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Basic nutritional investigation

Iron supplementation effectively suppresses gastrocnemius muscle lesions to improve exercise capacity in chronic heart failure rats with anemia

Peng Guan M.Sc. [a,b], Li Li M.D. [c], Mu-Qing Zhang M.M.S. [d], Shu-Juan Liu M.Sc. [b], Wen-Ya Li M.M.S. [a], Na Wang M.D. [a,*]

[a] Department of Physiology, School of Basic Medicine, Hebei College of Traditional Chinese Medicine, Hebei, China
[b] The Key Laboratory of Animal Physiology, Biochemistry and Molecular Biology of Hebei Province, Hebei Normal University, Hebei, China
[c] Department of Histology and Embryology, Hebei Medical University, Shijiazhuang, China
[d] Department of Nephrology, The Fourth Hospital of Hebei Medical University, Shijiazhuang, China

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ABSTRACT

Objective: For patients with chronic heart failure (CHF), exertional fatigue is one of the most common and debilitating symptoms. However, the poor relationship between heart dysfunction and exercise capacity has been ascribed to peripheral abnormalities. Several previous studies confirmed that iron supplementation could significantly improve the exercise capacity of patients with CHF, although they did not analyze effects in the musculoskeletal system. The aim of this study was to investigate the effect of iron treatment on gastrocnemius muscles of CHF rats with anemia.

Methods: Male Sprague-Dawley rats were subjected to coronary ligation to induce heart failure. At the same time, blood (1–1.5 mL) was withdrawn from the retro-orbital plexus once every week to induce anemia. After 6 wk of this process, iron dextran was administered to the CHF rats with anemia (CHFa rats) at the dose of 8, 16, 32, or 64 mg/kg every 2 d for 2 wk.

Results: Iron dextran (8 mg/kg every 2 d) effectively improved hemodynamic parameters ($P < 0.05$) compared with CHFa rats. Similarly, this dose of iron dextran significantly reduced the ratio of heart weight to body weight ($P < 0.01$), whereas it significantly increased the distance run (m) to exhaustion ($P < 0.01$). Iron dextran effectively inhibited sarcoplasmic vacuolation and muscle atrophy of gastrocnemius muscles in CHFa rats, as evaluated by pathologic examinations. Other iron treatments, however, were found to be ineffective on the same parameters, so particular focus was placed on the iron dextran (8 mg/kg every 2 d) group in subsequent analyses. Consistently, phospho-p38 in gastrocnemius muscles of CHFa rats was markedly suppressed by iron dextran. Additionally, iron dextran significantly decreased c-fos and c-jun and up-regulated cellular FLICE-inhibitory protein expression levels.

Introduction

Chronic heart failure (CHF) is a major cause of morbidity and mortality that affects more than 24 million individuals worldwide [1]. For patients with CHF, exertional fatigue is one of the most common and debilitating symptoms [2,3], but the etiology remains controversial. The underlying abnormalities in patients with CHF not only have been associated with the heart but also the skeletal muscle system [4]. Initially, the early onset of fatigue found in the CHF state was thought to be primarily the result of a reduced skeletal muscle blood flow response to exercise [5]. Nevertheless, abnormalities intrinsic to skeletal muscle have recently emerged as a new index linked to muscle fatigability [6].

Several previous studies also confirmed iron deficiency (ID) to be common among patients with heart failure [7]. A survey of 574 patients with self-reported heart failure showed ID in 73.2% of participants with anemia and in 56.4% of those without

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* Corresponding author. Tel.: +86 311 80787579; fax: +86 311 83930675.
E-mail address: wangna9503@126.com (N. Wang).

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anemia [8]. Iron is critical for oxidative metabolism, which is an obligate component of the major proteins responsible for oxygen transport, transfer, and utilization in skeletal muscle [9]. Therefore, ID may attenuate aerobic performance by decreasing oxygen transport, transfer, and utilization in skeletal muscle and result in diminished peak oxygen consumption and decreased ability to endure submaximal exertion [10].

Several investigators have studied the effect of iron supplementation in patients with heart failure with and without anemia. Anemic patients in heart failure who were treated with intravenous (IV) iron had improved exercise capacity (6-min walk distance from 242 ± 78 to 286 ± 72 m; $P = 0.01$) [11]. In another study, anemic heart failure patients with renal insufficiency showed a dramatic improvement in exercise capacity, renal function, and quality of life (QoL) when given IV iron supplementation compared with the control [12]. The effect of IV iron sucrose on exercise tolerance in patients with CHF was also studied, and the results showed that both the absolute peak VO_2 and peak VO_2/kg significantly improved in the treadmill exercise duration test [13]. The effect of ferric carboxymaltose (FCM) in CHF patients with ID, with or without anemia has been studied and it was demonstrated that maximal exercise capacity and QoL assessments were restored dramatically ($P < 0.001$). Importantly, these results were similar in patients with and without anemia [14].

However, previous studies on exercise performance in patients with CHF did not consider related effects in the musculoskeletal system. The purpose of this study was to directly validate the hypothesis that CHF is associated with intrinsic skeletal muscle dysfunction and to test the physical and molecular effects of iron supplementation. Experimental rats with CHF and anemia (CHFa rats) with or without additional iron were submitted for exercise testing, and the p38 MAPK and apoptotic pathways were investigated in their isolated gastrocnemius muscles by histopathology and biochemical analysis. By clarifying the effect of iron supplementation on simple endurance capacity in CHFa rats, we may gain a better understanding of the abnormalities intrinsic to skeletal muscle in CHF.

Materials and methods

Animal preparation, groups, and treatments

Adult male Sprague-Dawley (SD) rats were used in all experiments. All rats (weight 210 ± 10 g) were purchased from the Experimental Animal Center, Hebei Medical University, China. Rats were kept under a 12-h light/dark cycle (lights on 0800 h) in a temperature- and humidity-regulated facility. Animals were provided free access to food and water ad libitum. All animal handling procedures were in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals and were approved by the animal Ethics and Use Committee of Hebei Science and Technical Bureau in the People's Republic of China. All efforts were made to reduce the number of animals used and their suffering.

The rat coronary ligation model was employed to induce heart failure [15]. After acclimating for 1 wk in the animal facility, the SD rats were anesthetized with 2% isofluorane, intubated, and maintained on a Harvard rodent ventilator. A left thoracotomy was performed at the fifth intercostal space, and while the heart was exteriorized the main left coronary artery was ligated 1 to 2 mm from its origin with a 7.0 suture (CHF group, n = 60). In the sham-operated group (n = 6), the same procedure was employed, but the suture was not tied. The heart was then repositioned, and the muscle and skin layer were closed with a purse-string suture. Moreover, 1 to 1.5 mL blood was withdrawn from the retro-orbital plexus of each rat in the CHF group once every week to cause anemia. In the CHF group, operative mortality was 25% (n = 45), and all sham-operated rats (n = 6) survived until 6 wk postsurgery. Transthoracic echocardiography (Vevo 770, Visual Sonics, Bethune International Peace Hospital) was performed to evaluate cardiac parameters and to select the CHF rats at the same time. Blood samples were drawn from the retro-orbital plexus of the CHF rats and divided into several aliquots. One aliquot was taken immediately for determination of red blood cell (RBC) count, hemoglobin (Hb) concentration and hematocrit (Hct) by an automatic blood analyzer (MEK-6318 K, Japan). CHFa rats (n = 32) were identified for use in the following experiment (Supplementary Table 1).

CHFa rats were divided into five groups (Fig. 1): untreated CHFa group (n = 6); 8 mg iron dextran-treated group (CHFa + Fe1 group, n = 6); 16 mg iron dextran-treated group (CHFa + Fe2 group, n = 6); 32 mg iron dextran-treated group (CHFa + Fe3 group, n = 7); 64 mg iron dextran-treated group (CHFa + Fe4 group, n = 7). All groups received intraperitoneal injections for 2 wk. The iron treatment groups were injected with iron dextran at the dose of 8, 16, 32 or 64 mg/kg every 2 d. The CHFa no-iron control group received the equivalent volume of physiological buffered saline (PBS; pH 7.4). All groups had free access to normal rat chow and drinking water.

Experimental procedures

Exercise testing

In the last week of the study, the rats were brought to the laboratory and allowed to adapt to the environment for 1 h. Subsequently, each rat was placed on a motorized treadmill to run continuously at 15 m/min for 5 min/d on each of 3 d. Such workload induces no training adaptations but familiarizes the rats with treadmill running [16].

On the last day, the surviving rats (CHFa + Fe1, n = 6; CHFa + Fe2, n = 4; CHFa + Fe3, n = 3; CHFa + Fe4, n = 3) ran continuously on a motorized treadmill at 15 m/min until exhausted. Exhaustion was operationally defined as the third time that the rat seemed to no longer keep pace with the speed of the treadmill and submitted to receiving a shock rather than continue running. At the moment of exhaustion, the current to the grid was stopped, and the rat was removed from the treadmill. The total distance run (m) to exhaustion was calculated and taken as the estimate of exercise capacity [17,18].

Hemodynamic and echocardiographic parameters

The rats were allowed to rest for 1 h after the treadmill exercise test and then anesthetized. Transthoracic echocardiography was recorded and analyzed. Subsequently, hemodynamic parameters were acquired as described previously [19]. A miniature pressure transducer (Chengdu Instrument Co., Chengdu, China) was inserted into the left ventricle (LV) via the right carotid artery. The heart rate (HR), left ventricular systolic pressure (LVSP), left ventricular diastolic pressure (LVDP), and first derivatives of the left intraventricular pressure (maximum rate of pressure development + dp/dt_{max} and maximum rate of pressure decrease $-dp/dt_{max}$) were monitored continuously, recorded, and analyzed after 10 min of stabilization by using a multichannel physiological signal acquisition and processing system (RM6240 C, Chengdu Instrument Co.).

Tissue samplings

After the rats were perfused with ice-cold PBS through the LV, the heart and gastrocnemius muscle samples were rapidly removed, weighed, immediately wrapped in aluminum foil, and frozen below −80°C for storage after treatment with liquid nitrogen.

Histologic analysis

Body weight (BW), heart weight (HW), and gastrocnemius muscle tissue weight were collected. The gastrocnemius muscle tissues were fixed with 10% paraformaldehyde in PBS for 2 d at 4°C, embedded in paraffin and cut into 5-μm

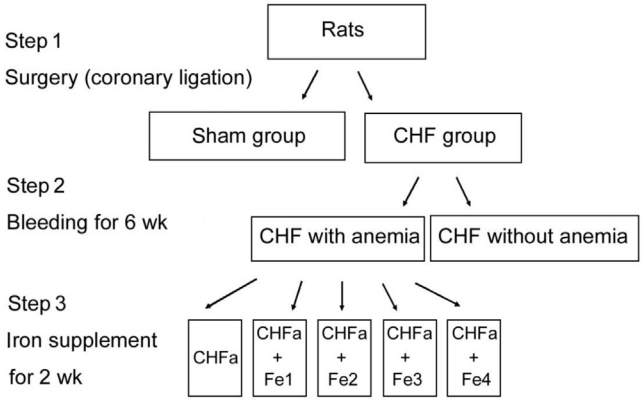

Fig. 1. Experimental protocol. Experiment is a three-step process. Step 1: rat coronary ligation model was employed to induce heart failure. Step 2: 1 to 1.5 mL blood was drawn from the retro-orbital plexus of each rat in the CHF group once per week to cause anemia. Step 3: CHFa rats were divided into five groups for iron supplement. CHF, chronic heart failure; CHFa, chronic heart failure with anemia.