijing North Institute of Biological Technology, China), according to the manufacturer' instructions.

2.4. Western blotting

2 μg lysate from thyroid tissue was loaded on each lane of 10% polyacrylamide gel, and then blotted onto a polyvinylidene difluoride (PVDF) membrane. After blocking with a PBST containing 5% nonfat dry milk, the membrane was incubated with antibodies against Caspase-3 and beta-actin (Abcam). Peroxidase-linked anti rabbit IgG (Abcam) was used as secondary antibodies. These proteins were visualized by using an ECL western blotting detection kit (Amersham Biosciences).

2.5. TUNEL assay

Terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) assay was performed by using an in situ cell death detection kit (Santa Cruz Biotechnology). Briefly, thyroid sections were fixed with 4% paraformaldehyde and permeabilized with 0.2% Triton X-100. Endogenous peroxidase was blocked with hydrogen peroxide (5%, v/v) for 20 min, Fragmented DNA was labeled with terminal deoxynucleotidyl transferase (TdT) at 37 °C for 1 h, and further incubated using the digoxigenin/antidigoxigenin antibody system. The final reaction was monitored with a microscope using DAB. Images were captured using a Nikon ECLIPSE 90i.

2.6. Transmission electron microscope

Thyroid tissues were sequentially fixed by 3% glutaraldehyde, 1.5% paraformaldehyde, and 1% osmic acid-1.5%potassium ferrocyanide. Then, tissues were dehydrated with ethanol-acetone. Samples were embedded in ethoxyline resin. Sections were stained for 20 min with 2% (w/v) aqueous uranyl acetate followed by

6 min with Reynolds lead citrate, which were later on examined in a PHILIPS EM208 transmission electron microscope.

2.7. Statistical analysis

Raw data were analyzed using SPSS19.0 software. Caspase-3 grey value was determined by Image J software. Thyroid hormone levels were presented as mean ± standard deviation. The difference among all the groups in thyroid hormone levels was determined by one-way ANOVA. TUNEL positive staining and caspase-3 expression were presented as median (p25, p75), and the comparison between multiple groups was analyzed by Kruskal-Wallis H test. Nemenyi test was used to determine the difference between two groups. A p value less than 0.05 is considered as significantly different.

3. Result

3.1. Thyroid hormone levels decreased in sepsis rat model

Compared to the control group, thyroid hormone levels decreased in sepsis rat model except for TSH, as shown in Table.1. The T3 level decreased at 12 h and 24 h, and significantly decreased at 36 h after surgery ($^*p < 0.05$). The T4 level significantly reduced at all three time points. The fT3 and fT4 levels significantly decreased at 24 h and 36 h, but not 12 h after surgery. By contrast, we did not observe any significant alteration in the TSH level.

3.2. Apoptosis was promoted in sepsis model

To determine whether the sepsis leads to the apoptosis in the rat model, we detected the caspase-3 expression by western blotting. The expression of Caspase-3 significantly increased at 12 h and 24 h after surgery, but decreased at 36 h. By contrast, the expression of internal control beta-actin was consistent among all the groups (Fig. 1). Statistically, the expression of capase-3 increased to 2.5 fold at 12 h, and to 4.9 fold at 24 h after surgery ($^*p < 0.05$). This ratio decreased to 1.8 fold at 36 h after surgery, as shown in Table.2. Next, we detected the ratio of TUNEL positive cells in the thyroid gland after the sepsis establishment. We found that the ratio of TUNEL positive cells increased at all three time point after surgery (Fig. 2). Furthermore, the increasing ratio in TUNEL positive cells was significant at 24 h and 36 h ($^*p < 0.05$), as shown in Table.2.

3.3. Ultrastructural alteration in thyroid in sepsis model

Ultrastructural change in thyroid after surgery was observed by transmission electron microscope. In the control group, thyroid