Acta Histochemica 116 (2014) 1216–1224

Contents lists available at ScienceDirect

Acta Histochemica

journal homepage: www.elsevier.de/acthis

Effects of eccentric exercise in rehabilitation of phasic and tonic muscles after leg immobilization in rats

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ARTICLE INFO

Article history:
Received 15 June 2014
Received in revised form 6 July 2014
Accepted 7 July 2014

Keywords:
Immobilization
Skeletal muscle
Rats
Eccentric training
Rehabilitation

ABSTRACT

Eccentric exercise is an essential resource for skeletal muscle rehabilitation following muscle disuse however, abnormalities linked to the tissue recuperation require further research. Our aim was analyze the adaptation ability of rehabilitated muscular tissue in rats during different periods of eccentric training after 10 days of limb immobilization. Twenty-seven Wistar rats were divided into six groups: immobilized 10 days, immobilized and eccentric trained for 10 days, immobilized and eccentric trained for 21 days, and three age-matched control groups. After sacrifice, soleus and plantaris muscles were frozen, cut and stained for general histology using hematoxylin and eosin and Gomori trichrome methods and immunohistochemical methods for fiber typing (mATPase, NADH2-TR), for capillaries (CD31) and intermediate filaments (desmin, vimentin) and high resolution microscopy of resin embedded material. Immobilization resulted in more intense morphological alterations in soleus muscles such as formation of target fibers, nuclear centralization, a reduction in the number of type I fibers, diameter of type I, IIA, IIAD fibers, and capillaries. After 10 days of eccentric training, increases in the nuclear centralization and the number of lobulated fibers were observed. This period was insufficient to reestablish the capillary/fiber (C/F) ratio and distribution of fiber types as that observed in the control group. However, 21 days of rehabilitation allowed the reversal of all morphological and quantitative abnormalities. For the plantaris muscles, 10-days of training restored their basic characteristics. Despite the fact that immobilization affected soleus and plantaris muscles, 10 days of eccentric training was insufficient to restore the morphological characteristics of soleus muscles, which was not the case observed in plantaris muscle.

Introduction

The maintenance of skeletal muscle cytoarchitecture requires a minimum number of repetitious actions. Muscle disuse resulting from use of limb immobilization devices leads to reduced mechanical transduction and a cascade of structural alterations, mainly in skeletal muscles with tonic characteristics such as the soleus (Pette and Staron, 2000). On the contrary, phasic muscles such as the plantaris are less affected.

Muscle fiber phenotypes are also modulated by stimuli transduction as in cases of increased/decreased neuromuscular activity and/or mechanical charge (Talmadge, 2000). Previous studies in which the soleus muscle of rats was submitted either to limb immobilization in shortened position (Loughna et al., 1990) or suspension (Stevens et al., 2000; Cornachione et al., 2008) showed that disuse resulted in atrophy and transition of myosin heavy chain (MHC) from slow to fast isoforms, causing an increase in MHCIIa and a reduction of MHCI.

Disuse can also cause changes in local blood flow and in the number of capillaries. These alterations are more evident in the

Abbreviations: Immob, immobilized group; C, control group; $IE_{(10)}$, immobilized and rehabilitated by eccentric exercise for 10 days; $C_{(10)}$, control group of the $IE_{(10)}$; $IE_{(21)}$, immobilized and rehabilitated by eccentric exercise for 21 days; $C_{(21)}$, control group of the $IE_{(21)}$; H.E, hematoxylin-eosin; mATPase, myofibrillar adenosine triphosphatase; NADH2-TR, reduced nicotinamide adenine dinucleotide tetrazolium reductase; I, fiber type I; IIA, fiber type IIA; IIB, fiber type IIB; IIC, fiber type IIC; IID, fiber type IID; C/F, capillary-to-fiber ratio.

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http://dx.doi.org/10.1016/j.acthis.2014.07.002
0065-1281/

first week of disuse, but they can persist for up to three weeks. Simultaneously, an increase in intramuscular connective tissue has been observed (Jozsa et al., 1990; Kannus et al., 1998). This process of disuse and connective tissue proliferation is associated with the obliteration of capillaries, which, in turn, initiates a vicious cycle leading to more intramuscular connective tissue and reduced blood flow (Jozsa et al., 1990). Cornachione et al. (2011a) observed a significant reduction in the capillary/fiber (C/F) ratio in the soleus muscle following hypokinesia.

Desmin and vimentin are important intermediary filaments (IFs) that help maintain cell architecture and transduce stimuli in both extracellular and intracellular spaces (Cizkova et al., 2009). Vimentin is a typical IF derived from mesenchyme (Franke et al., 1978), which disappears completely during tissue differentiation (Sejersen and Lendahl, 1993). Vimentin is also present in the initial developmental phase of new myoblasts and myotubes, and at a later stage, desmin appears and remains in skeletal muscle throughout the entire life of the animal (Vaittinen et al., 2001). The composition and organization of these IFs can be affected by neuromuscular diseases (Sarnat, 1992) or as a result of experimental procedures such as immobilization (Vater et al., 1992). These proteins seem to be important regeneration markers when muscles are induced to overload or engage in physical activity after a period of disuse.

Despite the deleterious structural and functional effects caused by immobilization, this procedure is still commonly used in clinical practice in the initial phases of treating musculoskeletal lesions. It is known that skeletal muscle regeneration occurs during a period of approximately 21 days composed of three sequential phases: (1) an inflammatory reaction characterized by the invasion of macrophages, (2) an activation phase in which satellite cells differentiate and consolidate, and (3) the maturation of recently regenerated myofibrils (Ciciliot and Schiaffino, 2010). During this recovery period, rehabilitation programs can help promote the reversal of lesions caused by primary musculoskeletal damage and secondary disuse and other additional lesions caused by the initial reloading process.

Eccentric exercise has been shown to induce muscular regeneration after periods of hypokinesia (Cornachione et al., 2008, 2011a). Eccentric training programs have produced increased tension levels over those found during concentric and isometric contractions (Olson et al., 1972). Some authors argue that stimuli triggered by eccentric contraction lead to increased protein synthesis and hypertrophy (Mayhew et al., 1995). Eccentric exercise is also related to increased tension in the myofilaments, which is determined by reduced recruitment of motor units (Mayhew et al., 1995), a situation that might lead to cellular damage (Evans et al., 1986). Proske and Morgan (2001) reported that cellular lesions appeared during the initial phase of eccentric training, but the tissue was able to adjust and minimize the occurrence of morphological and functional lesions when the stimulus was maintained for a long time.

Physical exercise can optimize the post-disuse recovery period. Based on this, our objective was to analyze the adaptation ability of muscle tissue of rats rehabilitated using different periods of eccentric training following 10-days of limb immobilization. The anti-gravitational attitude of quadrupeds favors the constant recruitment of the soleus muscle in maintaining static posture (Roy et al., 1991; Gregor et al., 2006). On the contrary, the plantaris assists in the phasic fast and rhythmical movements of plantar flexion during movement. Our main hypothesis was that only a period of time exceeding 10 days of eccentric training associated with free movement in a cage would favor the complete regeneration of the soleus muscle.

Materials and methods

Animal groups

The Animal Research and Care Committee of the Medical School of University of São Paulo approved all experimental protocols for this study. The protocols and procedures of this study were approved by the Ethics in Animal Experimentation Committee of the University of São Paulo Medical School at Ribeirão Preto (Process # 043/2007). The animals had free access to water and food. They were maintained at room temperature with 12-h light/12-h dark cycle with restricted movement of persons.

Twenty-seven Wistar rats (81 days old, mean body weight of 319 g) were divided into six groups: (1) immobilized for 10 days (Immob, $n=6$); (2) 91-day old control group (C, $n=3$); (3) Immobilized and rehabilitated by eccentric exercise for 10 days ($IE_{(10)}$, $n=6$); (4) 101-day-old control group of the $IE_{(10)}$ ($C_{(10)}$, $n=3$); (5) immobilized and rehabilitated by eccentric exercise for 21 days ($IE_{(21)}$, $n=6$); (6) 112-day-old control group of the $IE_{(21)}$ ($C_{(21)}$, $n=3$).

Immobilization procedure

The animals were immobilized following the model proposed by Coutinho et al. (2002) and Benedini-Elias et al. (2009). The special immobilization apparatus was composed of stainless steel mesh, cotton, impermeable surgical tape, adhesive tape, micropore tape, impermeable surgical tape, viscolycra fabric and a stapler. The lower right limb was immobilized, fixing the knee in extension position and the ankle in maximum plantar flexion (Cornachione et al., 2013). The contralateral (left) lower limb and the upper limbs were free and allowed the animals to move in the cage. The total immobilization period was 10 days.

Eccentric training program

The eccentric training program was performed on an electrical treadmill running with 16° declination (Takekura et al., 2001) and started 24 h after the removal of the immobilization apparatus. After immobilization for 10 days, the rats in groups $IE_{(10)}$ and $IE_{(21)}$ were submitted to a period of 10 days and 21 days running on a declined treadmill, respectively, following the protocol developed by Norman et al. (2000). Using this approach, the training exercise period started with a 10-min daily running session. Every day, an additional 5 min (adaptation) were added until 40 min of training per day was reached, at a speed of 17 m/min. The animals trained for three consecutive days followed by one day without training. This procedure was applied to avoid overtraining. After the experiment was concluded, the animals were weighed and euthanized by an overdose of thiopental anesthetics and the soleus and plantaris muscles were removed.

Histology and histoenzymology

For histological procedures, a fragment of the ventral portion of each muscle was removed. The fragments were dusted in talcum powder and frozen in liquid nitrogen. Another fragment from the central portion of each muscle was removed for high resolution microscopy and fixed in formol, dehydrated in alcohol, and embedded in Historesin® (Leica Instruments GmbH, Heidelberg, Germany). 5 μm-thick transverse frozen sections of soleus and plantaris muscles fragments were cut with a Leica CM 1850 UV cryotome (−25 °C) (Leica Instruments GmbH, Heidelberg, Germany). Sections were stained with Hematoxylin and eosin (H&E) and modified Gomori's trichrome techniques, and histoenzymological techniques for myofibrillar adenosine triphosphatase (mATPase)