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Thyroid hormones effects on oxidative stress and cardiac remodeling in the right ventricle of infarcted rats

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ABSTRACT

Right ventricle (RV) dysfunction post-myocardial infarction (MI) was associated with a worsened prognosis. In this scenario, reactive oxygen species (ROS) are related with the progression from MI to heart failure. Previous work showed that thyroid hormones (TH) are cardioprotective after MI.

Aims: This study aims to investigate the effect of T3 and T4 administration on oxidative stress and angiogenesis parameters in the RV after MI.

Main methods: Wistar rats were allocated into four groups: Sham-operated (SHAM), infarcted (AMI), sham-operated + TH (SHAMT), and infarcted + TH (AMIT). The treated groups received T3 (2 μg/100 g/day) and T4 (8 μg/100 g/day) by gavage for 26 days. After this, echocardiographic analysis was performed and the RV was collected to western blot and biochemical analysis.

Key findings: Infarcted treated rats showed RV hypertrophy compared with AMI and SHAMT. Hydrogen peroxide levels were decrease and SOD activity and expression were increased in the infarcted treated rats. Besides that, the hormonal administration increased eNOS expression and prevented the reduction of VEGF levels in AMIT rats.

Significance: In conclusion, TH seems to improve oxidative stress parameters, to promote physiological hypertrophy and to increase the expression of proteins involved with angiogenesis in the right heart.

1. Introduction

Myocardial infarction (MI) is a cardiac pathology that affects millions of people worldwide. This ischemic disease occurs by an impairment of cardiac perfusion, leading to the necrosis of the myocardial tissue [1,2]. In this context, the redox balance of the heart is an important factor, since reactive oxygen species (ROS) concentration can be related both with the promotion of cardiac damage [2], as well as with the activation of cardioprotective mechanisms [3,4]. In fact, a mild oxidative stress was related with an adaptative response of the cells, an effect called hormesis [4]. In order to regulate ROS levels, there are, in the heart, important enzymatic defenses, such as catalase and superoxide dismutase (SOD) [5,6]. Another important antioxidant enzyme is the endothelial nitric oxide synthase (eNOS). Besides its involvement with the antioxidant defense system, this enzyme presents an important role in myocardial perfusion [7]. Under physiological conditions, eNOS is the major source of NO in the heart [8], and its activation is mediated by the serine/threonine protein kinase B (Akt/PKB) [9]. Furthermore, eNOS has an essential role in the induction of angiogenesis [10]. In addition to eNOS, the vascular endothelial growth factor (VEGF) presents an important role in the angiogenesis scenario after myocardial infarction. This factor stimulates vascular regrowth within the infarction area, improving cellular perfusion [11].

It has been recently shown a positive role of thyroid hormones after MI [12,13]. The treatment of infarcted animals with T3 and T4 appears to present positive effects, leading to an improvement in functional and morphological parameters of the heart [12,13]. Thus, a recent work published by our group [14] analyzed the impact of thyroid hormones administration in a rat model of left ventricle (LV) infarction on parameters of oxidative stress. In this study, thyroid hormones promoted a cardioprotective effect on

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the LV, preventing redox imbalance and improving cardiac remodeling [14].

Although most studies using animal models of MI evaluate only the LV [2,13,14], the right ventricle (RV) also presents an important role in cardiac remodeling process after the ischemic injury [15]. Corroborating with this, a study showed that patients with inferior myocardial infarction presented a negative impact in the RV function, which was associated with a worsened prognosis [15]. Besides that, a study performed in a rat model of LV infarction showed that, 16 weeks after the injury, these animals presented a prejudice in RV hemodynamic parameters, as well as an increase in RV oxidative stress [16]. In another clinical study, the treatment with T4 was shown to improved RV function in patients with subclinical hypothyroidism [17].

Based on this, the present study comes across as an attempt to answer how the treatment with T3 and T4 can affect the RV of rats submitted to LV acute myocardial infarction, 28 days after the injury. Since it was previous demonstrated severe deterioration in the RV in a later period after infarction (16 weeks) [16], the present study performed a hormonal treatment in an early period (28 days), as an attempt to prevent the later damage of the right heart, which was associated with a worsened prognosis of this cardiac disease [15]. In view of that, the aim of this work was to study the effect of the administration of thyroid hormones on RV, in a rat model of LV myocardial infarction, in a time point when the right chamber has not yet been severely affected, analyzing oxidative stress and angiogenesis parameters.

2. Methods

2.1. Ethical considerations

All the animal care procedures were approved by the Ethics Committee for animal research at Federal University of Rio Grande do Sul (UFRGS) (process number: 23,262).

2.2. Experimental groups

The experimental animals were 90-day-old male Wistar rats (316 ± 59 g) obtained from the Central Animal House of the Federal University of Rio Grande do Sul (UFRGS), Brazil. The rats were housed in plastic cages and received pelleted food and water *ad libitum*. They were kept under standard conditions (21 °C, 12 h light/dark cycle). The animals were allocated into 4 groups: Sham-operated group (SHAM), sham-operated plus treatment group (SHAMT), acute myocardial infarction group (AMI) and acute myocardial infarction plus treatment group (AMIT).

2.3. Myocardial infarction procedure

The rats were anesthetized (xylazine 20 mg kg-1 i.p., ketamine 90 mg kg-1 i.p.). After this, the rats were submitted to a ligature of the descending anterior branches of the left coronary artery, or to a sham-operation [18]. Mortality rate of the infarcted rats after the surgical procedure and during the whole time of the protocol was 40%.

2.4. T4 and T3 administration

The rats were allowed to recover for 2 days after surgery. After recover, SHAMT and AMIT animals received, by gavage, T4 (8 μg/100 g/day) and T3 (2 μg/100 g/day) diluted in saline. SHAM and AMI groups received only saline. The rats received the hormonal administration for 26 days. The treatment protocol used was selected based on a previous study from our laboratory that showed positive effects after LV myocardial infarction [14]. The hormonal doses used in the present study were already shown to increase T3 plasmatic levels, without altering T4 levels, and to promote beneficial effects on the LV [14].

2.5. Echocardiographic evaluation

Echocardiographic measurements were evaluated 28 days after the surgical procedure. Philips HD7 XE ultrasound system with a L3-12 MHz transducer was used. The heart rate (bpm) was evaluated using M-Mode at three planes: apical, middle and basal [19]. In the transverse plane, it was measured the arch of the segments with infarction (I) and the total endocardial perimeter (EP), at end-diastole. The infarction size (IS) was calculated as % IS = (I/EP) × 100 [20,21]. Pulmonary artery acceleration time (AT) and ejection time (ET) were evaluated by Doppler echocardiography [22].

2.6. Morphometric parameters

Anesthetized rats were killed by cervical dislocation. The heart was rapidly excised, the scar area was discarded. The RV isolation was performed taking into consideration the limits between both cardiac chambers (LV and RV) and the intraventricular septum was not included. The RV was collected for Western blot and biochemical analysis, weighed and frozen in liquid nitrogen. RV hypertrophy was evaluated by RV weight to body weight (mg/g) ratio; RV weight to tibia length (mg/cm) ratio and RV to LV weight (mg/mg) ratio (Fulton index) [23]. Liver was rapidly excised and weighed. Liver congestion was evaluated by the organ weight to body weight ratio (g/g) [24].

2.7. Right ventricle tissue preparation

The right ventricle was homogenized (1.15% w/v KCl and phenyl methyl sulphonyl fluoride PMSF 20 mmol/L) in Ultra-Turrax. The suspension was centrifuged at 1000 X g for 10 min at 0–4 °C to remove the nuclei and cell debris and supernatants were used for the oxidative stress measurements [25].

2.8. Determination of total reactive oxygen species (ROS) levels

Total reactive oxygen species levels were evaluated by the oxidation of diacetate dichlorofluorescein (DCFH-DA) in 2,7-dichlorofluorescein, in the presence of these species. The samples were excited at 488 nm and the emission was collected in 525 nm in a spectrofluorimeter. The results were expressed as nmoles per mg of protein [26].

2.9. Hydrogen peroxide quantification

Hydrogen peroxide was quantified through the oxidation of phenol red mediated by horseradish peroxidase. The values were expressed as nmoles per milligram of protein [27].

2.10. Lipid peroxidation determination

Lipid peroxidation was evaluated through chemiluminescence using a liquid scintillation counter in the out-of-coincidence mode (LKB Rack Beta Liquid Scintillation Spectrometer 1215, LKB — Produkter AB, Sweden). Homogenized samples, with a protein concentration of 0.5–1.0 mg/mL, were placed in low-potassium vials in the reaction medium containing phosphate buffer (pH = 7.4). Tert-butyl hydroperoxide (3 mmol/L) were added to initiate the measurements. Data was expressed as counts per second per milligram of protein (cps/mg protein) [28].