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Cardioprotective effects of thyroid hormones in a rat model of myocardial infarction are associated with oxidative stress reduction

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ABSTRACT

Reactive oxygen species (ROS) are involved with progression from infarction to heart failure. Studies show that thyroid hormones (TH) present cardioprotective effects. This study aims to evaluate whether TH effects after infarction are associated to redox balance modulation. Male Wistar rats were divided into four groups: Sham-operated (SHAM), infarcted (AMI), sham-operated + TH (SHAMT), and infarcted + TH (AMIT). During 26 days, animals received T3 (2 μg/100 g/day) and T4 (8 μg/100 g/day) by gavage. Echocardiographic parameters were assessed and heart tissue was collected to biochemical analysis. AMIT rats presented absence of lung congestion, less cardiac dilatation, and normalization in myocardial performance index, compared with AMI. AMI rats presented an increase in hydrogen peroxide levels and in lipid peroxidation and a decrease in GSH/GSSG. TH prevented these alterations in AMIT. In conclusion, TH seem to reduce the levels of ROS, preventing oxidative stress, and improving cardiac function in infarcted rats.

1. Introduction

Several studies have shown that reactive oxygen species (ROS) are involved in the process of cardiac remodeling after acute myocardial infarction (Schenkel et al., 2012; 2010; Singal et al., 1999). As early as 48 h after the ischemic event, there is evidence of changes in oxidative stress parameters in the myocardial tissue (Tavares et al., 2012). After injury, neurohumoral mechanisms start to act in order to maintain cardiac function and the heart goes into a compensated stage of hypertrophy (Francis et al., 2001). In this phase, cellular antioxidant reserve increases (Singal et al., 1999). However, in a decompensated stage of heart muscle, this reserve is reduced, resulting in the occurrence of oxidative stress (Singal et al., 1999). This was demonstrated in experimental models of acute myocardial infarction, 28 days after the ischemic insult (Schenkel et al., 2010). In this work, the reduction in the fractional area change of the heart was positively correlated with a decrease in the reduced to oxidized glutathione ratio (GSH/GSSG) in heart tissue (Schenkel et al., 2010).

It has been recently shown that thyroid hormones may present a protective effect after myocardial infarction (Forini et al., 2011; Mourouzis et al., 2013b; Pantos et al., 2008; 2007). In a heart failure stage, plasma levels of these hormones decrease and/or the expression of both thyroid hormone receptors (TRs), TRα, and TRβ, in the myocardium decreases (Forini et al., 2011; Pantos et al., 2011). This scenario is detrimental for the heart, since these receptors, especially TRα1, seem to be relevant for the cardiomyocytes response against stress (Mourouzis et al., 2013a). Based on this, many studies have evaluated the hypothesis that the administration of thyroid hormones could exert beneficial effects on the heart (Forini et al., 2011; Mourouzis et al., 2013b; Pantos et al., 2008; 2007). Treatment with T3 and T4, in the post-infarction period, could prevent the development of tissue hypothyroidism and, thereby, mitigate deleterious changes in the cardiac tissue (Pantos et al., 2011; 2010a,b). The main mechanism through which thyroid hormones could promote a cardioprotective effect is by a genomic action, involving the binding of these hormones to its intracellular receptors and the modulation of protein synthesis in the cardiomyocyte (Pantos et al., 2011; 2010a,b). T3 and T4 administration, after myocardial infarction, could reduce

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the expression of beta-myosin heavy chain (β-MHC) and increase the expression of alpha-myosin heavy chain (α-MHC), preventing fetal-like phenotypic changes in the heart and improving cardiac contractility (Pantos et al., 2008). These hormones can also increase the expression of vascular endothelial growth factor (VEGF) receptor-2, inducing angiogenesis in the ischemic heart (Makino et al., 2009), and decrease the expression of collagen type I, preventing fibrosis (Ziegelhoffer-Mihalovicova et al., 2003). Echocardiographic analysis of infarcted rats treated with thyroid hormones revealed an improvement in the left ventricular ejection fraction two weeks after the ischemic event (Pantos et al., 2008). Also, 28 days after the infarction, rats treated with T3 presented a preserved left ventricular systolic function, when compared with non-treated animals (Forini et al., 2011).

Although thyroid hormones modulate significantly cardiac tissue redox balance (Araujo et al., 2006; Fernandes et al., 2011), to our knowledge, there are no studies that show the participation of ROS and redox status in the cardioprotective effect of these hormones after myocardial infarction. Due to the significant role of ROS in the progression from infarction to heart failure (Singal et al., 1999), it would be interesting to evaluate its involvement in T3 and T4 positive effects. Based on this, in the present study, we investigated whether the beneficial effects of the administration of T3 and T4, during the post-infarction period, could involve a modulation of redox balance and levels of ROS in the heart.

2. Materials and methods

2.1. Ethical approval

The study and animal care procedures were approved by the Ethics Committee for animal research at this University (Universidade Federal do Rio Grande do Sul – UFRGS; process number 23262).

2.2. Animals

Male Wistar rats (347 ± 48 g) were obtained from the Central Animal House of the Universidade Federal do Rio Grande do Sul, Brazil. Animals were housed in plastic cages and received water and pelleted food *ad libitum*. They were maintained under standard laboratory conditions (controlled temperature of 21 °C, 12 h light/dark cycle). They were allocated into 4 groups: Sham-operated (SHAM), infarcted (AMI), sham-operated plus treatment (SHAMT), and infarcted plus treatment (AMIT).

2.3. Surgical procedure

Animals were anesthetized (ketamine 90 mg kg^{-1}; xylazine 20 mg kg^{-1}, i.p.), and myocardial infarctions were produced by a method similar to that previously described (Johns and Olson, 1954). Animals were submitted to a surgical procedure of ligature of the descending anterior branches of the left coronary artery, or to a sham-operation in which all surgical procedures were performed, except the suture around the coronary artery. The mortality, evaluated 24 h after the surgical procedure and during the protocol period, was approximately 40%.

2.4. Thyroid hormones administration

After surgery, animals were allowed to recover for 2 days. After this, the treated groups, SHAMT and AMIT, received T3 (2 μg/100 g/day) and T4 (8 μg/100 g/day), diluted in saline by gavage, while the control groups, SHAM and AMI, received just saline. The period of treatment was 26 days after the recovery period.

2.5. Measurement of thyroid hormones in plasma

Anesthetized animals were submitted to blood collection from retro-orbital plexus. The blood was centrifuged at 1000*g* for 10 min. Plasma L-thyroxine and 3,5,3′triiodothyronine quantitative measurements were performed by chemiluminescence using ROCHE cobas e-411 analyzer kits at TECSA Veterinary Laboratory.

2.6. Morphometric analysis

Anesthetized animals were killed by cervical dislocation. The heart, lungs, and liver were rapidly excised and weighted. The cardiac hypertrophy was evaluated by the heart weight (in mg) to body weight (in g) ratio (Araujo et al., 2006). The scar area of the left ventricle was removed and weighted. The left ventricle was separated for biochemical and western blot analysis and was immediately frozen in liquid nitrogen. Liver and lung congestion were evaluated by wet to dry weight ratio (g/g) (Fernandes et al., 2011; Tavares et al., 2010).

2.7. Echocardiographic analysis

Cardiac function was analyzed by echocardiography, 28 days after the surgery. Rats were anesthetized and placed in left lateral decubitus position (45°) to obtain cardiac images. Philips HD7 XE ultrasound system with a L12-13 MHz transducer was used. Left ventricular systolic and diastolic transverse areas (cm^2) were obtained by tracing the endocardial border at three levels: basal, middle, and apical. Left ventricular posterior wall thickness (cm) and heart rate (beats/min) were measured using the M-Mode, in the previously described three planes (Nozawa et al., 2006). Left ventricular ejection fraction was calculated using Simpson's rule (Nozawa et al., 2006; Tavares et al., 2010). On each echocardiographic transverse plane the arch corresponding to the segments with infarction (I) and the total endocardial perimeter (EP) were measured at end-diastole. Infarction size (IS) was estimated as% IS = (I/EP) × 100 (Peron et al., 2006; Tavares et al., 2010). Final value for each animal was obtained by taking the average of all three planes (Nozawa et al., 2006; Tavares et al., 2010). Left ventricular myocardial performance index (MPI) was calculated through Doppler echocardiography (Cury et al., 2005).

2.8. Tissue preparation

The left 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*g* for 10 min at 0–4 °C to remove the nuclei and cell debris and supernatants were used for the oxidative stress measurements (Llesuy et al., 1985).

2.9. Determination of hydrogen peroxide levels

Hydrogen peroxide was measured via its horseradish peroxidase-mediated oxidation of phenol red. The results were expressed in nmoles of H_2O_2 per milligram of protein (Pick and Keisari, 1980).

2.10. Determination of oxidized and reduced glutathione concentration

To measure oxidized (GSSG) and reduced glutathione (GSH) concentration, the heart tissue was deproteinized with 2 mol/L perchloric acid, centrifuged for 10 min at 1000*g*, and the supernatant was neutralized with 2 mol/L potassium hydroxide. The determination of GSH was based on the reaction with 5.50 dithiobis (2-nitrobenzoic acid), was catalyzed by glutathione reductase and the absorbance values were measured at 420 nm. To measure