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Exercise training versus T3 and T4 hormones treatment: The differential benefits of thyroid hormones on the parasympathetic drive of infarcted rats

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ABSTRACT

Aims: This study aimed to investigate whether beneficial effects of thyroid hormones are comparable to those provided by the aerobic exercise training, to verify its applicability as a therapeutic alternative to reverse the pathological cardiac remodeling post-infarction.

Materials and methods: Male rats were divided into SHAM-operated (SHAM), myocardial infarction (MI), MI subjected to exercise training (MIE), and MI who received T3 and T4 treatment (MIH) (n = 8/group). MI, MIE and MIH groups underwent an infarction surgery while SHAM was SHAM-operated. One-week post-surgery, MIE and MIH groups started the exercise training protocol (moderate intensity on treadmill), or the T3 (1.2 μg/100 g/day) and T4 (4.8 μg/100 g/day) hormones treatment by gavage, respectively, meanwhile SHAM and MI had no intervention for 9 weeks. The groups were accompanied until 74 days after surgery, when all animals were anesthetized, left ventricle echocardiography and femoral catheterization were performed, followed by euthanasia and left ventricle collection for morphological, oxidative stress, and intracellular kinases expression analysis.

Key findings: Thyroid hormones treatment was more effective in cardiac dilation and infarction area reduction, while exercise training provided more protection against fibrosis. Thyroid hormones treatment increased the lipoperoxidation and decreased GSHPx activity as compared to MI group, increased the t-Akt2 expression as compared to SHAM group, and increased the vascular parasympathetic drive.

Significance: Thyroid hormones treatment provided differential benefits on the LV function and autonomic modulation as compared to the exercise training. Nevertheless, the redox unbalance induced by thyroid hormones highlights the importance of more studies targeting the ideal duration of this treatment.

1. Introduction

The indices of morbidity and mortality directly caused by acute myocardial infarction (MI) are still rising. One of the major concerns is the maladaptive remodeling following MI, which declines the cardiac function, and at long term, can lead to heart failure and reduction of lifespan [1,2]. The aerobic exercise training is an efficacious coadjutant method to improve the cardiac function and autonomic balance after MI [3]. However, in cases of exercise intolerance, other therapeutic approaches are necessary.

In this sense, many studies have shown that thyroid hormones treatment has diverse beneficial effects on the recovery of cardiac function after MI, since thyroid hormones may modulate intracellular pathways involved in cell survival [4–7]. Nevertheless, such benefits may be accompanied by adverse effects, including the modulation of autonomic system, through the increase in sympathetic drive and the heart rate, which in turn may impair the heart function and cause vascular dysfunction [8]. In addition, these function alterations may augment the oxygen consumption, provoking disruption of redox homeostasis by the elevation in mitochondrial activity and reactive

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oxygen species (ROS) formation [9].

In this context, many efforts have been made in order to find a safe and effective dose of thyroid hormones which is capable of improve the cardiac function with minimal adverse effects [10,11]. A low dose of only T3 was recently showed to be safe in female rats, post-infarction [10]. Moreover, we have recently reported a low dose combination of T3 and T4 hormones that presented similar results in male rats as well [12] and it could possibly be used as the alternative to the aerobic exercise training.

Therefore, the present study aimed to investigate whether T3 plus T4 hormone treatment (in lower doses than those reported in literature) provide benefits comparable, or even superior, to the ones stimulated by the aerobic exercise training. In this context, the major focus in this study was to analyze the sympathetic and parasympathetic drive, cardiac function, oxidative stress parameters, and intracellular kinases expression in order to guarantee a safe use of this hormonal therapy.

2. Materials and methods

2.1. Ethical statement

The present study was approved by the Ethics Committee on the use of animals of the Universidade Federal do Rio Grande do Sul under the protocol number 29521, and all their ethical principles as well as the ARRIVE guidelines and the National Institutes of Health guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978) were respected and followed during this study.

2.2. Experimental protocol

The Fig. 1 illustrates the experimental design of this study. A total of 40 male Wistar rats (190 ± 10 g) were provided by the Center for Reproduction and Experimentation of Laboratory Animals at the Universidade Federal do Rio Grande do Sul. They were maintained under standard laboratory conditions (controlled temperature of 21 °C, 12 h light/dark cycle, water and pelleted food ad libitum), and allocated in plastic cages (four per cage). 8 rats were subjected to SHAM surgery (SHAM group). The other 32 animals were subjected to MI induction surgery by suturing the left anterior descending coronary artery [13], in which 4 rats died. All rats were anesthetized before the surgery (Ketamine 90 mg/kg and xylazine 10 mg/kg, intraperitonially). Post-surgery analgesia included Tramadol (12.5 mg/kg, subcutaneously) and dipyrone (200 mg/kg, orally) twice a day for 2 days. After 48 h, the infarct size was measured by echocardiographic assessment, and 4 rats which had a small infarct area were excluded of the study. The remaining 24 rats were randomly divided into MI (myocardial infarction), MIE (myocardial infarction plus exercise training) and MIH (myocardial infarction plus thyroid hormones) groups (n = 8/group). Passed one week from surgery, animals into MIE group started the aerobic exercise training whereas MIH rats started to receive thyroid hormones. Both interventions were always performed at early-morning.

The aerobic exercise training protocol was executed on a treadmill (Imbramed KT-01) adapted for rats, with frequency of five times a week during 9 weeks, likewise in previous studies [14–16]. One week after the MI surgery, MIE group started an adaptation period by walking for 15 min on the treadmill with constant velocity (0.3 km/h), for four consecutive days. After the adaptation period, MIE group underwent a maximum speed test and started the training program. Duration and intensity of the exercise were gradually increased until reach 60 min of duration, and an intensity of 60% of the maximal oxygen consumption (VO_2 max) obtained in the maximum speed test [14,17]. The maximum speed test was repeated at the fourth week of protocol to adjust the workload.

The treatment with thyroid hormones consisted of T3 (1.2 µg/100 g/day) and T4 (4.8 µg/100 g/day) hormones (both from SIGMA-ALDRICH, USA), dissolved in NaOH 1 N and diluted in saline, with final pH adjustment to 7.0. The solution was prepared once a week and stored protected from light. Thyroid hormone treatment was administrated to MIH group as follows: in a room without direct light, one time a day, from Monday to Friday, for 9 weeks, via intragastric gavage, beginning one week after MI surgery; total dose overtime was of 54 µg T3/100 g of animal, and 216 µg T4/100 g of animal. Whereas SHAM, MI, and MIE groups received only vehicle (the same volume of NaOH 1 N diluted in saline and final pH adjusted to 7.0).

After 10 weeks of MI surgery, all animals were weighed, anesthetized, cardiac function parameters were evaluated by echocardiography. Autonomic modulation and baroreflex sensitivity were accessed by femoral artery catheterization, followed by euthanasia through anesthetic overload (ketamine 90 mg/kg and xylazine 10 mg/kg, intraperitonially) and confirmation of death by cervical dislocation. Left ventricle (LV) and tibia were collected, and used to morphometric analysis. After morphometric measurements, the scar of each LV was removed and the remaining LV tissue was separated in two parts. The first part was processed for histological analysis. The other part was immediately immersed in liquid nitrogen, stored at −80 °C, homogenized and used for oxidative stress and western blotting analyses.

2.3. Echocardiography

Transthoracic echocardiography was performed at 48 h (2 days, initial analysis) and 74 days (10 weeks, final analysis) after surgery. The animals were anesthetized by an intraperitoneal injection of ketamine

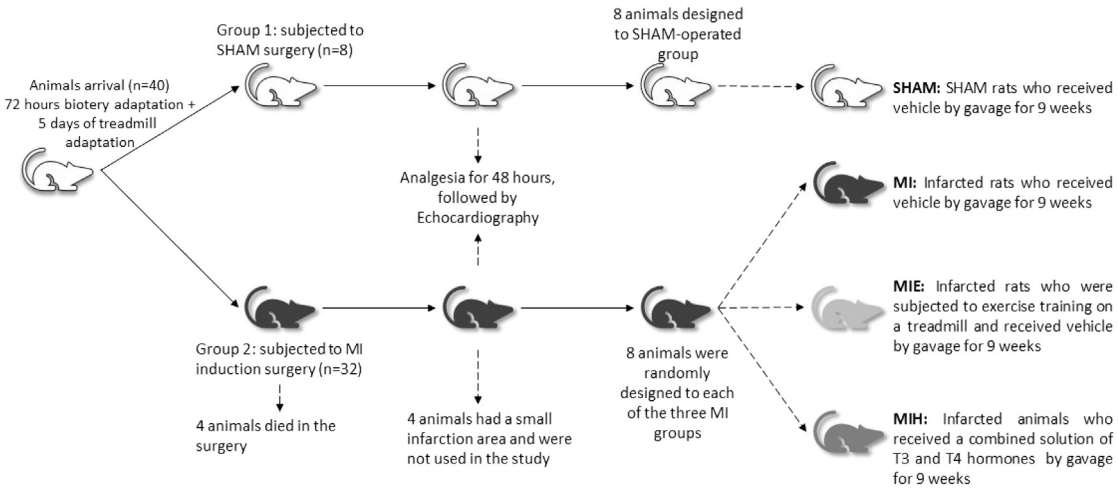

Fig. 1. Experimental design illustration.