Pathophysiology 23 (2016) 295–301

Contents lists available at ScienceDirect

Pathophysiology

journal homepage: www.elsevier.com/locate/pathophys

Omega-3 fatty acids decreases oxidative stress, tumor necrosis factor-alpha, and interleukin-1 beta in hyperthyroidism-induced hepatic dysfunction rat model

Asmaa M.S. Gomaa *, Ebtihal A. Abd El-Aziz

Department of Medical Physiology, Faculty of Medicine, Assiut University, Assiut, Egypt

ARTICLE INFO

Article history:
Received 21 April 2016
Received in revised form 19 October 2016
Accepted 20 October 2016

Keywords:
Hyperthyroidism
IL-1β
Liver
Omega-3
Oxidative stress
TNF-α

ABSTRACT

Hyperthyroidism is associated with abnormalities of the liver. Omega-3 polyunsaturated fatty acids, especially their long-chain forms: eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have beneficial health effects. The objectives of the present study were to assess hyperthyroidism-induced hepatic dysfunction in adult male rats and to evaluate the ameliorative effects of omega-3 on hyperthyroidism-induced hepatic dysfunction and the underlying mechanisms. Twenty four adult male rats were randomly divided into three equal groups; *control group* which received water for 6 weeks, *hyperthyroid group* which received L-thyroxine orally for 6 weeks and *hyperthyroid omega-3 treated group* which received L-thyroxine for 2 weeks and then co-treated with L-thyroxine and omega-3 oral compound containing 18% of EPA and 12% of DHA for 4 weeks. Hyperthyroid omega-3 treated group showed significantly increased final body weight and body weight gain, decreased liver weight to body weight ratio, decreased serum triiodo-L-thyronine level, increased serum thyroid stimulating hormone level, decreased serum levels of alanine transaminase, aspartate transaminase and tumor necrosis factor-alpha, increased hepatic levels of total antioxidant capacity and decreased hepatic levels of total peroxide and interleukin-1 beta when compared with the hyperthyroid group. Furthermore, histopathological studies revealed also marked improvement. We concluded that omega-3 had encouraging therapeutic effects against hyperthyroidism-induced hepatic dysfunction attributable to more than one mechanism: antioxidant, anti-inflammatory and anti-fibrotic effects.

1. Introduction

Thyroid hormones (THs) are considered one of the major endocrine regulators of cellular metabolic activity [1] including hepatocytes [2]. Many biological effects of THs depend on intracellular levels of 3,5,3′-triiodo-L-thyronine (T3) as it binds to nuclear receptors of thyroid hormones with the highest affinity. T3 is produced in peripheral tissues by outer-ring 5′-deiodination of thyroxine (T4), produced entirely in the thyroid gland [3]. The primary sites of conversion of T4 to T3 are the liver and kidneys [4]. One of the most common diseases which greatly affect the liver is the thyroid disease. Hyperthyroidism has been known to be associated with abnormalities of the liver including its biochemical markers and histology. The mechanism underlying the association between hyperthyroidism and hepatic dysfunction is unclear [5].

The production of the harmful reactive oxygen species (ROS) is stimulated by excess THs [6]. ROS and antioxidants are balanced in a healthy body, disturbance in this balance is known as oxidative stress [7]. Tumor necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β) are key inflammatory cytokines [8]. They are mainly produced by activated Kupffer cells or infiltrating neutrophils and macrophages. The production of TNF-α and IL-1β could result in increased defensive responses in parenchymal cells with activation of apoptosis. However, overwhelming of these defensive responses can result in death of cells by necrosis and thereby stimulate more inflammatory responses [9].

Omega-3 polyunsaturated fatty acids, especially their long-chain forms: eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have beneficial health effects [10]. Omega-3 is commonly found in oils extracted from fishes in cold regions such as salmon and tuna. Their beneficial effects could be attributed to

* Corresponding author at: Medical Physiology Department, Faculty of Medicine, Assiut University, Assiut, 71515, Egypt.
E-mail address: asmaagom3a@yahoo.com (A.M.S. Gomaa).

http://dx.doi.org/10.1016/j.pathophys.2016.10.001
0928-4680/

reduction in the production of proinflammatory cytokines, antioxidant ability as well as protection of the immune system [11].

Therefore, the objectives of the present study were to assess hyperthyroidism-induced hepatic dysfunction in adult male rats and to evaluate the ameliorative effects of omega-3 on hyperthyroidism-induced hepatic dysfunction and the underlying mechanisms.

2. Materials and methods

2.1. Experimental animals

The experiment was carried out on 24 adult male Wistar Albino rats weighing 160–190 g each. Rats were housed in clean capacious cages (4 per cage) in the animal house of Faculty of Medicine, Assiut University. They were maintained at room temperature and natural 12:12-h light-dark cycle in an aerated room with food (standard rat pellets) and water available *ad libitum*. The experimental protocol was approved by the Ethics committee, according to "Guidelines of Experiments on Animals" at the Faculty of Medicine, Assiut University, Egypt.

2.2. Experimental protocol

After 1 week of acclimatization, rats were randomly divided into three groups; eight animals each. ***Control group:*** animals received tap water and sacrificed after 6 weeks. ***Hyperthyroid group:*** hyperthyroidism was induced in animals by administration of 0.1 μg/g/day L-thyroxine (Sigma-Aldrich, USA) by orogastric tube for 6 weeks [12]. ***Hyperthyroid omega-3 treated group:*** animals were treated with 0.1 μg/g/day L-thyroxine for 2 weeks, then rats were received a combined treatment of 0.1 μg/g/day L-thyroxine and 3 g/kg/day of omega-3 oral compound containing 18% of EPA and 12% of DHA for 4 weeks (Doppelherz, Germany) [13].

At the end of the experiment, final body weights (BW) were measured and BW gain was calculated from the difference between BW at the start and at the end of the experiment. Blood samples were taken from the retro-orbital vein of each rat. Then, all animals were decapitated under anaesthesia. Blood samples were centrifuged at 3000 rounds per min (rpm) for 15 min. The clear, non hemolyzed supernatant sera were removed and kept at −20 °C until use for analysis. Liver was rapidly removed, weighed and washed with ice-cold phosphate buffer saline (PBS), and part is immediately frozen in liquid nitrogen and kept at −80 °C for further analysis and another part is used for histological study. Body and liver weights were used to calculate liver weight to body weight ratio (LW/BW).

2.3. Biochemical analysis

2.3.1. Measurement of serum triiodo-L-thyronine (T3) and thyroid stimulating hormone (TSH)

The levels of both T3 and TSH in the serum of rats was measured using enzyme-linked immunosorbent assays commercial kits (BioSource, Europe, Belgium) with monoclonal antibodies against each substance and following the instructions attached with each kit.

2.3.2. Measurement of serum alanine transaminase (ALT) and aspartate transaminase (AST)

The levels of both ALT and AST were assayed using commercial kits (Chema, Italy) according to the manufacturer's instructions. The activity of serum enzymes was expressed as units/l (U/L).

2.3.3. Measurement of serum tumor necrosis factor-alpha (TNF-α)

Serum level of TNF-α was assayed using specific rat's TNF-α ELISA kit (Koma Biotech, Seoul, Korea) and following the instructions attached with the kit. The results were plotted on a standard curve.

2.3.4. Measurement of hepatic total antioxidant capacity (TAC), total peroxide (TP) and calculation of oxidative stress index (OSI)

The liver tissue samples were weighed and homogenized in an ice-cold PBS. The tissue homogenates were centrifuged at 3000 rpm for 10 min at 4 °C. The resulting supernatant was used for analysis of levels of TAC and TP. TAC was measured colorimetrically using the commercially available kit (Bio-Diagnostics, Egypt) according to Koracevic et al. [14]. Total peroxide concentration was determined colorimetrically according to Harma et al. [15] through an enzymatic reaction that involves the oxidation of ferric–xylenol orange into a coloured product. The OSI is used as an indicator for oxidative stress. It is the percentage ratio of TP to TAC; each measured as μM/L (OSI = TP, μM/L/TAC, μM/L X 100) [16].

2.3.5. Measurement of hepatic interleukin-1 beta (IL-1β)

The hepatic homogenate was used for estimation of the IL-1β level using a rat's IL-1β ELISA kit (Koma Biotech, Seoul, Korea) and following the instructions attached with the kit. The results were plotted on a standard curve.

2.4. Histopathology

Liver specimens were fixed in 10% neutral buffered formalin, dehydrated and embedded in paraffin. Coronal sections (5 μm) were cut and stained by Haematoxylin and Eosin (H&E) for general histological structure, Masson's trichrome stain for detection of collagen fibers and Periodic acid-Schiff for detection of glycogen content [17]. Histopathological examination was conducted using light microscope (Olympus CH).

2.5. Statistical analysis

Data were analyzed using SPSS version 16 (SPSS Inc., Chicago, Ill., USA). Multiple comparison testing was done using the nonparametric Mann-Whitney *U* test. Data were presented as mean ± standard deviation (SD). Differences were considered statistically significant if $p \leq 0.05$.

3. Results

The hyperthyroid animals showed a significant decrease of both final BW and BW gain and a significant increase of LW/BW ratio when compared with the control group. Concurrent treatment of hyperthyroid animals with omega-3 significantly increased final BW and BW gain and significantly decreased LW/BW ratio in comparison with the hyperthyroid group. Final BW and BW gain were significantly lower and LW/BW ratio was significantly higher in hyperthyroid omega-3 treated animals than the control group (Table 1).

A significant increase in serum T3 and a significant decrease in serum TSH levels were observed after administration of L-thyroxine when compared to the control group, suggesting the success of hyperthyroidism induction in rats of the present study. Administration of omega-3 resulted in a significant improvement in the hormonal profile in the form of a significant decrease in T3 and a significant increase in TSH levels when compared to the hyperthyroid group. However, the level of T3 was significantly higher and the level of TSH was significantly lower in the hyperthyroid omega-3 treated group than the control group (Table 1).