CONSEQUENCES OF ADAPTIVE EFFORT ON SKELETAL AND CARDIAC MUSCLE STRUCTURE IN RAT

M. L. Kiss¹, R. Moldovan¹, V. Rus², Flavia Ruxanda², Adriana Mureșan¹

¹„IULIU HAȚIEGANU” UNIVERSITY OF MEDICINE AND PHARMACY, FACULTY OF MEDICINE, DEPARTMENT OF MEDICAL EDUCATION, CLUJ-NAPOCA, ROMANIA; ²UNIVERSITY OF AGRICULTURAL SCIENCES AND VETERINARIAN MEDICINE, FACULTY OF VETERINARIAN MEDICINE, CLUJ-NAPOCA, ROMANIA

Summary

The purpose of this study was to assess the histological changes that might appear in skeletal and cardiac muscle in rats, after physical effort. 25 male Wistar rats were taken into study and divided in 5 groups as follows: I (control animals), II (control animals supplemented with flavonoids), III (effort trained animals), IV (animals supplemented with flavonoids and effort trained) and V (animals supplemented with flavonoids and effort trained with 15% weigh loading). After 21 days, the animals were sacrificed through euthanasia and samples of skeletal and cardiac muscles were harvested. After histological processing, the samples were analyzed through light microscopy (Olympus BX51 microscope). The results showed a moderate muscle cell hyperplasia (in the skeletal muscle), in one of the animals from group IV. The rest of the animals did not show any histological changes in the harvested muscles. Animals taken into study did not show any significant histological changes in the myocardium and skeletal muscle, which demonstrates a good ability to adapt to physical exercise conducted between physiological limits. Flavonoid supplementation does not determine structural changes perceptible through light microscopy, in the muscles taken into study.

Keywords: flavonoids, hypertrophy, myocardium, physical effort, skeletal muscle

Introduction

A series of studies have highlighted histological changes in skeletal muscle after effort: fragmentation of the myofibrils after forced lengthening contractions (Komulainen et al., 1998), volume changes and increased number of myonuclei (James and Cabric, 1981), satellite cell proliferation (Smith et al., 2001), inflammatory precocious changes and degenerative changes (Armstrong, 1986; Hesselink et al., 1996). Intracellular changes of the sarcotubular system in effort were highlighted in the myocardium (Thomas, 1985).

It was believed that the skeletal and cardiac muscle tissue in adults is incapable of proliferating and that the volume augmentation of these tissues is entirely the result of hypertrophy. Currently, there is more and more evidence that these types of cells are capable of proliferation, as well as repopulating from precursor cells, besides hypertrophy (Kumar et al., 2010).

Hypertrophy is the result of increased production of cell proteins. Most of the studies and the knowledge gathered until now on the cell hypertrophy, were conducted on the heart. Hypertrophy can be induced by simultaneous action of some mechanical sensors (which are triggered by physical effort), growth factors (including TGF-β, insulin-like growth factor-1 [IGF-1], fibroblast growth factor) and vasoactive agents (like α-adrenergic agonists, endothelin-1 and angiotensin-II). Truly, the mechanical sensors induce the production of some growth factors and their agonists. All these stimuli synergically operate to increase the muscle protein synthesis, which is responsible for the muscle hypertrophy. The two main biochemical
pathways involved in muscle hypertrophy seem to be phosphoinositide-3-kinase/akt (postulated to be more important in the physiologically induced hypertrophy, for example the effort hypertrophy, as in our experiment) and signal decrease for G protein-coupled receptors (induced by some growth factors and vasoactive agents; which is believed to have a more important role in pathological hypertrophy).

Muscle hypertrophy can also be associated with a replacement of adult forms contractile proteins with foetal or neonatal ones. For example, during the muscle hypertrophy, α-myosin heavy chain isoform is replaced with β–myosin heavy chain isoform, which gives a slower contraction, which is more economic, energetically speaking. Moreover, some genes which are expressed only during early development, reexpressed in muscle cells hypertrophy, and the products of these genes, participate in cellular stress response (Kumar et al., 2010).

Material and methods

Studies were conducted on male Wistar rats, with an average weight of 200-220 g, purchased from the biobase of „Iuliu Hatieganu” University of Medicine and Pharmacy, in Cluj-Napoca. Animals were kept in appropriate vivarium conditions, standardized at the Department of Physiology biobase.

Researches were performed on 5 groups of rats (n= 5 animals/group):
- group I – control animals;
- group II – control animals supplemented with flavonoids;
- group III – effort trained animals;
- group IV – animals supplemented with flavonoids and effort trained;
- group V – animals supplemented with flavonoids and effort trained with 15% weigh loading.

The flavonoids (at 5% concentration) were administered through buccopharyngeal gavage, in dose of 90µl/rat. „Grape Seed Oil TX-008041” product from Textron Técrica SL Company was used.

The effort capacity was established based on the running time. The effort time was monitorized for 21 days. The aerobic effort capacity was measured in the Experimental Research Laboratory in the Physiology Department, « Iuliu Hatieganu » University of Medicine and Pharmacy, Cluj-Napoca. In order to determine the effort capacity, the running treadmill test was conducted. The length of the test was timed (seconds), from the start of the running treadmill until the exhaustion of the rats, the moment the running ceased (refusal to run). The speed of the treadmill belt was 3.2 km/h.

The following samples were harvested for histological examination: myocardium (left ventricle) and skeletal muscle (femoral biceps), immediately after euthanasia, at the end of the experiment (day 21). The samples were processed through paraffin technique. Hematoxylin and Eosin (H&E) staining was used, which allows a good differentiation of the cell types. Examination and processing of the samples were made using an Olympus image processing system, namely an Olympus BX51 microscope and Olympus Cell B basic imaging software.

Results and discussions

After the compared histopathologic examination, we observed that animals from groups I, II, III and V did not show histological changes in the organs taken into study (Fig. 1). In group IV, there was a certain muscle cell hyperplasia, in the skeletal muscle, in one of the animals from this group (Fig. 2).

The change was moderate, representing one of the muscle fiber’s adaptive methods to effort. Microscopically, the rounding of the affected muscle fibers appears, with an increased eosinophilic hue. This was the only microscopical change in the skeletal muscle investigated.
Muscle hypertrophy refers to an increasing volume of the cells, resulting in an increase of the affected organ. The hypertrophied muscle does not contain new cells, only larger sized cells. The increase in cell is due to the synthesis of new structural compounds. The main stimulus for muscle hypertrophy is represented by the increase in the muscular effort. For example, the rounded muscles in bodybuilders, represent muscle fibers engaged in “iron pumping” and result from increase in size of the individual muscle fibers as response to an increased solicitation. In cardiac muscle, the stimulus for hypertrophy is usually chronic hemodynamic overload, after hypertension or valvular defects. In both kinds of tissues, muscle cells synthesize more proteins and the myofilament number increases. This leads to an augmentation of the force generated by every myofiber, thus increasing the strength and working capacity of the whole muscle (Kumar et al., 2010).

The myocardium showed no changes in any animal from the investigated groups (Fig. 3).

Venditti et al. (1997) have studied the effect of the physical effort on the antioxidant defense and susceptibility to damage induced by intense effort on adult Wistar rat tissues. They have observed that the membrane integrity was unaffected both in skeletal muscle and myocardium. After the study, the authors concluded that the lesions induced by free radicals produced in the muscles during exercise may play an important role in appearance of muscle fatigue. Sen et al. (1992), in a study on Wistar rats, have showed that prolonged physical exercise has contributed to increased oxidant and detoxification status in muscle and liver.

Our results show the lack of significant histological changes in myocardium and skeletal muscle, which shows that animals subjected to physical exercise tests, showed a good adaptability.

Conclusions

Animals taken into study did not show any significant histological changes in the myocardium and skeletal muscle, which demonstrates a good ability to adapt to physical exercise conducted between physiological limits.

Flavonoid supplementation does not determine structural changes perceptible through light microscopy, in the muscles taken into study.
Fig. 3. Group III – cardiac muscle, normal aspect (H&E)

References