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Original article

Thyroxine-induced cardiac hypertrophy: Role of ascorbic acid in treatment

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ABSTRACT

The heart is a major target organ for thyroid hormone action and marked changes occur in cardiac function in the case of hypo- or hyperthyroidism. Hyperthyroidism is a common metabolic disorder with prominent cardiovascular manifestation. We studied the changes in the heart structure and functions of hyperthyroid rat and ameliorating and protective role of ascorbic acid in treatment. Fourty male albino rats were equally divided into five groups; the first and second groups were the control and ascorbic acid groups respectively. Third group was the hyperthyroid rat group while 4th and 5th groups were co- and post-treated hyperthyroid rat with ascorbic acid respectively. Serum T_3 and T_4 levels were significantly increased also; TSH levels were significantly decreased in hyperthyroid rat as compared to control rat groups. Cholesterol, triglyceride, LDL-c and VLDL-c were significantly decreased when compared with control group. Many of abnormalities as severe hydrophobic changes of myofibrillar structure with striations, hypertrophy, cytoplasmic vacuoles and marked increase in desmin immunoreactivity were observed in left ventricle in hyperthyroid rats. Treatment with ascorbic acid helps in improving the adverse effect of hypothyroidism and also the histopathological and desmin immunoreactivity results confirms this finding.

1. Introduction

Thyroid hormones regulate all metabolic activities such as growth rate, sodium/potassium pump, cholesterol secretion in the bile, heart rate, blood pressure, respiration, oxygen consumption, digestion strength, lipid, carbohydrate and protein metabolism, central nervous system function, and the actions of other endocrine glands [1–13]. Thyroid status is also an important determinant of cardiovascular function [3,5,13,14]. Thyroid hormone lowers systemic vascular resistance, increases blood volume, and has inotropic and chronotropic effects on cardiac function [15,16]. In the heart, the thyroid hormone enhances the total protein synthesis, in addition to, it regulates the transcription of several specific proteins that are critical for cardiac function [17].

Hyperthyroidism is commonly associated with increased food consumption, parallel with a loss of body weight and decreased serum cholesterol level [18]. The liver is central in cholesterol metabolism, balancing hepatic cholesterol synthesis and hepatic uptake of plasma lipoproteins from the circulation against the excretion of hepatic cholesterol and bile acids in the bile [19]. T_3 can influence the metabolism of cholesterol at several critical steps in the liver [20]: the low-density lipoprotein (LDL-c) receptor (LDL-c-R), which mediates cholesterol uptake from the circulation, 3-hydroxy-3-methylglutaryl coenzyme A reductase, controlling cholesterol biosynthesis, and cholesterol 7α-hydroxylase (CYP7A1), the rate-limiting enzyme in the synthesis of bile acids where cholesterol is used as substrate [21].

Hyperthyroid patients have a high output circulation state, whereas hypothyroid patients have low cardiac output, decreased stroke volume and increased systemic vascular resistance [22]. These changes in cardiac function by thyroid hormones ultimately depend on the regulation of target genes within the heart and indirect effects due to hemodynamic changes by thyroid hormones.

Short-term hyperthyroidism is characterized by a high cardiac output state with a remarkable increase in heart rate and cardiac preload, and a reduction in peripheral vascular resistance, resulting in a hyper dynamic circulation [23]. The improvement in diastolic relaxation in the presence of T_3 is due to the down regulation of phosplolamban by increasing its phosphorylation and the up regulation of SERCA2 [17]. Hyperthyroidism is present in 1.3% of the United States population (overt in 0.5% and subclinical in 0.7%), and hypothyroidism in 4.6% of the population (overt in 0.3% and subclinical in 4.3%). Heart failure occurs in 6% of hyperthyroid patients [24]. Nonetheless, only half of those with hyperthyroidism-related heart failure have impaired left ventricular systolic function [25].

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Most patients with hyperthyroidism experience cardiovascular manifestations, and the most serious complications of hyperthyroidism occur as a result of cardiac involvement.

Ascorbic acid (vitamin C) is an essential nutrient for humans and certain other animal species. In living organisms, ascorbate acts as an antioxidant by protecting the body against oxidative stress. It is also a cofactor in at least eight enzymatic reactions including several collagen synthesis reactions that, when dysfunctional, cause the most severe symptoms of scurvy [26]. Multiple vitamin deficiencies have been repeatedly observed in the experimental animal rendered hyperthyroid by the administration of large doses of thyroid hormone.

Heart disease is the number one killer in the United States. For those with existing heart disease, that blockage of heart arteries could actually be reversed by supplementing with 6000 mg of ascorbic acid and 6000 mg of lysine (a common amino acid) taken in divided doses throughout the day. Ascorbic acid supplementation both lowers serum cholesterol levels and repairs lesions of arterial walls. Supplementing with ascorbic acid and vitamins E significantly reduces the risk of developing arteriosclerosis [27]. Simultaneous use of ascorbic acid and vitamins E was associated with a lower risk of total mortality and coronary mortality after adjusting for alcohol use, smoking history, aspirin use, and medical conditions [28].

Desmin is a specific cell protein of the myocardium, striated muscles, and smooth muscles. The desmin cytoskeleton in cardiomyocytes forms three dimensional scaffold that maintains cellular organelles in normal arrangement and plays additionally mechanical and regulatory functions [29]. Desmin, along with tubulin and actin, belongs to the basic cytoskeletal proteins and is the major muscle-specific, type III intermediate filament (IF) protein. Desmin plays a role in muscle cells, fulfilling mechanical, structural, and control functions. It comprises approximately 2% of the weight of myocardial cells, and 0.35% of the cell weight in skeletal and smooth muscles [30]. Also, desmin might be involved in the regulation of gene expression, myofibrillogenesis and intercellular signaling [31], and be responsible for shape and tension of the cell membrane and other organelles [32]. Changes of the cytoskeleton expressed as decreased or increased desmin immunostaining are known feature related to myocardial tissue injury. These have been shown in experimental studies [33] and human heart diseases [30,34,35]. There is little information about the relation between hyperthyroidism, the changes in heart structure and desmin experssion. So, the present study represents a contribution to declare the alternation on the biochemical, histopathological and immunohistochemical changes in the heart of hyperthyroid rat, in addition to the ameliorating and protective role of ascorbic acid.

2. Materials and methods

The experiments were performed on 40 male albino rats (*Rattus norvigicus*) weighing 120 ±10 g and of 8 week's age. They were obtained from the farms in medical research institute, Alexandria University, Egypt. The rats were kept in the laboratory for one week before the experimental work and maintained on a standard rodent diet (20% casein, 15% corn oil, 55% corn starch, 5% salt mixture and 5% vitaminzed starch; Egyptian Company of Oils and Soap Kafr-Elzayat Egypt) and water available *ad libitum*. The temperature in the animal room was maintained at 23 ± 2 °C with a relative humidity of 55 ± 5%. Light was on a 12:12 h light–dark cycle. The experimental protocol was approved by Local Ethics Committee and Animals Research. The rats were randomly and equally divided into five groups (8 animals each):

- G_1: control group in which animals did not received any treatment;
- G_2: ascorbic acid group in which animals received ascorbic acid daily (El Nasr Pharmaceutical Chemicals Co., at 420 mg/kg of body weight) for four weeks [26];
- G_3: hyperthyroid rats group in which rats received L-Thyroxin sodium (100 μg/kg, 4 weeks) administration daily in drinking water according to [13];
- G_4: co-treated group in which animals received L-Thyroxin sodium daily in drinking water and ascorbic acid simultaneously according to El Barbary et al. [26];
- G_5: post-treated group in which animals received L-Thyroxin sodium daily in drinking water for four weeks and then ascorbic acid for another four weeks according to El Barbary et al. [26].

At the end of the experimental period, rats were euthanized with interperitoneal injection with sodium pentobarbital and subjected to a complete necropsy.

2.1. Biochemical assays

Blood samples were individually collected from the inferior vena cava of each rat in non-heparinized glass tubes to estimate biochemical parameters. Serum was separated from non-heparinized blood by centrifugation at 3000 rpm for 15 minutes. The collected serum was stored at −18 °C until analysis. Estimation of thyroxine (T_4), triiodothyronine (T_3) and thyrotropin (TSH) according to the methods of Thakur et al. [36], the kits for these hormones were obtained from Calbiotech INC (CBI), USA.

Serum total cholesterol was determined according to the method of Allain et al. [37] and serum triglycerides were determined according to the method of Fossati and Prenciple [38] using kits supplied by Human. Serum HDL-cholesterol (HDL-c) was determined according to the method of Lopes-Virella et al. [39] using kits supplied by Human. Also low density lipoprotein cholesterol (LDL-c) and very low denisity lipoprotein cholesterol (VLDL-c) were calculated according to Friedewald and Robert [40] as following: LDL-c = total cholesterol − HDL-c -VLDL-c VLDL-c = TG/5.

2.2. Histological investigation

Immediately after decapitation animals were dissected, heart (left ventricle) were removed from different groups were fixed in 10% neutral buffered formalin. After fixation, specimens were dehydrated in an ascending series of alcohol, cleared in two changes of xylene and embedded in molten paraffin (mp. 50–58 °C). Sections of 5 microns thickness were cut using rotary microtome and mounted on clean slides. For histopathological examination, sections were stained with Ehrlich's haematoxylin and counterstained with eosin as a routine method after Bancroft and Stevens [41] and the rest were used for immunohistochemical studies.

2.3. Desmin expression

Paraffin sections (5 μm thick) from ventricle were mounted on positive gelatin chromalum–coated glass slides were used for desmin immunohistochemical staining method and the activity of the endogenous peroxidase was blocked using 3% H_2O_2. Subsequently, the paraffin sections were subjected to boiling in a microwave oven (250 W, 15 min) in the Antigen Retrieval Solution to unblock the antigenic determinants. For the detection of the desmin expression in the paraffin sections, mouse monoclonal antibodies (clone DE-R-11, DakoCytomation, Denmark) diluted 1:50 were used. The studied antigens were visualised using the LSAB2 reagent set and diaminobenzidine (DAB). Finally, the sections were washed with PBS, counterstained with methyl blue