Effect of Head-Down Hanging on the Course of Degenerative Process in the Hind Paw Muscles of 12-Month-Old MDX Mice

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Effect of functional unloading on the course of degenerative process in muscles was studied in dystrophin-deficient mdx mice. Head down-hanging of animals led to a significant decrease in the cross-section area of muscle fibers, increase in the percentage of fibers with centrally located nuclei and of Evans blue-stained fibers. Gravitation unloading of 12-month-old animals with pronounced manifestations of muscular dystrophy did not inhibit this pathological process.

Key Words: *mdx mice; Duchenne dystrophy; gravitation unloading*

Despite active studies, some aspects of the pathogenesis of Duchenne muscular dystrophy remain disputable. By the present time several main stages in the development of dystrophinopathies are distinguished. Mutation of the gene encoding dystrophin synthesis leads to the appearance of defective structure of the sarcolemmal cytoskeleton in muscle fiber. Membrane integrity is impaired and excessive calcium ions enter into the muscle fiber leading to activation of calcium-dependent proteinases and destruction of muscle proteins [8]. The relationship between degenerative processes and contractile activity of mutant mdx animals was analyzed in some studies, but the results were contradictory. Long-term muscle overstrain induced by the synergist removal led to progressive myodegeneration in mice and increased the size of degeneration zone in the muscle [3]. Increased liability of muscles to necrotic changes caused by eccentric exercise was observed in mutant mice [9]. However, the use of less intensive exercise had a favorable impact on contractile characteristics of mutant animals [5]. Immobilization of young mdx mice inhibited the development of degenerative process [6]. On the other hand, the status of muscles of dystrophic mice under conditions of gravitation unloading has not been described. Hence, some problems related to the role of contractile activity in the pathogenesis of muscle dystrophinopathies remain unclear. Our study was aimed at evaluating the type of contractile activity of muscles on the course of pathological process in dystrophinopathy. The main task of the study was comparison of the reaction of normal animals and animals genetically defective by dystrophin to functional unloading of the muscles. It was expected that functional unloading, preventing working tension and, hence, deformation of the sarcolemma, would inhibit the development of dystrophic process. The study was carried out on 12-month-old animals with signs of muscular dystrophy.

MATERIALS AND METHODS

Experiments were carried out in accordance with standard regulations for handling laboratory animals and were approved by Biomedical Ethics Committee of the Institute. We used 12-month-old male

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mdx (n=13) and C57Bl (n=14) mice. The animals were divided into groups. Eight mdx mice and 7 C57Bl mice were exposed to head-down hanging after Morey-Holton [7] over 9 days. Control groups consisted of animals (6 per strain) left in cages. The animals received water and fodder *ad libitum*. Twenty-four hours before sacrifice all animals were intraperitoneally injected with 1% Evans blue (1 ml/100 g) [4]. The animals were sacrificed by nembutal overdosage. The complex of soft tissues of the shin with bones was dissected and frozen in liquid nitrogen. Transverse cryostat sections (10 μ) were stained with hematoxylin and eosin, the percentage of fibers of centrally located nuclei was evaluated and cross-section area was estimated for at least 300 fibers using Leica software for image analysis. Connective tissue area was estimated as the difference between the total area of the section and area occupied by muscle fibers. Some sections were fixed in cold (-20°C) acetone for 2 min [4] and analyzed under a fluorescent microscope (for detecting fibers stained by Evans blue).

RESULTS

Morphological study of biopsy specimens of the hind paw muscles from C57Bl mice not exposed to hanging showed unchanged round muscle fibers with marginally located nuclei and solitary fibers with central nuclei. No signs of leukocytic infiltration and connective tissue growth were seen. Generally, the specimens corresponded to normal histological picture.

In C57Bl mice exposed to hanging muscle fibers shrank, the round shape and marginal location of the nuclei were retained; a little number of fibers with central location of the nuclei appeared; connective tissue growth was noted.

Histological signs of degeneration were detected in all sections from mdx mice: muscle fibers varied in diameter, with central location of the nuclei in many fibers, necrotic foci, connective tissue growth, and leukocytic infiltration (Fig. 1).

Typical muscular dystrophy was observed in mdx mice not exposed to hanging. Analysis of muscle tissue from dystrophin-defective mice with muscular dystrophy exposed to hanging showed decreased cross-section area of fibers and increased number of fibers with central position of the nuclei.

Unloading significantly decreased cross-section area of muscle fibers in both groups (p<0.05): by 18% in C57Bl mice and by 33% in mdx mice (Table 1). The percentage of centrally located nuclei in animals of both strains increased significantly during skeletal unloading (Table 1).

Fibers intensely stained with Evans blue were unevenly distributed in the section from mdx mice not exposed to hanging, the share of these fibers not surpassing 3-4% of the total number of fibers. After hanging the content of these fibers increased significantly in the greater part of animals, the irregularity of their distribution being even more pronounced: along with intact sites, there were zones with 20-40% stained fibers, the location of intensely stained sites predominantly coinciding with the interface of separate muscles (Fig. 2).

We analyzed the course of dystrophy in mdx mice. The cross-section area of muscle fibers decreased significantly during hanging in all C57Bl animals and in mice with muscular dystrophy. The

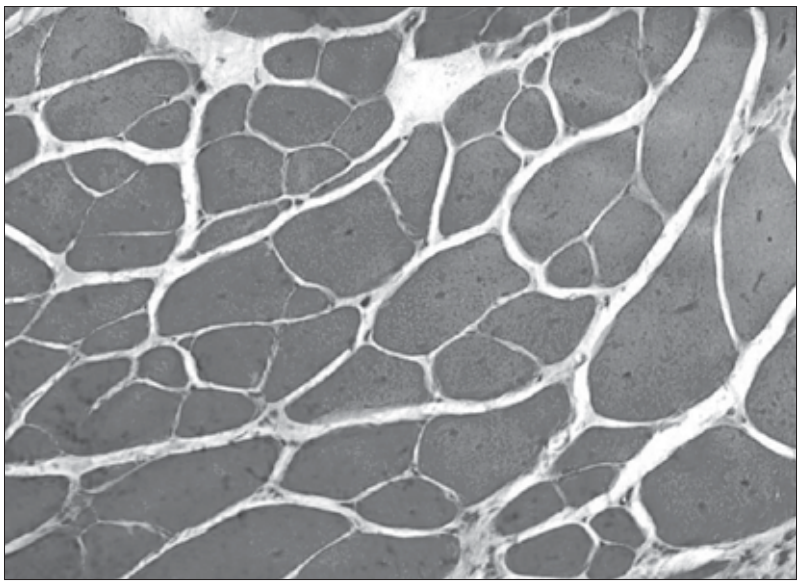

Fig. 1. Transverse section of mdx mouse hind paw muscles. Central location of nuclei in muscle fibers. Hematoxylin and eosin staining, ×20.

TABLE 1. Morphometric Characteristics of Experimental Groups

Parameter	mdx mice		C57Bl mice	
	control	hanging	control	hanging
Central location of nuclei, %	82±4	95±1	0	5±1
Cross-section area, μ^2	2277±124	1531±86	1994±118	1627±79

decrease in the cross-section area was more pronounced in mdx mice (the fibers of animals with dystrophin synthesis defect were more sensitive to functional unloading). The percentage of fibers with central location of the nuclei was significantly higher after hanging exposure than in the control. Numerous centrally located nuclei in mdx mice is a result of active regeneration aimed at replacement of necrotic fibers [1]. Due to species specificity of these animals, the regeneration is effective during a long time in them, and necrotic fibers are replaced by connective tissue and pseudohypertrophy and muscular dystrophy develop in old 10-12-month-old animals. The first appearance of these nuclei at the age of 5-7 weeks indicates activation of the degenerative process and regeneration. Immobilization of the limbs in young mdx animals performed before dystrophy activation delayed the onset of the first degeneration/regeneration peak [6]. The number of centrally located nuclei was decreased significantly in immobilized muscles in comparison with the control [6]. The increase in the content of centrally located nuclei in our study probably attests to activation of regeneration under conditions of functional unloading, but the appearance of these nuclei in C57Bl mice exposed to hanging indicates that long-term relaxation is paralleled by destructive processes stimulating the development of pathology in mdx mice.

Increased permeability of muscle fiber membranes plays an important role in the development of dystrophy. The percentage of fibers with sarcolemma permeable for macromolecular tracers reaches 2-15% in mdx mouse fibers, while in normal muscles these fibers are absent [2]. The percentage of these fibers after treadmill eccentric exercise is about 3% in normal animals and 30% in dystrophic ones, which indicates higher sensitivity of degenerative muscles to the destructive effect of eccentric exercise [2]. In our experiment the content of fibers intensely stained by Evans blue increased in the majority of animals exposed to immobilization by hanging (muscle fiber membrane permeability increased after the exposure, that is, degenerative process augmented).

Hence, the results cannot confirm the hypothesis on inhibition of degenerative process under conditions of reduced contractile activity of muscles in animals with manifest signs of muscular dystrophy. It seems that the absence of mechanical violations of the sarcolemma in old animals under conditions of gravity unloading in the presence of progressive dystrophic process is no longer a factor capable of modulating the disease development.

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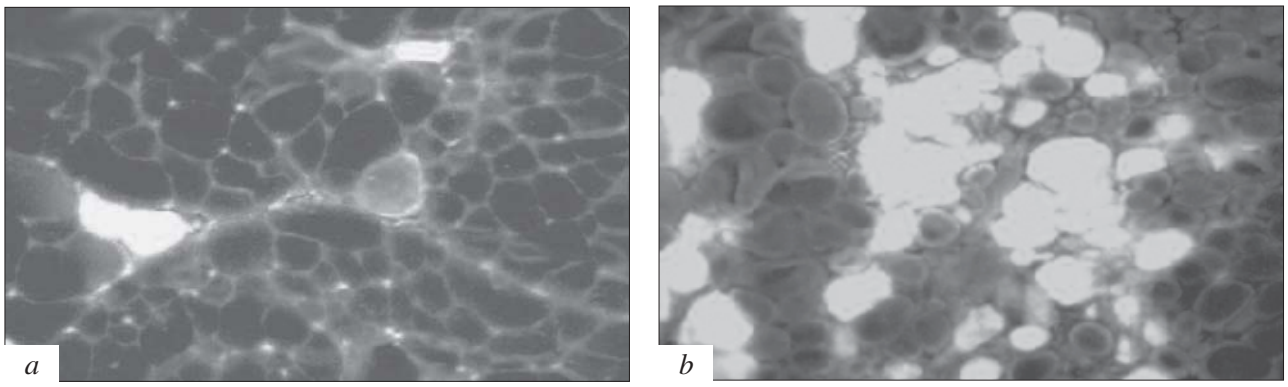

Fig. 2. Transverse section of mdx mouse hind paw muscles. Muscle fibers positively stained by Evans blue. *a*) control; *b*) hanging, ×20.

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