DOI 10.26724/2079-8334-2019-2-68-215-219
UDC 616.748–003.93–06:616.153.922]–092.9

I. I.Yuryk, P. Ya. Bodnar, Ya. Ya. Bodnar, Yu. M. Orel
HSEE "I. Horbachevsky Ternopil State Medical University, MOH of Ukraine", Ternopil

FEATURES OF REMODELING CROSS-STRIATED MUSCLES OF HIND LIMBS IN RATS UNDER CONDITIONS OF EXPERIMENTAL HYPERCHOLESTEROLEMIA

e-mail: iyuryk@ukr.net

The study was performed with the purpose to study the features of cross-striated muscles remodeling of the femoral, knee and lower leg areas of the hind limbs in rats of pre-reproductive and reproductive age under conditions of experimental hypercholesterolemia. It was established that remodeling of muscle fibers in all terms of the experiment was manifested by loss of striation, homogenization, sarcoplasmolysis with myocytolysis and contracture changes. Submicroscopically degenerative and destructive changes of mitochondria, cytoplasm vacuolation, pycnosis of hemocapillar endotheliocytes' nucleoplasm were observed. The most significant remodeling occurs in the cross-striated muscles of reproductive age animals in the lower leg area, in animals of pre-reproductive age - in the femoral and knee areas.

Key words: hypercholesterolemia, remodeling, skeletal muscles, contracture changes, myocytolysis.

The work is a fragment of the research project "Prevention and treatment of postoperative complications in planned and urgent surgery", state registration No. 0116U003354.

Atherosclerotic injury of the lower extremities' arteries remains an important medical and social problem of the present day. According to epidemiological studies, the overall prevalence of peripheral arterial disease makes 3-10%, and among patients aged over 70 it makes 15-20% [8, 9]. The problem's topicality is aggravated by an increase in the share of the elderly persons among the population. In such individuals, the incidence of occlusive lesions in the lower extremities arteries may reach 23%, among which 20-40% develop chronic critical ischemia of the lower extremities (CCILE). Over 90% of patients with CCILE during the first year after diagnosis are performed amputations, reconstructive or angioplasty operations. Only during the first year after the CCILE diagnosis, 25% of patients require high level lower limb amputation and another 25% die [7]. Atherosclerosis is a systemic disease, so injury of a single vascular pool increases the incidence of asymptomatic disease or clinical manifestations of other localizations. In the REACH studies, in 65% of patients with diagnosed lower limb artery disease, clinical manifestations of other vascular pools were detected. A significant problem remains the presence of "multistoried" lesions of the lower extremities' vessels, which is found in about 70% of patients with critical ischemia [2, 4]. However, the conditions of surgical treatment do not always take into account the morphofunctional state of the patient's cross-striated muscles. At the same time, the cross-striated muscles play a significant role in providing hemodynamics in the lower extremities. Proceeding from the above, the study of the muscle and hemomicrocirculatory bed remodeling features under the conditions of atherosclerotic lesion of the lower extremities arteries is a topical problem for development of efficient methods of prevention, diagnosis, correction and treatment of this pathology.

The purpose of the study was to establish the peculiarities of the hind limb cross-striated muscles remodeling in rats of the pre-reproductive (PRA) and reproductive age (RA) under experimental hypercholesterolemia (HC).

Materials and methods. HC simulation was performed in 16 white rats by feeding cholesterol in the dose of 0.5 g/kg with warmed vegetable oil and using Mercazolil in the dose of 10 mg/kg [3, 5, 6]. The mixture was injected with a probe intragastrically. Animals with biochemically verified HC were divided into 2 groups: the first included 8 animals aged 3-4 months, weighing 150 - 170 grams, and the second - 8 rats, which reached 11-12 months of age, weighing 230-250 grams. The control group included rats at the age of 4 and 12 months. Each group comprised 8 animals.

The rats keeping and all experiments were carried out in accordance with the provisions of the European Convention for the Protection of Vertebrate Animals used for Experiments and Other Scientific Purposes (Strasbourg, 1986), the General Ethical Principles of Animal Experiments adopted by the First National Congress on Bioethics (Kyiv, 2001), Helsinki Declaration of the World Medical Association (2000), the Order of the Ministry of Health of Ukraine No. 281 of November 1, 2000. Removal of laboratory rats from the experiment was carried out by bleeding after intraperitoneal administration of sodium thiopental in the dose of 50 mg/kg body weight after 15, 30 and 45 days of the study.

For histological and polarization studies, soft tissues were excised with vascular-nerve bundles up to 0.5 cm thick from the femoral, knee, and lower leg areas of the hind limbs. Fixation, paraffin sealing and histological sections were made according to standard techniques. Dewaxed sections were

stained with hematoxylin and eosin, Hart fuxelin, picro-fuchsin by the van Gieson method, iron hematoxylin by the method of Heydengain. The preparations were studied in a Granum microscope. The images from the microscope were displayed to the computer monitor using the VISION Color CCD Camera and the InterVideoWinDVR software.

Microscopic-polarization study of the samples was performed in a trinocular microscope with a software image processing and polarization camera (SEO). Sections for an electron microscopic study were prepared according to standard procedures [1]. The semifine sections 1-2 μm thick were made using LKB-3 ultramicrotome (Sweden) and stained with methylene blue. Ultrathin sections were contrasted with lead citrate by the Reynolds method and studied with the PEM-125K electron microscope [1].

Results of the study and their discussion. Light-optically, in muscles of the pre-reproductive age animals after 15 days of experimental HC stasis, erythrocyte aggregation, partially exfoliated endothelium, swelling and fraying of perivascular stroma were revealed. Among the unchanged muscle fibers there are unevenly colored, without expressive cross-striation, there are no cytolysis phenomena. Polarization microscopy has shown the predominance of the I and II stage fiber injuries of the contracture type. In 4 animals' knee area muscles, an A-discs fusion was observed, which corresponded to the stage III injury.

In the reproductive age animals, these changes were more often reported in the lower leg area, where the frequency of stages III and IV injuries increased. Muscle fibers in this period were usually having a homogeneous structure, their nuclei were clearly defined under the myolemma, orienting their long axes in parallel with it. The cytolysis phenomena were manifested sporadically.

Sub-microscopic changes in the muscle fibers of animals in the both age groups in this period more concerned the energy apparatus. In mitochondria, a focal cristae destruction and the matrix clearing were revealed. Mitochondria were located evenly. Other organelles with the changed structure alternated with little changed ones. Other myofibrils changes reported by us in the first series of the experiment were not observed. Myofibrils had the correct location, but with areas of myofilaments fraying. Sarcomeres, as a rule, were distinctly noticeable.

Hemocapillar endotheliocytes are densely located, their cytoplasm shows an increase in the number of pinocytotic vesicles and the presence of excrescences on the lumen surface. The cytoplasm is heterogeneous with small osmophilic inclusions. The nuclei are swollen, with single superficial invaginations (fig. 1).

After 30 days of experimental HC, the manifestations of structural changes in the skeletal muscles of the rat hind limbs significantly increased in animals of the both studied groups and were manifested by aggravation of the hemomicrocirculatory disorders that became systemic. As in the previous studies, the structural reorganization of the knee and femoral vessels was characterized by a hypertrophic remodeling. The polarization study of the muscles in this group of the pre-reproductive age (PRA) animals showed both types of myofibril injuries. With the prevalence of contracture type injuries, cases of their third stage and myocytolysis with less pronounced regeneration were more frequent.

In samples stained with hematoxylin and eosin, there were areas of homogenization and fragmentation. The characteristic rectilinear arrangement of myofibrils often varies in an unevenly wave-like manner. Lymphohistiocytic infiltrates were formed around the aggressive cells.

All the structural components of muscles underwent the similar changes in the reproductive age animals. The peculiarity was the presence of round cell infiltrates in the vascular wall and in the perivascular area with a tendency to spread on peri- and endomysium. We observed the most pronounced changes in the lower leg area.

On the semifine sections, along with the unchanged muscle fibers, there were fibers with signs of myofibrils fraying, their decay and deformed directivity.

At the submicroscopic level, the manifestations of structural changes in muscle fibers were increasing. The number of mitochondriawas growing, indicating the activation of compensatory mechanisms. They were getting polymorphic, most of the organelles were concentrated under the myolemma and were destructively changed, and the secondary lysosomes were detected between them. The myolemma was partially exfoliated and had a hilled appearance. Sarcomeres of muscle fibers varied in size, disintegration of their anisotropic disks was clearly expressed. More distinct destructive changes were observed. Hemocapillars studies also showed an increase in destructive changes. The basement membrane was thickened, the cytoplasm of the endothelial cells was heterogeneous, cleared, the nucleui were pyknotic. In the perivascular spaces, there were edema phenomena due to the increase in the content of the electron-illuminated amorphous component of the fluffy connective tissue (fig. 2).

Microscopic analysis of structural changes in skeletal muscle and hemomicrocirculatory bed of

PRA and RA rats after 45 days of experiment showed the same patterns of morphological changes development as those described above. In general, their progress was observed, but the intensity of growth was somewhat reduced. As in the previous periods, the dependence on the age of rats was observed: the above changes prevailed in the reproductive age animals.

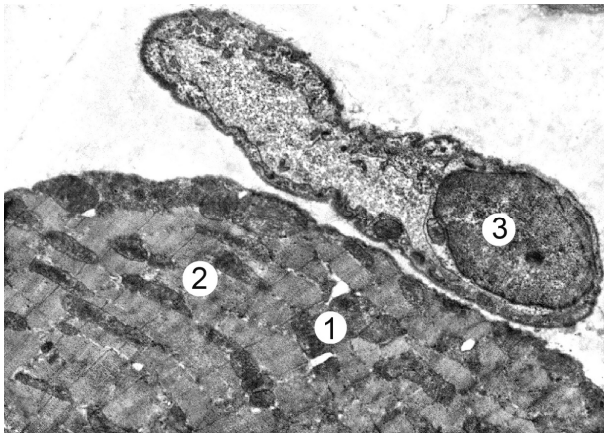

Fig. 1. Ultrastructure of of the lower leg skeletal muscle fiber in a rat of reproductive age after 15 days of experimental hypercholesterolemia. Mitochondria (1), myofibrils (2), endotheliocyte's nucleus (3).Magnific.: × 12000.

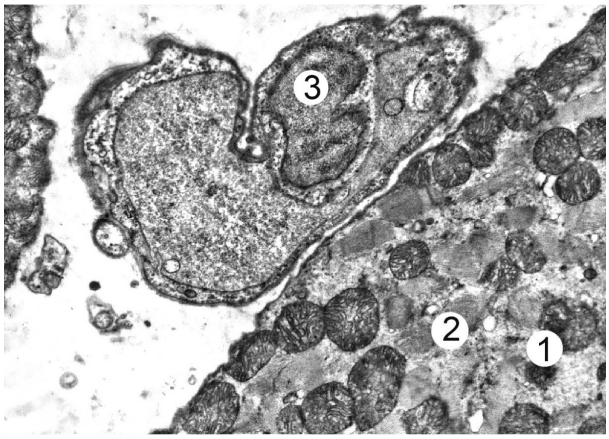

Fig. 2. Ultrastructure of the lower leg skeletal muscle fiber in the reproductive age rat after 30 days of experimental hypercholesterolemia. Mitochondria (1) and myofibrils (2) with destructive changes, endotheliocyte nucleus (3). Electronic microphotography.Magnific.: × 19000.

The study of muscle in polarization light in PRA animals in the knee area revealed all types of contracture injuries. In half of animals, along with the III-IV stages injuries, the phenomenon of intracellular myocytolysis occured.

In the RA animals, similar as to the injury severity changes were found in the lower leg muscles, where contracture I-II stages changes occurred in all the rats, and III-IV stages lesions with intracellular myocytolysis were observed in most animals.

The femur muscles were less damaged. Most frequently, the contracture type I-II stages lesions, less frequently - stages III and IV were observed. Myocytolysis in the lower leg was detected in 3 rats out of 8 ones studied (fig. 3).

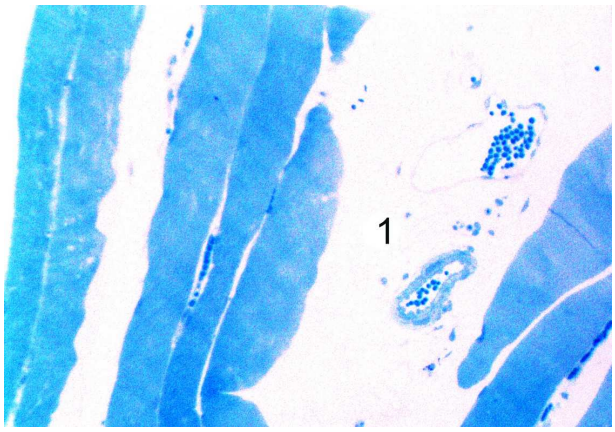

Fig. 3. Myocytolysis of the lower leg skeletal muscle a reproductive age rat after 45 days of experimental hypercholesterolemia. Perivascular edema (1). Semifine section. Staining with methylene blue. Magnific.: × 1200.

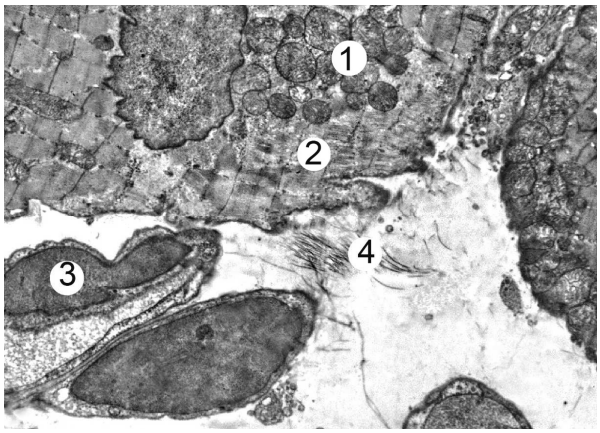

Fig. 4. Ultrastructure of the skeletal muscle fiber of the femur area in a pre-reproductive age rat after 45 days of experimental hypercholesterolemia. Mitochondria (1) and myofibrils (2) with destruction phenomena, endotheliocyte nucleus (3), collagen fibers (4). Electronic Microphotography. Magnific.: × 12000.

Submicroscopically further enhancement of destructive changes in muscle fibers and blood vessels of the hemomicrocirculatory bed was established. Most of the mitochondria had a bright matrix and reduced cristae, the outer membrane was occasionally damaged. Cytoplasm of the of hemocapillars endotheliocytes was cleared and contained small osmiofilic lobules. A small number of micropinocytic vesicles and single small excrescences on the luminal surface showed a decrease in transcapillary metabolism. Proliferation of collagen fibers was detected perivascularly. In the muscle fibers there was a submyolemma edema. Myolemma is partially exfoliated and has a hilly appearance. Decompositions of necrotic muscle fibers have been observed in separate sites (fig. 4).

A review of modern literature sources suggests that hypercholesterolemia is an important

hereditary, pathobiochemical and pathophysiological factor that can change the course of the disease, and also affect remodeling of its structural components. Analyzing in general the obtained results of the study it can be argued that in the skeletal muscles and hemomycocirculatory channels in the conditions of experimental hypercholesterolemia there is a complex of progressively unspecific morphological changes at all levels of their structural organization. Metabolic disturbances cause toxic-hypoxic damage to the vascular endothelium, which creates preconditions for plasma vascular percolation and accumulation of glycosaminoglycans. The final phase of this process development is hyalinosis and sclerosis of arterioles. Under these conditions, inadequate blood supply causes damage to the structure of the muscular component in the form of contractural and cytolytic changes, which have a clear age dependence in depth of their manifestation, which is reflected in the works of many researchers [2, 4, 7, 9]. Thus, in animals of preproductive age, remodeling in all series of the experiment was weaker than that of RA animals. Studies of histological sections of skeletal muscles in the polarized light permitted to detect various contracture damages of muscle fibers. It should be noted that contracture changes of stage III became systemic at the late stages of the experiment and prevailed in animals of the older age group. The presence of dystrophic-necrotic changes in skeletal muscles was confirmed submicroscopically.

Thus, under the conditions of experimental hypercholesterolemia in the skeletal muscles, compensatory and adaptive changes develop, which is a manifestation of trophic insufficiency.

Conclusions

1. Under the conditions of experimental hypercholesterolemia in the muscles and hemomicrocirculatory bed of the experimental animals' hind limbs, a complex of nonspecific changes is developed, characterized by a combination of dystrophic, degenerative, inflammatory and regenerative-hypertrophic processes.

2. The main morphological manifestations of the muscle fibers remodeling at all stages of the experiment were the loss of myolemma cross-striation, its cloddy disintegration and myocytolysis, contracture changes.

3. The most pronounced structural changes were found in the lower legs of reproductive age animals; in animals of pre-reproductive age the changes occurred in the femoral and knee areas.

Prospects for further research: to clarify the features of remodeling the hind limbs skeletal muscles in the rats of pre-reproductive and reproductive age in combination with experimental hypercholesterolemia and hyperuricemia.

References

1. Bahriy MM, Dibrova VA, Popadynets OH, Hryshchuk MI. Metodyky morfolohichnykh doslidzhen. Vinnytsya: Nova Knyha; 2016. 328 s. [in Ukrainian]

2. Klymenko VM, Baldin IA. Revaskulyarizatsiya nyzhnikh kintsivok pry krytychniy ishemiyi. Materialy KHKHIII zyizdu khirurhiv Ukrayiny; 2015, zhovtnya 21-23; Kyiv. 2015. [Internet]: Dostupno na: http://surgery.org.ua/data/ upload / content / main / ua / mainPage / program_zyzdu_21-23.10.2015.pdf [in Ukrainian]

3. Krepkova LV. Ispolzovaniye modeli giperlipidemii i ateroskleroza u krys v toksikologicheskom eksperimente. Biomeditsina. 2011;3:103-6. [in Russian]

4. Nikulnykov PI, Bytsay AN, Yatsenko AI. Mezhdystsyplinarnyi podkhod k lecheniyu patsiyentov s okklyuzionno-stenotycheskim porazheniem arterialnykh sosudov nizhnikh konechnostey. Materialy XXIII zyizdu khirurhiv Ukrayiny; 2015, zhovtnya 21-23; Kyiv. 2015. [Internet]: Dostupno na: http://surgery.org.ua/data/ upload / content / main / ua / mainPage / program_zyzdu_21-23.10.2015.pdf [in Russian]

5. Piskun RP, Hrynchak NM. Osoblyvosti mikromorfometrychnykh zmin struktur sertsya pry eksperymentalnomu aterosklerozi ta yoho hennoyi korektsiyi. Visnyk problem biolohiyi ta medytsyny. 2011; 2 (3): 147-50. [in Ukrainian]

6. Polyakov LM, Lushnykova EL, Nepomnyashchykh LM, Ruskykh HS, Byushkina NKh, Klynnikova MH. ta in. Pokaznyky metabolizmu lipidiv ta bilkovoho skladu lipoproteyiniv plazmy krovi hipotyreoyidnykh shchuriv pry eksperymentalniy hiperkholesterynemiyi. Fundamentalni doslidzhennya. 2014; 10: 342-5. [in Ukrainian]

7. Cherniak VA. Surgery of critical ischemia of the lower extremities. Heart and blood vessels. 2013;1:54-63.

8. Gardner AW, Parker DE, Montgomery PS, Khurana A, Ritti-Dias RM, Blevins SM. Gender differences in daily ambulatory activity patterns in patients with intermittent claudication. J. Vasc. Surg. 2010 Nov;52(5):1204-10.

9. Hirsch AT, Duval S. The global pandemic of peripheral artery disease. Lancet. 2013 Oct 19;382(9901):1312-4.

Реферати

ОСОБЛИВОСТІ РЕМОДЕЛЮВАННЯ ПОСМУГОВАНИХ М'ЯЗІВ ЗАДНІХ КІНЦІВОК ЩУРІВ ЗА УМОВ ЕКСПЕРИМЕНТАЛЬНОЇ ГІПЕРХОЛЕСТЕРОЛЕМІЇ

Юрик І.І., Боднар П.Я., Боднар Я.Я., Орел Ю.М.

Дослідження проведено з метою вивчення особливостей ремоделювання посмугованих м'язів

ОСОБЕННОСТИ РЕМОДЕЛИРОВАНИЯ ПОПЕРЕЧНОПОЛОСАТЫХ МЫШЦ ЗАДНИХ КОНЕЧНОСТЕЙ КРЫС В УСЛОВИЯХ ЭКСПЕРИМЕНТАЛЬНОЙ ГИПЕРХОЛЕСТЕРОЛЕМИИ

Юрик И.И., Боднар П.Я., Боднар Я.Я., Орел Ю.М.

Исследование проведено с целью изучения особенностей ремоделирования поперечнополосатых мышц

стегнової, колінної і гомілкової ділянок задніх кінцівок щурів дорепродуктивного та репродуктивного віку в умовах експериментальної гіперхолестеролемії. Встановлено, що ремоделювання м'язових волокон на всіх термінах експерименту проявлялося втратою посмугованості, гомогенізацією, розпадом саркоплазми з міоцитолізом і контрактурними змінами. Субмікроскопічно доведено дегенеративно-деструктивні зміни мітохондрій, вакуолізація цитоплазми, пікноз нуклеоплазми ендотеліоцитів гемокапілярів. Найбільш істотне ремоделювання проявляється в скелетних м'язах тварин репродуктивного віку в гомілковій ділянці, у тварин дорепродуктивного віку - у стегновій та колінній ділянках.

Ключові слова: гіперхолестеролемія, ремоделювання, скелетні м'язи, контрактурні зміни, міоцитоліз.

Стаття надійшла 20.04.18 р.

бедренной, коленной и голенной областей задних конечностей крыс дорепродуктивного и репродуктивного возраста в условиях экспериментальной гиперхолестеролемии. Установлено, что ремоделирование мышечных волокон на всех сроках эксперимента проявлялось потерей полосатости, гомогенизацией, распадом саркоплазмы с миоцитолизом и контрактурными изменениями. Субмикроскопически доказаны дегенеративно-деструктивные изменения митохондрий, вакуолизация цитоплазмы, пикноз нуклеоплазмы эндотелиоцитов гемокапилляров. Наиболее существенно ремоделирование проявляется в скелетных мышцах животных репродуктивного возраста в голенной области, у животных дорепродуктивного возраста - в бедренной и коленной областях.

Ключевые слова: гиперхолестеролемия, ремоделирование, скелетные мышцы, контрактурные изменения, миоцитолиз.

Рецензент Шепітько В.І.