Hereditary Muscular Dystrophy in MDX Mice as a Homologous Model for Introduction of Cell Technologies in the Treatment of Progressive Muscular Dystrophies in Humans

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Life-time monitoring of the main clinical and laboratory manifestations of hereditary muscular dystrophy in mdx mice confirmed the presence of mutation in exon 23 of dystrophin gene and the absence of this protein in skeletal muscles of mutant animals. Muscular dystrophy in mice was similar to human progressive muscle disorder, which allows the use of this model for the development of cell technologies for the treatment of hereditary muscular diseases in humans.

Key Words: *mdx mice; progressive muscular dystrophy; model*

Progressive muscular dystrophies (PMD) are highly prevalent; the most severe of them is Duchenne-Becker muscular dystrophy occurring in one per 3500 newborn boys [1]. The clinical picture of the degenerative process develops from the moment of birth, rapidly progresses, and eventuates in death from secondary infection, respiratory or cardiac failure at the end of the second or at the beginning of the third decade of life. The known methods for the treatment of PMD (metabolic therapy, hormone therapy, spa therapy) are ineffective and are aimed just at elimination of some symptoms of the disease.

The emergence and progress of the disease is now attributed to molecular defect in the gene located in chromosome X (Xp21) and responsible for the production of dystrophin (427 kDa protein). This protein ensures flexible connection of the dystrophin complex proteins with non-muscular F actin in muscle tissue [9]. Some other tissues (peripheral nervous system, brain, retina, platelets) contain short isoforms of dystrophin (58-260 kDa), but their functional role is little studied [8,12].

Modeling of pathological processes is widely and for a long time used in biomedical studies as an approach to disclosing the etiology and pathogenesis of many diseases. It is obvious that illness only more or less approximating the studied human disease can be simulated in such model studies.

Several experimental PMD models are known. Myopathy in 129 Re/J mice is regarded as a PMD analog: this disease is autosomal and is not paralleled by dystrophinopathy because dystrophin gene is not involved. MDX mice in which muscular dystrophy was caused by point mutation in exon 23 of the dystrophin gene located in X chromosome were bred in 1984 [14]. For this reason, muscular dystrophy in these mice is regarded as a process homologous to human PMD. A reproductive colony of mdx mice has been created and maintained over several years at the Russian State Medical University. Today stem cell therapy is an alternative to the traditional methods for the treatment of hereditary neuromuscular diseases. The use of stem

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cells is aimed at incorporation of injected cells (after differentiation) into muscle cell ensemble.

MATERIALS AND METHODS

The reproductive colony of mdx mice was kept on a standard ration under conditions of maximum isolation from the vivarium. Experiments were carried out on adult male 10-12-month-old animals.

The restriction site for VSP1 restrictase (ATTAAT) was detected in mdx mice using specially selected primers for DNA diagnosis of the mutation carriership. The presence of the AAT triplet (stop-codon during transcription of full-length dystrophin mRNA) was regarded as a specific characteristic of mdx mouse genome [3].

Dystrophin was detected by immunohistochemical analysis of cryostat sections of skeletal muscles [3]. Dys1 and Dys2 antibodies (Novocastra) to epitopes of the dystrophin core and C-terminal fragments were used.

Activities of creatine phosphokinase (CPK) and lactate dehydrogenase (LDH) in the blood were measured by the "dry chemistry" method on a Reflotron biochemical express analyzer.

The state of the cardiovascular system was evaluated by recording ECG in three standard and amplified unipolar leads. The amplitude-frequency characteristics of *P*, *Q*, *R*, *S*, *T* waves and the corresponding intervals were analyzed. Intracardiac conduction of stimulation, heart rate, and arterial pressure were evaluated [2].

Motor activity of mice was evaluated by the results of swimming in a 20×25×14 cm basin (35°C water temperature). Water surface was 1.5-2.0 cm below the edge of the basin. The time between the beginning of active movements and first plunging of the mouse into water, the number and duration of rest periods when the animal floating in the water in vertical or horizontal position did not move its paws were recorded.

RESULTS

DNA diagnosis detected animals with point mutations in exon 23 of the dystrophin gene and normal mice without mutation. Human PMD is characterized by

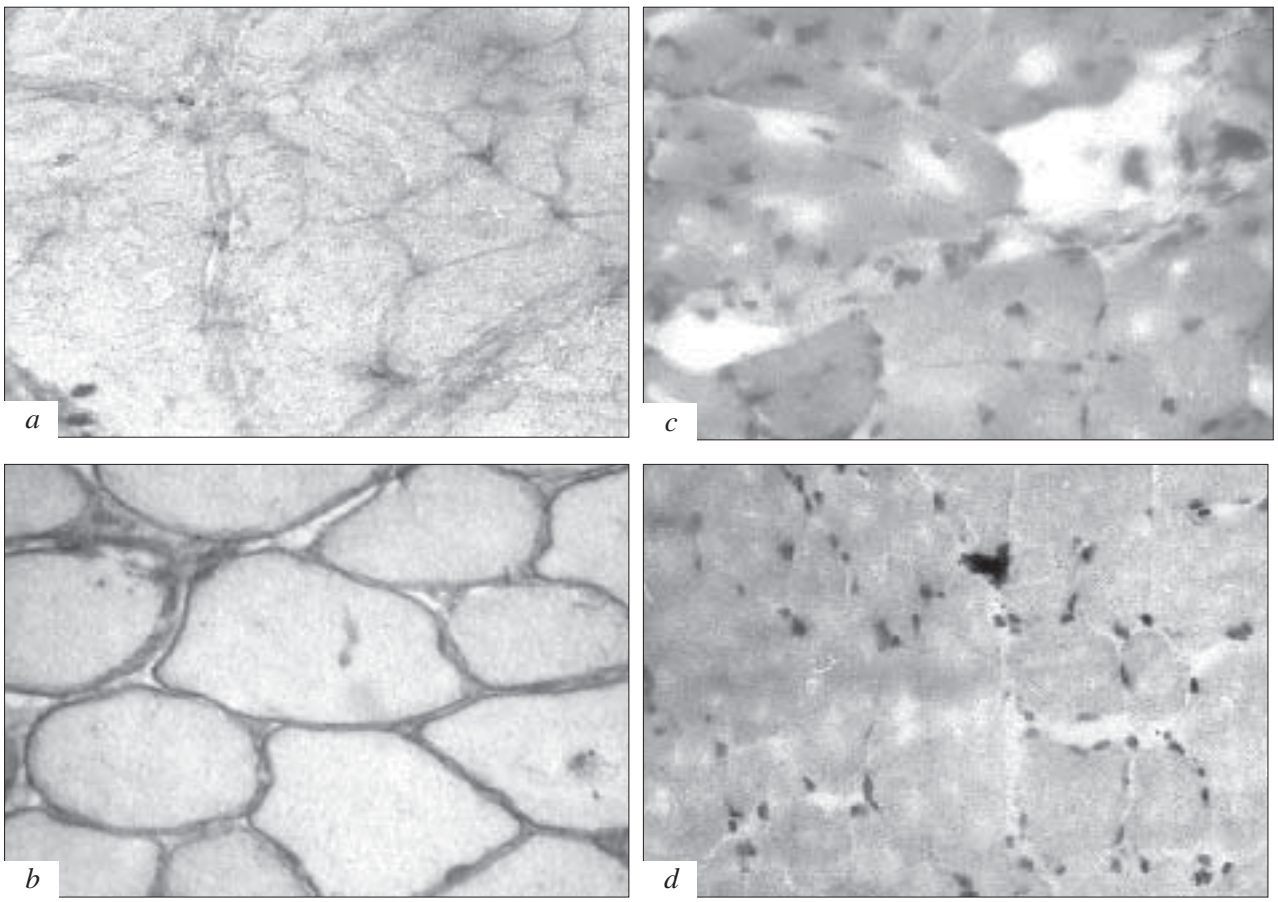

Fig. 1. Immunohistochemical detection of dystrophin in muscle tissue of mdx mice. *a*) muscle tissue of mutant mice. Staining for dystrophin, ×400; *b*) muscle tissue of normal animals. Staining for dystrophin, ×600; *c*) histology of muscle tissue in mutant animals. Giemsa staining, ×400; *d*) muscle tissue histology in normal animals. Giemsa staining, ×400.

TABLE 1. Motor Activity of MDX Mice in the Swimming Test (*M*±*m*)

Group	Swimming duration, min	Number of rest periods	Intensity of movements during swimming
Control	6.22±1.34	0.40±0.13	High
Mutants	3.95±0.68*	5.86±2.50*	Low

Note. *$p<0.05$ compared to the control.

point mutations and deletions in the dystrophin gene "hot spots". Hence, the molecular defects of dystrophin gene lead to the same result in mice and humans: the absence of full-length dystrophin in skeletal muscles and its isoforms in other tissues.

Immunohistochemical analysis of muscle tissue with changed structure (central position of the nuclei, high variability of fiber diameters) confirmed the absence of dystrophin in myofibrils of mdx mice and its presence in control animals (Fig. 1). This result agrees with the results of comparison of biopsy specimens from PMD patients and healthy boys.

Many-fold increase in serum activities of CPK, LDH, and some other enzymes is a key factor for the diagnosis of PMD. It is believed that the increase in enzyme activities is caused by their "leakage" from muscle tissue because of increased permeability of the muscle fiber sarcolemma. High CPK activity of the blood is detected in patients immediately after birth and in their mothers (carriers of the mutant gene) [7].

Activity of CPK in adult mutant male mice from our colony was appreciably increased (like in PMD patients) in comparison with normal animals (4400± 1200 *vs.* 1000±257 U/liter, respectively). LDH activity in mutant mice (1279±273 U/liter) also surpassed the normal level. Hence, CPK and LDH activities in mice are informative parameters, similarly as in patients with PMD, and can be used for long-term monitoring of this pathological process.

Patients with PMD often develop dilatation cardiomyopathy associated with arrhythmia [3], and biopsy specimens of the myocardium contain no dystrophin [6]. The same changes were detected in mdx mice [13]. Results of analysis of the cardiovascular system in animals from our colony were reported previously [2]. The amplitudes of ECG waves were changed in mutant animals: widened *S* wave, transition of the descending *R* wave branch into *T* wave, U-shaped *ST* segment and its shift from the isoelectric line by 1.5-2.0 mm, prolonged *PQ* interval, and decreased heart rate. The rate of conduction via the conduction system was slow in mdx mice, coronary circulation was changed, and congestion phenomena were detected in the right heart.

Bradycardia in mdx mice is not in line with the clinical picture in humans and with the data of some authors investigating mdx mice [1,5]. This disagreement can be explained by the absence of clear-cut universally acknowledged criteria for discriminating between the normal and abnormal heart rates in mice, on the one hand, and by different methodological conditions of measurements, on the other. It is also possible, that decreased heart rate is a specific feature of mice from our colony. In this respect these animals resemble cardiomyopathic hamsters (strain B10 14.6), in which this parameter is 30% lower than in control hamsters [10].

The dystrophic process in skeletal muscles and heart of PMD patients develops from the moment of birth. By the age of 7-8 years the child has to move in a wheel-chair because of severe disorders in motor activity and contractures in the limbs. In mdx mice the motor activity is almost completely retained even in old age. Presumably, utrophin protein, related to the absent dystrophin, contributes to retention of the motor activity; the level of utrophin in mdx mice surpassed that in normal animals [15].

Changes in motor activity of mice manifested in the swimming test (Table 1). The mean duration of swimming for mice carrying the mutant dystrophin gene was significantly shorter than for normal animals. Control mice swam without rest, while the mutants were alternating active movements with periods of rest, the duration of these periods increasing by the end of the swimming session. The intensity of movements in mutants was visually much slower than in the controls. These results agree with the data of other authors [11] and necessitate investigation of the behavioral reactions of mdx mice.

Comprehensive evaluation of various manifestations of muscular dystrophy in mdx mice became possible after we adapted the complex of methods used in clinical laboratory studies in PMD patients to experiments on mice. The possibility of repeated studies on the same animals is an important advantage. Our study showed high homology of muscular dystrophy in mdx mice and the pathological process in PMD patients.

The possibility of recording manifestations of muscular dystrophic process in this PMD model make it a convenient instrument for the development of new technologies for the treatment of human hereditary

muscular dystrophies. The results obtained in our studies on mdx mice [3] confirm good prospects of this trend of investigations.

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