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Resistance training attenuates inflammation and the progression of renal fibrosis in chronic renal disease

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ABSTRACT

Patients with chronic kidney disease (CKD) have progressive renal fibrosis, inflammation, and reduced muscle mass and strength. Resistance training (RT) has been suggested to mitigate the loss of muscle mass, of strength and the inflammation in CKD, but the mechanisms are unknown. The aim of this study was to evaluate the influence of RT on renal fibrosis, renal cytokine expression, creatine kinase levels, and muscle mass and strength in CKD rats. A CKD model was obtained by 5/6 nephrectomy (Nx). Fifteen 8-week-old male rats were divided into 3 groups: Sham (control), Nx SED (CKD sedentary) and Nx RT (CKD trained). The RT consisted of ladder climbing at 70% of the animal's maximal carrying capacity for 10 weeks. Muscle strength, creatine kinase levels, renal fibrosis and mRNA interleukin (IL)-4, IL-6 and IL-10 were analyzed after the RT protocol. There was significant improvement in the muscle strength and creatine kinase levels in the Nx RT group. Moreover, renal fibrosis and inflammation were attenuated, with increased IL-4 and IL-10 expression and reduced IL-6 expression in the Nx RT group compared with that in the Nx SED group. No difference in muscle mass was observed among the groups. In conclusion, RT was effective in reducing fibrosis and inflammation, in addition to increasing muscle strength and creatine kinase levels, in rats with CKD, independent of muscle mass.

1. Introduction

Chronic kidney disease (CKD) has a high incidence and prevalence worldwide, is progressive and irreversible and is associated with an increased risk of cardiovascular death [1]. Conservative treatment of CKD consists of clinical strategies (medication, dietary, physical activity and lifestyle modifications) that may delay the progression of renal dysfunction and symptoms and prevent associated complications, such as inflammation [2,3].

CKD increases the inflammatory profile in renal tissue, causing collagen accumulation and fibrosis [4]. Increased renal fibrosis interferes with renal function, leading to the terminal stage (end stage) of the disease in which dialysis therapy is required, consequently decreasing the quality of life [5]. At this stage, the condition appears to be associated with skeletal muscle wasting and decreased strength since myofibroblast transdifferentiation may induce glomerulosclerosis and tubule-interstitial fibrosis [4].

Evidence suggests that exercise training, specifically resistance training (RT), promotes considerable benefits for neuromuscular parameters, such as increases in strength, power and muscle mass [6,7]. In experimental models of kidney disease, some evidence indicates that aerobic exercise (treadmill and swimming) could promote a decrease in inflammation and apoptosis [4,8]. Nevertheless, the effects of RT on renal tissue inflammation and fibrosis in CKD and the roles of muscle mass and strength in this process are still not clear.

Thus far, the literature has suggested that RT can be used as a

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complementary strategy in CKD treatment to prevent decreases in muscle strength [9]. In addition, RT was demonstrated to counterbalance inflammation by modulating immune system functioning [10]. Several possible treatment strategies have been tested in rats with CKD induced by subtotal nephrectomy [11,12]. After 5/6 nephrectomy (Nx), rats have high systolic blood pressure, high urinary protein and serum creatinine levels, muscle mass loss, and kidney fibrosis; however, there are still no studies with RT as a supporting treatment for CKD induced by Nx [11].

Since two of the problems faced by CKD patients are the loss of muscle mass and increased inflammation [4], the use of RT can be an important tool for the treatment and/or prevention of this condition. Nevertheless, the effects of RT on renal tissue in the CKD model have not yet been evaluated. Thus, the aim of this study was to investigate the influence of RT on renal fibrosis and pro/anti-inflammatory gene expression in CKD rats. Our hypothesis is that RT would attenuate inflammation, muscle mass loss, decreased strength and renal fibrosis in rats with CKD induced by nephrectomy.

2. Materials and methods

2.1. Animals

Fifteen male Munich-Wistar rats (8 weeks old) from the breeding colony of the School of Medicine, University of São Paulo (FMUSP São Paulo, Brazil), with an initial weight of approximately 240 ± 20 g, were used. The animals had free access to water and chow and were kept in collective cages (5 rats per cage) at a constant temperature (23 ± 2 °C) with a 12-h/12-h light/dark cycle (lights on at 06:00 a.m.). The animals were fed with a standard rat chow diet (Purina, Descalvado, São Paulo, Brazil). This research was approved by the Committee for Experimental Animals of the Federal University of São Paulo (protocol No. 0243/12). All animal procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council 1996).

2.2. Renal ablation

Renal ablation was performed by 5/6 nephrectomy (Nx), which was performed after ventral laparotomy under anesthesia (ketamine - 50 $mg \cdot kg^{-1}$ and xylazine 10 $mg \cdot kg^{-1}$; Syntec®, São Paulo, Brazil). The right kidney was removed, and two-thirds of the left kidney was infarcted. Sham-operated rats underwent anesthesia and manipulation of the kidney pedicles, without removal of the kidneys. After surgery, the animals received 5 $mg \cdot kg^{-1}$ of enrofloxacin (Schering-Plough®, São Paulo, Brazil) and returned to their original cages, which were heated for 24 h. After full recovery, the animals received ad libitum access to tap water and regular rodent chow. The protocol was performed as described by Arias and colleagues [13]. Four weeks after renal ablation, the animals were randomly distributed into the following 3 experimental groups (5 animals per group): control sedentary (Sham), Nx sedentary (Nx SED), and Nx resistance training (Nx RT).

2.3. Maximal weight carried test

To evaluate the muscle strength in all groups, the maximal weight carried (MWC) test was used. Before the test, rats were subjected to familiarization trials consisting of climbing a vertical ladder (5 climbs per day without a load) on 5 consecutive days. After 3 days of familiarization trials, the MWC test was applied. The test consisted of 4–8 ladder climbs, with progressively heavier loads added for each climb; the load was attached to the proximal portion of the tail with an adhesive tape as described by Neves and colleagues [14]. The initial climbing load for the first test was set at 75% of the animal's body mass; afterward, an additional load of 30 g was added for each subsequent climb, with a 120 s interval between climbs, until the animal failed to complete the climb. Failure was considered when the animal could no longer climb the ladder after 3 successive stimuli (using tweezers on the tail). The highest load successfully carried on the ladder was considered the rat's MWC, which was used to determine the training load. During the training program, the MWC test was performed every 2 weeks to readjust the training load for the Nx RT group and to evaluate the behavior of muscle strength in the Sham and Nx SED groups. For the subsequent MWC tests, the first four loads calculated to begin the test were 50%, 75%, 90% and 100% of the last MWC, with an interval of 120 s between climbs; afterward, an additional load of 30 g was added for each climb until the animal reached failure. The highest load successfully carried on the ladder was considered the rat's new MWC.

2.4. Resistance training program

After determination of the MWC, the resistance training started. The training was performed for 10 weeks, three times a week, on non-consecutive days, for a total of 30 sessions of training; the sessions lasted approximately 12 min per animal. The training sessions consisted of 8 ladder climbs, with two climbs each at 30%, 50%, 60% and 70% of their MWC and 60 s of rest between each ladder climb. The length of the ladder induced the animal to perform 8–12 dynamic movements per climb as described by Neves and colleagues [14].

2.5. Metabolic cage

Forty-eighty hours after the last training session, the animals were placed in a standard circular metabolic cage with a wire mesh floor, and urine was collected in an acrylic tube in a funnel system at the bottom of the metabolic cage. For all animals, diuresis, food and water intake values were collected for 24 h in the metabolic cage [15]. The urine collected in the acrylic tube was quantified in a graduated cylinder to calculate the diuresis of each animal. To measure the water and food intake, each animal received a standard portion of 30 g of rodent chow and 100 mL of water; after 24 h of collection, the chow weight and water volume differences were determined by subtracting the remaining portion from the initial portion.

2.6. Renal tissue collection and biochemistry

Euthanasia of the animals occurred 96 h after the last training session to prevent any influence of the acute exercise session on the physiological parameters studied. The animals were anesthetized with ketamine and xylazine (50 and 10 $mg \cdot kg^{-1}$, respectively, intramuscularly), and kidneys were collected, wherein one midcoronal renal slice was postfixed in buffered 4% formaldehyde for subsequent histomorphometric analysis [13]. The other slice of renal tissue was stored in a −80 °C freezer for gene expression analysis. A blood sample was collected from the abdominal aorta for measurement of total creatine kinase (CK) levels following the manufacturer's instructions (Labtest Diagnostica, São Paulo, Brazil).

2.7. Histomorphometric analysis of renal fibrosis

To evaluate fibrosis, the kidneys were embedded in paraffin, and picrosirius red staining of the sections was performed on the left renal tissue. An objective of 20× was used under polarized light to calculate fibrotic tissue. For this measurement, an AxioPhot I microscope (Carl Zeiss, Gottingen, Germany) combined with a ICc 3 Axiocam camera were used to examine the slides. To obtain the images, the AxioVision software v 4.7.2.0 capture system (Carl Zeiss, Goettingen, Germany) was utilized. The bright Sirius Red areas were quantified by using the software ImageJ 1.43a, 64-bit. The subcapsular cortex, the medulla, and the vessels were avoided when acquiring the images. The result of the analysis is given in square micrometers and was used to determine the percent area (%), which refers to the proportion of the interstitial