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ORIGINAL RESEARCH

Exercise training improves the IL-10/TNF-α cytokine balance in the gastrocnemius of rats with heart failure

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KEYWORDS
Skeletal muscle;
Inflammation;
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Abstract
Objective: This study examined the effects of exercise training (ExT) upon concentration of tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6) and interleukin-10 (IL-10) in the gastrocnemius of rats with heart failure (HF) induced by left coronary artery ligation.
Methods: Adult male Wistar rats submitted to myocardial infarction (MI) or sham surgery were randomly allocated into one of four experimental groups: trained HF (Tr-HF), sedentary HF (Sed-HF), trained sham (Tr-Sham) and sedentary sham (Sed-Sham). ExT protocol was performed on treadmill for a period of 8 weeks (60 m/days, 5×/week, 16 m/min), which started 6 weeks after MI. Cardiac hemodynamic evaluations of left ventricular end-diastolic pressure (LVEDP) and morphometric cardiac were used to characterize HF. The hemodynamic variables were recorded and gastrocnemius muscle was collected. TNF-α, IL-6 and IL-10 protein levels were determined by multiplex bead array.
Results: Sed-HF group presented increase of TNF-α level when compared with the Sed-Sham group (mean difference, MD 1.3; 95% confidence interval, CI −0.04 to 2.5). ExT reduced by 59% TNF-α level in Tr-HF group (MD −1.7; 95% CI −2.9 to −0.3) and increased IL-10 (MD 15; 95% CI 11–26) when compared with the Sed-HF group. Thus, the gastrocnemius muscle IL-10/TNF-α ratio was increased in Tr-HF rats (MD 15; 95% CI −8 to 47) when compared with the Sed-HF rats.
Conclusion: These results demonstrate that ExT not only attenuates TNF-α level but also improves the IL-10 cytokine level in skeletal muscle of HF rats.

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Introduction

Heart failure (HF) is a complex and multifactorial syndrome associated with disability, morbidity and mortality.[1,2] Beyond neurohumoral activation, the inflammatory component plays an important role in the development of left ventricular dysfunction and peripheral myopathy, which in turn impair the functional capacity of patients with HF.[3] Plasma TNF-α and interleukin-6 (IL-6) levels are correlated with severity of HF symptoms and oxygen consumption capacity upon exercise.[4] The development of an anabolic-catabolic imbalance, with reduced anabolism and enhanced catabolism, is related to abnormalities in the neurohormonal systems and the activation of pro-inflammatory cytokines.[5] Skeletal myopathy seems to be an important factor associated with exercise intolerance, fatigue, and dyspnea in HF patients.[2]

TNF-α may affect muscle metabolism and strength by stimulating expression of inducible nitric oxide synthase (iNOS) via nuclear-factor-kappa-B (NF-kB).[6] Indeed, contractile dysfunction can result from TNF-α overexpression that signals via nitric oxide to decrease strength of skeletal muscle.[7] Moreover, an increased expression of TNF-alpha and IL-6 was observed in skeletal muscle biopsies from patients with stable HF.[8] As previously reported, seven-day subcutaneous administration of recombinant human IL-6 to rats resulted in a dose-dependent respiratory and peripheral skeletal muscle atrophy.[9] Nevertheless, physical training decreases the expression of TNF-α in skeletal muscle that was accompanied by a reduction of atrophy in HF rats[10] and humans.[8]

Exercise training (ExT) is associated with improvement of sympathetic and parasympathetic dysfunction in patients with HF.[1] Recent studies have established a critical role of the sympathetic nervous system (SNS) in mediating interactions between the nervous and immune systems.[11] Interleukin 10 (IL-10) could contribute to mediate the anti-inflammatory effects of ExT. Accumulating evidence suggests that ExT promotes anti-inflammatory benefits in HF experimental models[12,13] and clinical studies.[14,15] Although IL-10 is increased in plasma[16] and soleus muscle[13] in post-MI HF rats after treadmill endurance training, the effect of ExT on this cytokine in gastrocnemius muscle of HF rats is unclear.

Therefore, considering the important role of skeletal muscle in the release of cytokine, and the potential advantage of ExT in attenuating the loss of muscle mass and inflammation in chronic diseases, we evaluated the effects of ExT upon muscle mass, expression of TNF-α, IL-6 and IL-10 in the white gastrocnemius of rats with HF induced by left coronary artery ligation. In addition, we examined the balance between IL-10 and TNF-α production as an anti-inflammatory indicator following ExT.

Methods

Animals

Experiments were performed on 28 male Wistar rats weighing 220–270 g, obtained from the Animal Breeding Unit at the *Universidade Federal de Ciências da Saúde de Porto Alegre* (UFCSPA), Porto Alegre, RS, Brazil. The animals were allocated in groups of three to a cage with free access to water and pellet rodent chow diet and were maintained under standard conditions of temperature (22 °C). All procedures were performed in accordance with the *Guideline for the Care and Use of Laboratory Animals* (NIH Publication n° 85-23, revised 1996). All procedures outlined in this study were approved by the by the UFCSPA Ethics and Research Committee (protocol no. 39/11).

Experimental design

To induce myocardial infarction (MI), rats were anaesthetized with xylazine (12 mg/kg IP) and ketamine (90 mg/kg IP), intubated and artificially ventilated (SamWay VR 15) with a breathing rate of 60 breaths/min and oxygen inspired fraction of 100%. After thoracotomy, coronary artery ligation (CAL) was performed to induce MI and, subsequently HF.[12] During the first 48 h, the animals were treated for post-operative pain with subcutaneous buprenorphine (0.15 mg/kg) and given a single dose of penicillin (20,000 U IP). After surgery, the rats remained six weeks in home cage for evolution of MI. This period is necessary to develop HF state.[17] It was reported that in six weeks following MI the renal sympathetic nerve activity and left ventricular end-diastolic pressure (LVEDP) increase, and baroreflex control decreases,[17] all of these changes are associated with chronic HF. Rats were randomly allocated into four experimental groups: sedentary sham-operated (Sed-Sham, $n = 7$), trained sham-operated (Tr-Sham, $n = 7$), sedentary HF (Sed-HF, $n = 7$) and trained HF rats (Tr-HF, $n = 7$).

Aerobic exercise training protocol

One week before the aerobic exercise training protocol the adaptation period was started to familiarize the rats with running on the treadmill with low speed (10 m/min) and short duration (10 min per session). Rats in the training groups (Sham and HF) performed an aerobic ExT program for 8 weeks (5×/week)[16,18] with speed at 16 m/min for 60 min/day.[19] This intensity was maintained at a constant level throughout the experiment. Previous study demonstrated that this exercise protocol improved the aerobic capacity and citrate synthase activity in post-myocardial infarction rats.[20] Only rats that ran steadily with little or no prompting were used in the study. The total duration of the experiment was 14 weeks (5 resting in home cage, 1 in adaptation to treadmill and 8 of aerobic ExT protocol).

Cardiac hemodynamic evaluation, infarct size, cardiac hypertrophy and pulmonary congestion

After a rest period of 48 h from the last training session, the cardiac hemodynamic evaluation was performed. The animals were anesthetized with xylazine (12 mg/kg IP) and ketamine (90 mg/kg IP), and a small incision in the anterior cervical region was performed for the insertion of a polyethylene catheter (PE-50) into the right carotid artery. The arterial pressure (AP) was recorded first during a 5-min period by connecting the arterial cannula to

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