МЕДИЦИНСКИЕ НАУКИ

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MORPHO-FUNCTIONAL OUTCOMES OF PACLITAXEL-INDUCED PERIPHERAL NEUROPATHY COMBINED WITH 2-ETHYL-6-METHYL-3-HYDROXYPYRIDINE SUCCINATE ADMINISTRATION ON THE SPINAL CORD ANTERIOR HORN NEURONS

Summary. Paclitaxel-induced peripheral neuropathy (PIPN) is a major debilitating side effect of paclitaxel in patients with cancer with no fully known mechanisms. The aim of the study was to investigate the fine sub-microscopic structure of the spinal cord anterior horn neurons in PIPN combined with 2-ethyl-6-methyl-3-hydroxypyridine succinate administration. The experiment was performed on 80 white rats, which were administered intraperitoneally with Paclitaxel (Actavis, Romania), pre-dissolved in an isotonic saline at a dose of 2 mg / kg body weight four times a day to achieve a dose of 8 mg / kg. Then 48 of these animals were injected intraperitoneally 2-ethyl-6-methyl-3-hydroxypyridine succinate (Armadine) at a dose of 10 mg / kg (32 rats received intraperitoneally water for injection). Observation periods were 1, 7, 14, 21, 28 days. We found that 2-ethyl-6-methyl-3-hydroxypyridine corrects the morpho-functional state of the motor neurons of the spinal cord and revealed a positive metabolic effect on them. This was manifested by the improvement of the electron microscopic picture of the neuronal structures responsible for their protien-synthetic (granular endoplasmic reticulum, ribosomes and polysomes), respiratory (mitochondria), and protective (lysosomes) functions.

Key words: paclitaxel, paclitaxel-induced peripheral neuropathy, spinal cord, 2-ethyl-6-methyl-3-hydroxypyridine succinate.

Introduction. Paclitaxel-induced peripheral neuropathy is a common side effect of this anti-cancer treatment and causes severe pain in patients, leading to cessation of treatment or a particularly severe rehabilitation period. To study the pathogenesis of neuropathy, the researchers proposed experimental models of Paclitaxel-induced peripheral neuropathies, and with the introduction of the drug animals found sensory neuropathy with the phenomena of hypoalgesia [1,2].

The structural bases of pathomorphogenesis of paclitaxel-induced neuropathies in mice is given by Wozniak et al. [3]. After 2 weeks, neurophysiological and behavioral tests revealed a dose-dependent effect of damage to the pericarion of sensitive neurons and myelin fibers of the sciatic nerve, and no evidence of a regenerative process. The authors proved that the pericarions of pseudounipolar neurons of the cerebrospinal ganglia of the large diameter are affected most deeply, morphological changes are less pronounced in pericaries of medium and small diameter [4]. However, it should be noted that the above papers do not contain systematic studies of the structure of the peripheral nerves and their segmental centers.

Studies have shown that paclitaxel causes altered calcium signaling, release of neuropeptides and growth factors, damage to mitochondria and the formation of reactive oxygen species, and can activate ion channels that mediate response to extracellular signals. Recent studies also suggest a role for matrix metalloproteinase 13 in mediating neuropathy. These various changes may be secondary to the disruption of microtubule transport caused by paclitaxel [5].

Experimental data indicate that up-regulation of the chemokine CXCL1 and its CXCL1 receptor is important for the development and maintenance of neuropathic pain caused by paclitaxel in mice. Therefore, blocking spinal CXCL1 / CXCR2 signaling may be a new innovative therapeutic approach to treat it [6]. Spinal cord stimulation demonstrated efficacy to attenuate some neuropathic pain states, prevented mechanical and cold hypersensitivity caused by paclitaxel, and modulated spinal cord gene expression in rats [7].

Some metabolic drugs with antioxidant, antihypoxic and membrane-stabilizing properties are widely used for the correction of numerous neuropathies. Among them is 2-ethyl-6-methyl-3-hydroxypyridine succinate, which belongs to heteroaromatic phenols, in particular to derivatives of 3-oxypyridine and succinic acid [8,9].

Known about the potential stimulating effect on carcinogenesis the question of the use of 2-ethyl-6-methyl-3-hydroxypyridine succinate in oncology has been widely debated. But it has been found that 2-ethyl-6-methyl-3-hydroxypyridine succinate also inhibits spontaneous metastasis both in monotherapy and in combination with some anticancer drugs [10,11]. Therefore, using 2-ethyl-6-methyl-3-hydroxypyridine succinate as a correction of paclitaxel-induced neuropathy, it is possible to achieve a direct impact on the known pathophysiological mechanisms of development of this neuropathy, as well as the suppression of spontaneous metastasis of the underlying pathology.

The aim of the study is to investigate the fine sub-microscopic structure of the spinal cord anterior horn neurons in paclitaxel-induced peripheral neuropathy combined with 2-ethyl-6-methyl-3-hydroxypyridine succinate administration.

Materials and methods. The experiment was performed on 80 white 150-200 g randomized male rats

that were kept under the same standard vivarium conditions at constant temperature, under normal light regime (day-night), and on a standard diet. Paclitaxel (Actavis, Romania) was administered intraperitoneally, pre-dissolved in an isotonic saline at a dose of 2 mg / kg body weight four times a day to achieve a dose of 8 mg / kg according to the model proposed by R.C. Polomano et al. [12]. Subsequently, the animals were randomly assigned to the experimental (48 animals) and control (32 animals) groups. In the experimental group, animals were injected intraperitoneally with 2-ethyl-6-methyl-3-hydroxypyridine succinate (Armadine, manufactured by LLC Microchem, Ukraine-Spain) at a dose of 10 mg / kg body weight over the next 10 days, pre-dissolving 0.5 ml of water for injection. Animals of the control group were intraperitoneally injected with water for injection in an equivalent volume for a similar period. The electron microscopy of the spinal cord was determined on 10 intact animals.

In the experiment, the retention of rats and all manipulations were performed in accordance with bioethical requirements. Animals were removed from the experiment by degreasing using etheric anesthesia. The study material (anterior horns of the spinal cord) was collected 1, 7, 14, 21, 28 days after the last administration of armadine. Electron microscopic examination was performed according to conventional methods and studied in an electron microscope PEM-125K, took pictures at magnification 4000-12000 times.

Results. The heterogeneity of the morpho-functional state of the neurons of the anterior horns of the spinal cord was observed immediately after the 10-day administration of 2-ethyl-6-methyl-3-hydroxypyridine succinate (Fig. 1). Spherical nuclei with a clearly contoured nuclear envelope were identified, in which the outer and inner nuclear sections were distinguished. Perinuclear space was not changed. A nucleolus is identified in the center of the nucleus. According to the degree of electron density of the neuroplasm we can observe 2 types of neurons – with "light" (Fig. 1A) and "dark" (Fig. 1B) neuroplasma. In "light" neurons, more mitochondria are quantitatively identified, characterized by a rounded or elongated shape, with distinct external and internal mitochondrial sections. Intra-mitochondrial cristae are localized in their matrix evenly. Some of the cristae have extensions in their length and cross-sections look like bubbles. In the neuroplasm, few lysosomes and autophagosomes occur. The granular endoplasmic reticulum looks like flat cisterns with a narrow lumen.

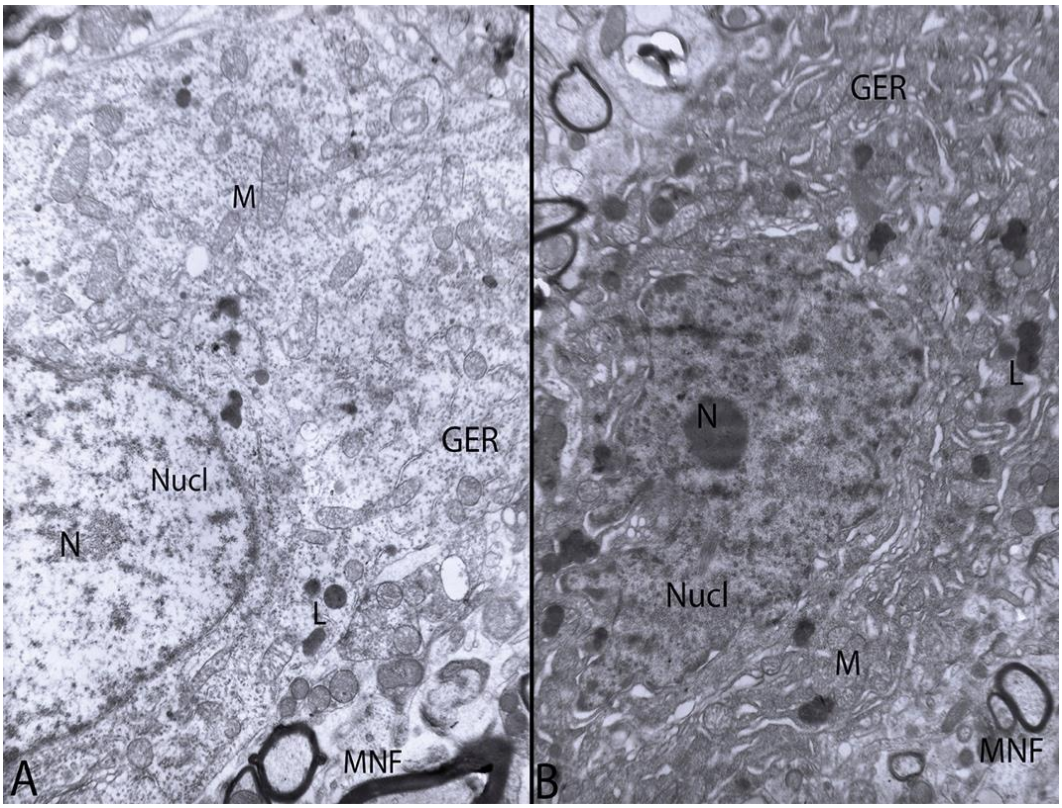

Fig. 1. The heterogeneity of the morpho-functional state of different neurons of the spinal cord anterior horn immediately after the 10-day administration of 2-ethyl-6-methyl-3-hydroxypyridine succinate: A – "light" neuron; B – "dark" neuron. Designation: GER – granular endoplasmic reticulum, Nucl – nucleus, N – nucleolus, L – lysosome. M – mitochondria, MNF – myelin nerve fiber. Electronic micrographs. Magnification: A – 4000x, B – 6400x.

Nuclei with a dense fibrous part predominantly emerge in "dark" neurons. The perinuclear space in some areas is unevenly expanded and interfaces with the cisterns of the granular endoplasmic reticulum. The hypertrophy of the granular endoplasmic reticulum and the Golgi complex is observed in these neurons.

Hyaloplasm is saturated with ribosomes and polyribosomes. Lysosomes and autophagosomes are widely present in the neuroplasm. It means that 10-day administration of 2-ethyl-6-methyl-3-hydroxypyridine succinate positively affects the morpho-functional state of mitochondria and activates synthetic processes in neurons.

7 days after the course of correction with 2-ethyl-6-methyl-3-hydroxypyridine succinate the increasement in the activity of the granular endoplasmic reticulum is observed in neurons (Fig. 2). Ribosomes attached to the sacs and cisterns of the endoplasmic reticulum provide proteosynthesis to restore protein microstructures of the neuron.

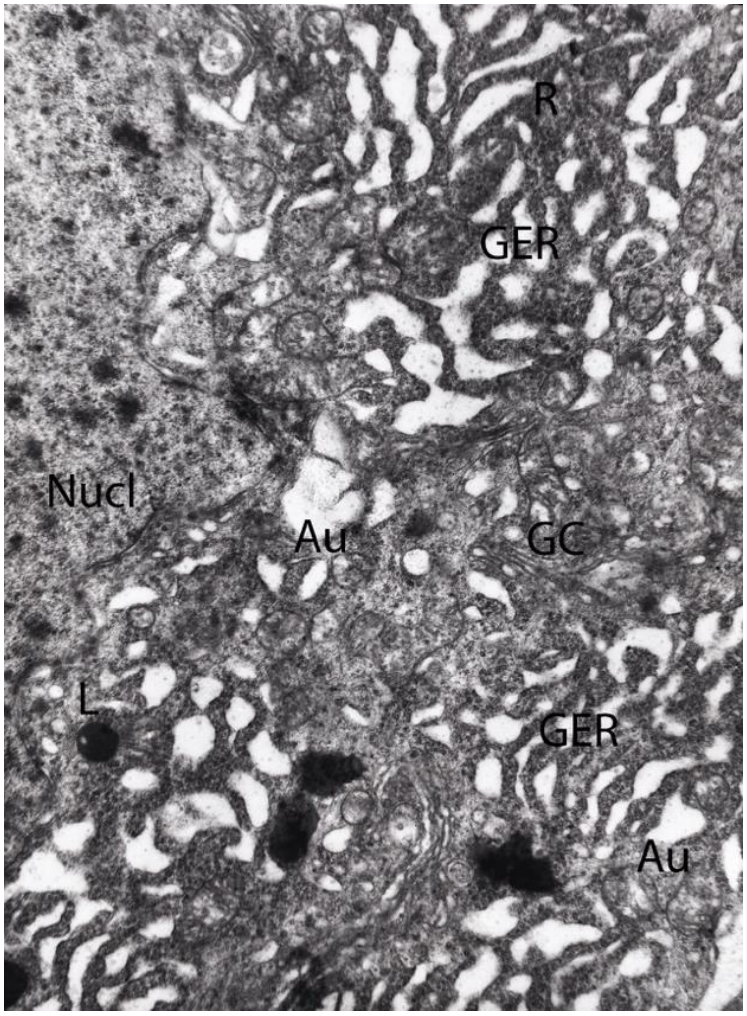

Fig. 2. Increasement in activity of granular endoplasmic reticulum (GER), hypertrophy of the Golgi complex (GC) in neurons of the anterior horn of the spinal cord. The term of the experiment is 7 days. Designation: Au – autophagosome, L – lysosome, R – ribosomes, Nucl – nucleus. Electronic micrograph. Magnification 6400x

Numerous ribosomes and polyribosomes are observed in the neuroplasm. At the same time, hypertrophy of the Golgi complex was observed, which creates the conditions for the formation of the apparatus for the purification of neurons from toxic and harmful products in order to accelerate the complete regeneration of these cells. Confirmation of this fact is the presence in the neuroplasm numerous lysosomes and autophagosomes.

The 14th day of the experiment is characterized by a stable state of mitochondria in neurons (Fig. 3). Mitochondria retain a rounded and oval shape. Their outer mitochondrial membrane is preserved and intact.

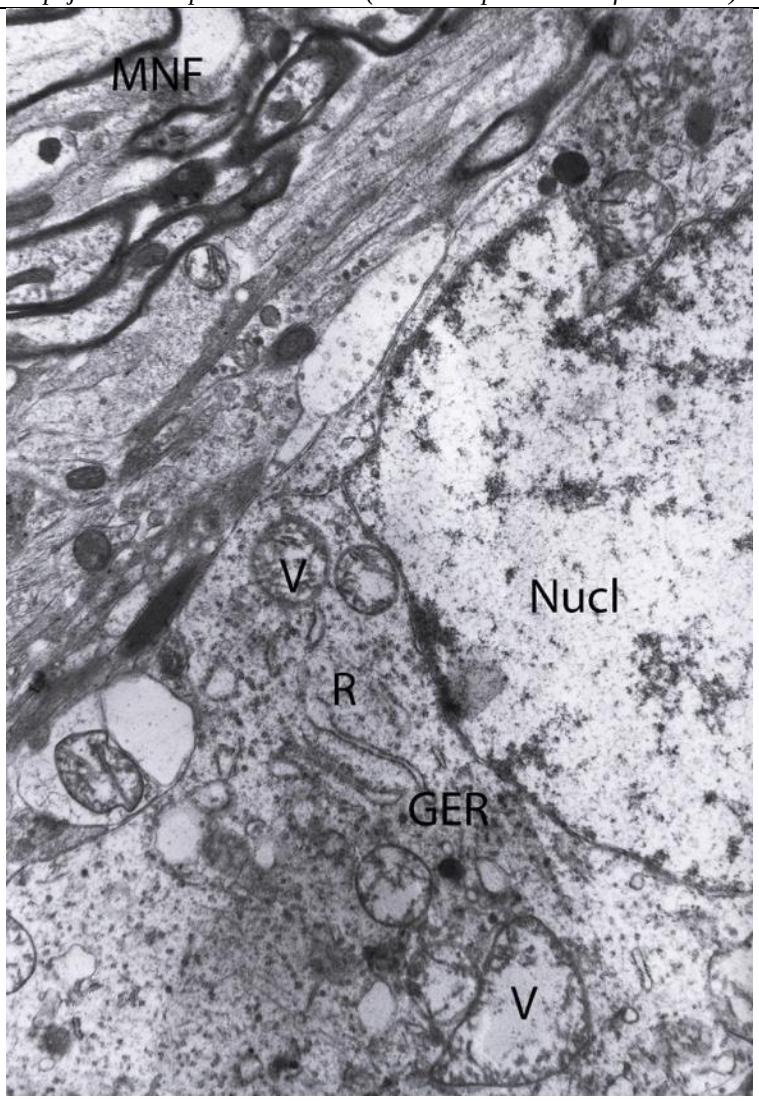

Fig. 3. Stable state of mitochondria in neurons, sacs of granular endoplasmic reticulum (GER) of rounded shape with uniformly attached ribosomes (R). The term of the experiment - 14 days. Designation: V – vacuole, MNF – myelin nerve fibers, Nucl – nucleus. Electronic micrograph. Magnification: 6400x

Cristae of the inner mitochondrial membrane become rarer. The mitochondrial matrix is light, electronically transparent. Some of those mitochondria are vacuolated with shortened cristae, prone to formation of vacuoles of different size with flake-like content. Close to them single lysosomes and autophagosomes are located. In the neuroplasm, the sacs of a granular endoplasmic reticulum with rounded ribosomes are attached. There is a small amount of free ribosomes and polyribosomes. Cross-sectional and tangential cross sections of thin myelin nerve fibers are observed near the neurons. They have a round or elongated shape with slight protrusions. Their myelin sheath has several layers of lamellar structures that fit snugly together. Their axial cylinders define numerous microfilaments, microtubules, single mitochondria, between which vacuoles occur.

On the 21st day (Fig. 4), after the last administration of 2-ethyl-6-methyl-3-hydroxypyridine succinate in the neurons of the anterior horns of the spinal cord, further changes are observed, as evidence of its high restorative potential. The nuclei are located in the center of the cell and have deep invaginations into the nucleoplasm.

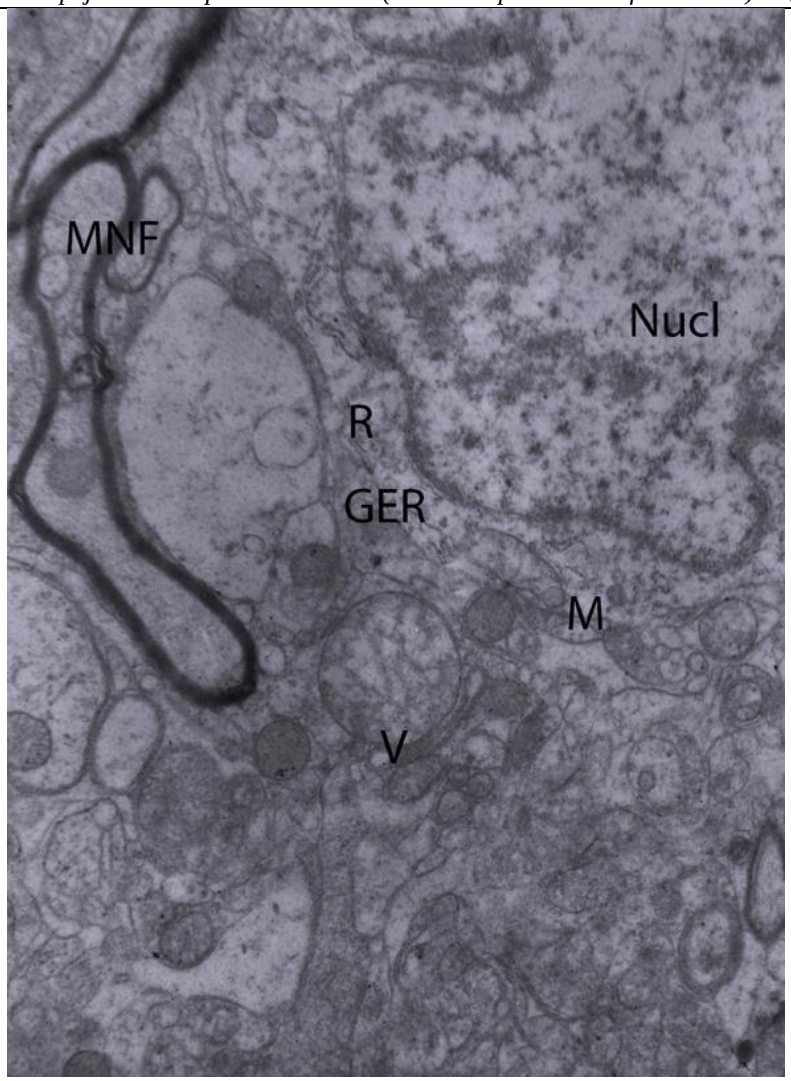

Fig. 4. High neuronal restorative potential: saturation of the neuroplasm with mitochondria (M), elongated granular endoplasmic reticulum (GER) with numerous attached free ribosomes (R), single vacuoles (V) with a flake-like content. The term of the experiment - 21 days. Designation: MNF – myelin nerve fibers, Nucl – nucleus. Electronic micrograph. Magnification: 4000x.

The nuclear envelope detects the outer and inner nuclear membranes. Euchromatin prevails in nucleoplasm. Neuroplasm is saturated with mitochondria of different sizes. Small mitochondria contain long straight cristae. Large mitochondria are determined by the presence of a clearly identified external mitochondrial membrane. The mitochondrial matrix is electronically transparent. The cristae of such mitochondria are not straight but twisted. Cisterns of the granular endoplasmic reticulum are elongated with numerous attached ribosomes. Free ribosomes and polysomes are traced between them. Sometimes occur large vacuoles with flake-like contents.

On the 28th day of experiment (Fig. 5) the manifestations of positive changes in the state of most neurons persist. The "light" and "dark" neurons are distinguished. In the cytoplasm of "light" neurons normal mitochondria, numerous ribosomes, polysomes and elements of the endoplasmic reticulum are detected. In "dark" neurons, the neuroplasma looks electron-dense due to the presence of expanded, hypertrophied cisterns and sacs of the granular endoplasmic reticulum, which testify high rates of protein biosynthesis. Among them, normal mitochondria and numerous ribosomes and polysomes are identified. Along the perimeter of the nucleus, the connections of the sacs of the endoplasmic reticulum with the perinuclear space are observed. The nucleus approaches an oval shape with clear outlines of the outer and inner nuclear membranes. Mostly euchromatin is present in its nucleoplasm. There are different granular and fibrillar components in the nucleus, which is located in the center of the cell.

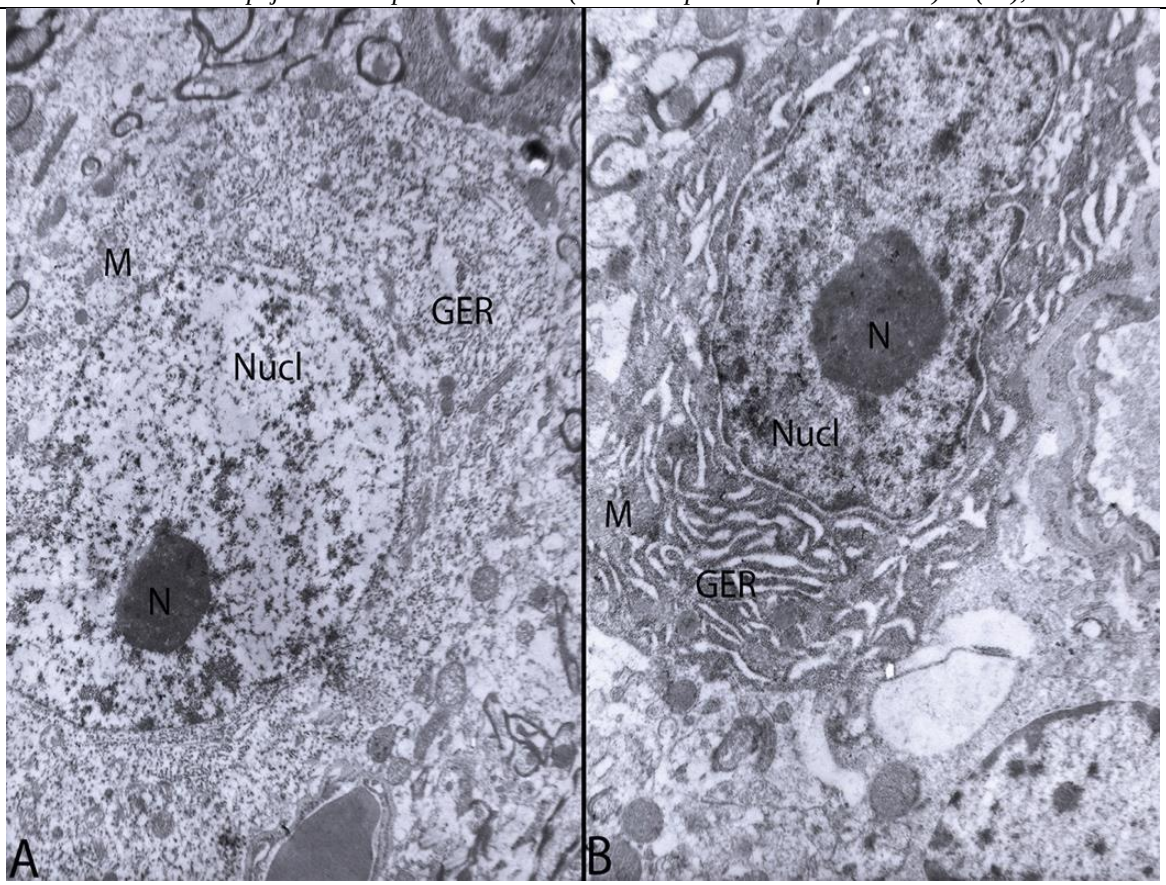

Fig. 5. Manifestations of positive changes in the morpho-functional state of "light" (A) and "dark" (B) neurons. The term of the experiment - 28 days. Designation: GER – granular endoplasmic reticulum, Nucl – nucleus, N – nucleolus, M – mitochondria. Electronic micrographs. Magnification: A – 4000x, B – 6400x

It is known that in paclitaxel-induced neuropathy the sensory and motor components of the segmental centers of the peripheral nerves are damaged. But the pathological process in them develops slowly and is characterized by inactive recovery processes [13]. This requires targeted correction with drugs that affect pathogenesis of neuropathy by their pharmacodynamic properties. Our results have shown that 10-day course of 2-ethyl-6-methyl-3-hydroxypyridine succinate has a positive effect on motor neurons of the anterior horns of the spinal cord, which provide and coordinate motor function in the segmental centers of the peripheral nerves.

Conclusions 1. Immediately after the 10-day administration of 2-ethyl-6-methyl-3-hydroxypyridine succinate in the electron microscopic picture of meurons of the spinal cord anterior horns, the heterogeneity of the morpho-functional state of different neurons was observed. The degree of electron density of the neuroplasm distinguishes neurons with "light" and "dark" neuroplasm. In "light" neurons, more mitochondria are quantitatively identified, characterized by a rounded or elongated shape, with distinct outer and inner mitochondrial membranes. In the neuroplasm, few lysosomes and autophagosomes occur. The granular endoplasmic reticulum looks like flat cisterns with a narrow lumen. In "dark" neurons, hypertrophy of the granular endoplasmic reticulum and the Golgi complex is observed. Hyaloplasm of neurons is saturated with ribosomes and polyribosomes. Lysosomes and autophagosomes are present in the neuroplasm. It means that 10-day administration of 2-ethyl-6-methyl-3-hydroxypyridine succinate positively affects the morpho-functional state of mitochondria and activates synthetic processes in neurons.

2. 7 days after the course of correction with 2-ethyl-6-methyl-3-hydroxypyridine succinate, an increase in the activity of the granular endoplasmic reticulum in neurons is observed, which provide protein biosynthesis for the restoration of neuronal microstructures (saturation of the neuroplasm by ribosomes and polyribosomes). At the same time, the hypertrophy of the Golgi complex was observed, which creates the conditions for the formation of the apparatus for the purification of neurons from toxic and harmful products in order to accelerate the complete regeneration of these cells (lysosomes and autophagosomes).

3. During the 14th to 21st days, a high restorative potential is observed in neurons, which is evidenced by the stable state of the mitochondria and the structural components of the granular endoplasmic reticulum. But individual mitochondria are vacuolated with shortened cristae, prone to formation of vacuoles of different size with flake-like content. Single lysosomes and autophagosomes are located close to them. There is a small amount of free ribosomes and polyribosomes.

4. On the 28th day of experiment positive changes in the state of most neurons persist. The "light" and "dark" neurons are distinguished. In the cytoplasm of

"light" neurons normal mitochondria, numerous ribosomes, polysomes and elements of the endoplasmic reticulum are detected. In "dark" neurons hypertrophied cisterns and sacs of the granular endoplasmic reticulum are present. Among them, normal mitochondria and numerous ribosomes and polysomes are identified.

5. The use of 2-ethyl-6-methyl-3-hydroxypyridine succinate (armadine) to correct the morpho-functional status of the motor neurons of the spinal cord revealed a positive metabolic effect on them. This was manifested by the improvement of the electron microscopic picture of the neuronal structures responsible for their protien-synthetic (granular endoplasmic reticulum, ribosomes and polysomes), respiratory (mitochondria), and protective (lysosomes) functions.

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