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BIOCHEMISTRY, BIOPHYSICS AND MOLECULAR BIOLOGY

Genomic Analysis of Mouse Lumbar Spinal Cord after 30-Day Space Flight on Biosatellites Bion-1M

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One of the adverse factors affecting human in space is microgravity. This is not surprising, because the evolution of all living systems proceeded under conditions of the Earth's gravity. The negative effect of microgravity develops in space flight but is clinically expressed after the return of astronauts to the Earth, under normal gravity conditions. All body systems adapted to the flight conditions, where the weight is virtually absent, are unable to function normally under conditions of the Earth's gravity for a long time.

The effect of microgravity manifests itself especially strongly in the disturbance of the function of the musculoskeletal system, leading to the development of a pathological state known as the "hypogravitational motor syndrome" (HMS), when the muscle strength and endurance are reduced, the structure and properties of bones change, and the functions of the systems responsible for the construction of movements are disrupted. The experience of long-term space flights showed that the most effective method to preserve the health of astronauts and prepare them for the return to the Earth is to regularly perform a complex of physical exercises during the flight under conditions simulating the Earth's gravity [1]. However, even a diligent implementation of specially designed preventive complexes does not completely prevent the development of HMS. Apparently, the success of long-term interplanetary missions will largely be determined by the advances in the study of the HMS pathogenesis at the molecular, cellular, and tissue levels. An important role in this direction is played by the studies performed with the animals that participated in space flights on biosatellites or were kept under simulated microgravity [2–4]. For example, a number of studies showed that an important starting point in the development of HMS is the disturbance of afferent impulsation from the extremities. This is due to the fact that their regions that are most sensitive to mechanical stimuli do not experience the action of support in microgravity [5, 6]. Our study of the state of skeletal muscles, myoneural synapses, and spinal motoneurons led us to conclude that an important role in the HMS pathogenesis in modeling the consequences of hypogravity on the Earth is played by demyelination of motor nerves [7], which reduces the velocity of conduction of action potentials in the injured nerve fibers. Molecular biological studies have shown that the myelination of axons is disrupted as a result of changes in the expression of the genes encoding myelin sheath proteins [8, 9]. However, it is clear that the data obtained on the Earth cannot be extrapolated to the conditions of orbital flights.

The comparison of data on the mechanism of HMS development in the animals with pathology caused by hypogravity simulation on the Earth and the animals that participated in space flight was made possible due to implementation of the Bion-M1 project. The aim of which was to study the mechanisms of HMS development. Within this project, we performed a genome-wide study of the lumbar section of the spinal cord of mice after 30 days of space flight.

Experiments were performed with male C57BL/6J mice (obtained form the Pushchino nursery of laboratory animals, Pushchino, Moscow oblast). The animals were divided into two groups—"flight" (mice that participated in a 30-day space flight ($n = 2$)) and "control" (mice that remained on the Earth, $n = 2$).

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The spinal cord of mice of the "flight" group was taken 14 h after landing the biosatellite. Simultaneously, the spinal cord of mice of the control group was taken. Then, the lumbar section of the spinal cord was frozen in liquid nitrogen and stored at −80°C until study. Total RNA was isolated from samples using the RNeasy kit (Qiagen, Germany) according to the manufacturer's instructions. The total RNA (2 μg) was used in the reverse transcription reaction and subsequent analysis of gene expression in the "control" and "flight" groups of mice using the Mouse Development Microarray Kit, 4 × 44000 (Agilent, United States). The results of the study were processed using the SubioPaltform software package (Japan). A more than twofold increase in gene expression in the animals of the "flight" group was taken as statistically significant.

Out of the 39 486 genes present on the microarray platform, the expression of 135 genes significantly increased and the expression of 42 genes significantly decreased in the lumbar section of the spinal cord of mice of the "flight" group as compared to the animals of the control group.

The analysis of affiliation of the genes that responded to the flight to different functional groups using the DAVID Bioinformatics Resource database showed a significant increase in the activity of the genes encoding the receptors associated with G proteins (36 genes), transmembrane proteins (38 genes), hydroxylation enzymes (3 genes), serine protease inhibitors (3 genes), and proteinases (6 genes).

Among the genes with a reduced expression, the genetic cascade responsible for the synthesis of sarcomeric proteins, the deficiency of which causes hypertrophic cardiomyopathy, is of interest. In addition, the expression of the genes whose products contain the domains that are specific for the proteins regulating the synthesis of immunoglobulins (five genes) significantly reduced. The expression of the genes encoding the enzymes involved in the synthesis and metabolic of nucleotides (six genes), muscle proteins (two genes), glycoproteins (nine genes), kinases (three genes), signaling proteins (eight genes), and enzymes associated with the synthesis and processing of ATP (five genes) also decreased.

The study of spinal cord fragments containing different cells does not allow us to postulate that the responded genes belonged to a certain type of cells; however, a preliminary analysis of the transcriptome suggests that different functional groups of genes were involved in the HMS development. On the basis of understanding the mechanisms that ensure reliable functioning of the neuromuscular system, special attention should be given to the drastic decline in the expression of the genes encoding calcium channel proteins (NM_031169, NM_007582, etc.).

Earlier, we studied the transcriptome of the lumbar section of the spinal cord of mice after 30 days of antiorthostatic hind limb hanging [9].

The comparison of results of genome-wide analysis of the spinal cord sections where the motor neurons innervating the muscles of the lower extremities are located (including the postural muscles, which are most sensitive to hypogravity) in the animals that participated in the orbital flight and the animals with HMS simulated on the Earth revealed significant differences. For example, the expected changes in the expression of the genes encoding myelin proteins were not confirmed. Our data strongly suggest that the pathogenesis of locomotor disorders in microgravity and in its simulation on the Earth can vary significantly. However, the fact of a small amount (due to its uniqueness) of the "flight" material cannot be ruled out, which could be the cause of distinctions in the results of "model" and "flight" studies.

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