

Structural damage of myelin in experimental Parkinsonism and prospects for their drug correction in the clinic of Parkinson's disease

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Abstract. Changes in myelin ultrastructure under experimental Parkinsonism in the tissues of the medulla oblongata and striatum were performed under experimental Parkinsonism induced by rotenone administration in 30 adult rats of the Wistar line. Clinico-electromyographic studies were performed on patients with Parkinson's disease with a stage of disease 2.0 -3.0 (Hoehn a. Yahr).

Efficacy of Cerebrolysin has been shown to correct myelin abnormalities to elucidate the effect on the muscle reflex response to irritation of sensitive nerve fibres of the mixed nerve with subsequent monosynaptic activation of motor neurons and spinal cord neurons. One of the mechanisms associated with myelin damage in Parkinsonism is the development of mitochondrial dysfunction, in any case, its ultrastructural component. The use of Cerebrolysin leads to a significant elimination of mitochondrial dysfunction and myelin damage. It can be assumed that the positive effect of the drug lies in the antioxidant effect, which, in turn, effect the transmembrane conductivity, which should be considered one of the neuroprotective effects of the drug.

Keywords: experimental Parkinsonism; Parkinson's disease; myelin; medulla oblongata; striatum; mitochondrial dysfunction; transmembrane conductivity; cerebrolysin.

The question of possible mechanisms of myelin damage in Parkinson's disease (PD) has been actively discussed in recent years, as it is well known that in neurodegenerative diseases, including BP, there is a violation of the conduct of nerve impulses which is a consequence of neuronal and axonal damage, which can be largely due to demyelination [1, 2]. The clinical picture of myelin structure disorders can be observed, in addition to the expressed mental and developmental disorders of dementia, walking disorders, paresis, muscle weakness, and vegetative dysfunction, which are characteristic of both PD Parkinson's-like states [3, 4]. However, PD still does not belong to demyelinating diseases, although in recent years more and more objective evidence has emerged that myelin damage plays a key role in the pathogenesis of the disease [5, 6].

Thus, with diffusion tensor MRI imaging and MP-tractography allowing to estimate the diffusion of water molecules in biological tissues and, consequently, the degree of tissue hyperhydration, it has been shown that in patients with PD, significant lesions of functionally defining cortical and subcortical conduction pathways are identified [7, 8].

On the other hand, clinical studies have shown that in PD, the formation of Levi's bodies in the neuronal tissue is accompanied by impaired axonal conductivity with myelin damage, and such changes can also occur in the absence of Levi's bodies [6].

Fundamental studies of recent years have convincingly shown that mitochondrial dysfunction (MD) develops in PD associated with the accumulation of oxidized dopamine and α -synuclein, dysfunction of lysosomes, ubiquitin-proteosomal dysfunction, inflammation, apoptosis, etc. [9, 10].

At the same time, MD manifests itself, first of all, as a violation of dynamic processes in the mitochondrial apparatus, mainly with inhibition of mitochondrial fission, which negatively affects energy metabolism and, accordingly, structural changes occurring in neurons, thereby closing the metabolic vicious circle, which, in turn, leads to further progression of the disease [9, 11, 12]. However, this disease is still not included in the list of known mitochondrial pathologies.

It should be recently noted, that the key components of disorders of the nervous tissue, in particular, myelin, include both oxidative stress and mitochondrial dysfunction, which play several decisive roles in the life of oligodendrocytes (the latter in the CNS are responsible for the synthesis and maintenance of the integrity of myelin, and their damage contributes to the development of oxidative disorders [3, 13]. It has been proven that mitochondria (MC) are the main suppliers of carbon skeletons and energy for lipid synthesis [14]. According to these data, the study of the mechanisms of changes in myelin in parallel with the mitochondrial apparatus under neurodegenerative pathology can be considered obligatory for the search for preventive and therapeutic methods to correct developing disorders. Such approaches can be aimed both directly at neurodegeneration and at the development of oxidative stress and mitochondrial dysfunction.

It can be assumed that modern synthesized neuropeptides with nootropic activity will have this ability.

Currently, Cerebrolysin (Cer) is positioned as a nootropic drug with neurotrophic activity and is used in the treatment of neurological diseases, including cerebral vascular pathology, mixed dementia, and Alzheimer's disease [15, 16].

According to pharmacological properties, the neuroprotective ability of Cer includes the sum of all mechanisms directed against long-term and short-term endo- and exogenous neuro-directed effects. Endogenous neuroprotection includes two different mechanisms - absolute and relative. Absolute mechanisms always activate DNA expression, followed by induction of protein synthesis. Relative mechanisms provide neuroprotection by affecting the pre-expressed proteins of the cell membrane, cytosol, and organelles [17]. Absolute mechanisms are controlled mainly by neurotrophic factors and neurotrophic molecules, while relative mechanisms are mainly associated with the use of ion channel blockers, agonists, and antagonists of specific receptors, antioxidants, chelators of various metals, and a number of other substances [17].

The neuroprotective strategy of Cer is associated primarily with the activation of absolute mechanisms by biologically active oligopeptides. In addition, the product contains amino acids, trace elements, and vitamins, which also contribute to the activation of the relative mechanisms of neuroprotection. The neuroprotective effect of Cer manifests itself in a decrease in excitotoxicity, inactivation of the formation of free radicals, suppression of the inflammatory response, a decrease in edema, and inhibition of the apoptosis and necrosis processes [18]. It is the last set of parameters that becomes an objective prerequisite for the use of Cer in the treatment of PD, which can be realized in the possibility of restoring the integrity of myelin.

Clinical results of the efficacy of Cer in PD confirm the crucial role of trophic homeostasis disturbances in the mechanisms of this disease development. The therapeutic potential of Cer under various forms of Parkinsonism is also confirmed both in the experiment and in clinical practice [19, 20, 21]. Thus, this potential was fully demonstrated in the classical model of Parkinsonism in rats induced by injection of the neurotoxin 6-OHDA into the substantia nigra: administration of Cer to such animals was accompanied by the restoration of dopamine levels in the midbrain and striatum, normalization of oxidative stress indicators, and improvement in motor functions. In a model of rotenone Parkinsonism in rats, the use of Cer had a comparable clinical effect with the administration of sinemet and intravenous application of mesenchymal stem cells (although the effect of the latter has not been confirmed in clinical

practice). It has been established that Cer improves the survival of neurons under neurodegeneration of the "Parkinsonian type" in vitro and in vivo [19].

The aim of this study was to investigate the possibility of Cer use to prevent myelin damage in experimental Parkinsonism, and then to evaluate the effectiveness of the drug in the clinical manifestations of PD.

Materials and methods.

The studies were carried out on mature male Wistar rats ($n = 30$) weighing 220-270 g. The rats were divided into the following groups (each consisted of 10 animals): 1st - control group; group 2 - simulation of experimental parkinsonism (EP): s/c animals were injected daily for 2 weeks with Rotenone (Ro) at a dose of 0.03 mg/100 g of body weight [22]; group 3 - animals were injected with Cer at a dose of 0.2 mg/100 g of body weight 15 minutes before RO for 10 days.

After the end of the planned exposure, the rats were decapitated under mild ether anesthesia. All studies were carried out in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Purposes (Strasbourg, 1986) and in accordance with the principles of the Helsinki Declaration (2000).

After decapitation, tissue pieces of the medulla oblongata (8-12 mm from Bregma) and striatum were taken from rats. Fixation of the material was performed immediately by introducing tissue samples into a buffered 2.5% solution of glutaraldehyde. Additional fixation of the material was carried out using Caulfield's reagent (based on a 2% solution of osmium tetroxide, pH 7.3) (reagents from Sigma, USA); dehydration of the material was carried out in alcohols of increasing concentration, absolute alcohol, and acetone, followed by pouring into upon-Araldite (reagents from Fluka, Switzerland). Ultrathin sections 40–60 nm thick for viewing in an electron microscope were contrasted with 1% uranyl acetate solution and 0.4% lead citrate solution (reagents from Sigma, USA) according to the Reynolds method. These manipulations were performed according to generally accepted methods [23]. The preparations were viewed using a PEM-125K electron microscope (Ukraine).

Morphometric studies were carried out using the program for morphometric calculations Image Tool Version 3 (USA) on 120-150 fields for each exposure. The total number of mitochondria (nMC), and the number of structurally damaged mitochondria (dMC) were determined, as well as the assessment of structurally damaged myelin (M) - the area of its damage (in % of the total area of myelin fibers in the microscope field of view).

A clinical and electromyography study of the Cer course application effectiveness was carried out in patients with chronic pancreatitis aged 60-74 years with a disease stage of 2.0-3.0 (Hoehn a. Yahr). The patients were hospitalized in the Department of Extraparallel Diseases of the Nervous System of D. F. Chebotarev Institute of Gerontology NAMS of Ukraine. Patients signed an informed voluntary consent to participate in the study, which is based on the provisions of the Helsinki Declaration of the World Medical Association in agreement with the Ethics Commission of the D. F. Chebotarev Institute of Gerontology NAMS of Ukraine.

The diagnosis of PD was made according to the clinical diagnostic criteria of the British Brain Bank Society PD - UK Brain Bank Criteria. All patients were on stable therapy with levodopa-containing drugs. Cer was administered as an intravenous infusion (20 ml) daily for 10 days.

The study was carried out using the neurophysiological complex Neuro-MEP Neurosoft (Russia) according to the standard method. Registration of the H-reflex, as a reflex response of the muscles to irritation of the sensory fibers of the mixed nerve, followed by monosynaptic activation of the motor neurons of the spinal cord and motor fibers of the nerve, was performed using disposable surface electrodes placed in the projection of the posterior leg muscle group, with stimulation of the tibial nerve in the popliteal fossa. The dynamics of the amplitude of H- and M-responses (mV) were assessed with an increase in the strength of electrical stimulation of the nerve (mA), the threshold for the onset of the H-reflex (mA), the latency of the H-reflex period (ms), the ratio of the maximum amplitude H_{max} / M_{max} , %.

Statistical processing of the results was carried out using the STATISICA 6 program. Numerical data were presented as the mean values of the indicators (M) and the error of the standard error of the mean (m). This representation is correct in connection with a large array of digital material, since, in accordance with the Shapiro-Wilkey criterion, the results obtained fit into the normal distribution law. To

assess the reliability of the results obtained, a one-way ANOVA analysis of variance was applied using the comparative Post Hoc Student-Newman-Keuls test. The results were considered statistically significant at $p < 0.05$.

Results and discussion

In our previous studies aimed at identifying morphological and functional changes in such brain structures as the medulla oblongata and striatum in experimental Parkinsonism, pronounced damage in the myelin ultrastructure was established due to edema and/or destruction of the myelin sheaths in both studied tissues (Fig. 1) [24, 25].

The pathophysiological mechanisms in charge of the PD development and its clinical manifestations currently include changes in the white matter of the brain (associated with demyelination processes), which at the microstructural level is responsible for the formation of cognitive disorders, non-motor disorders corresponding to the severity of the disease according to the Hoehn a Yahr scale [26].

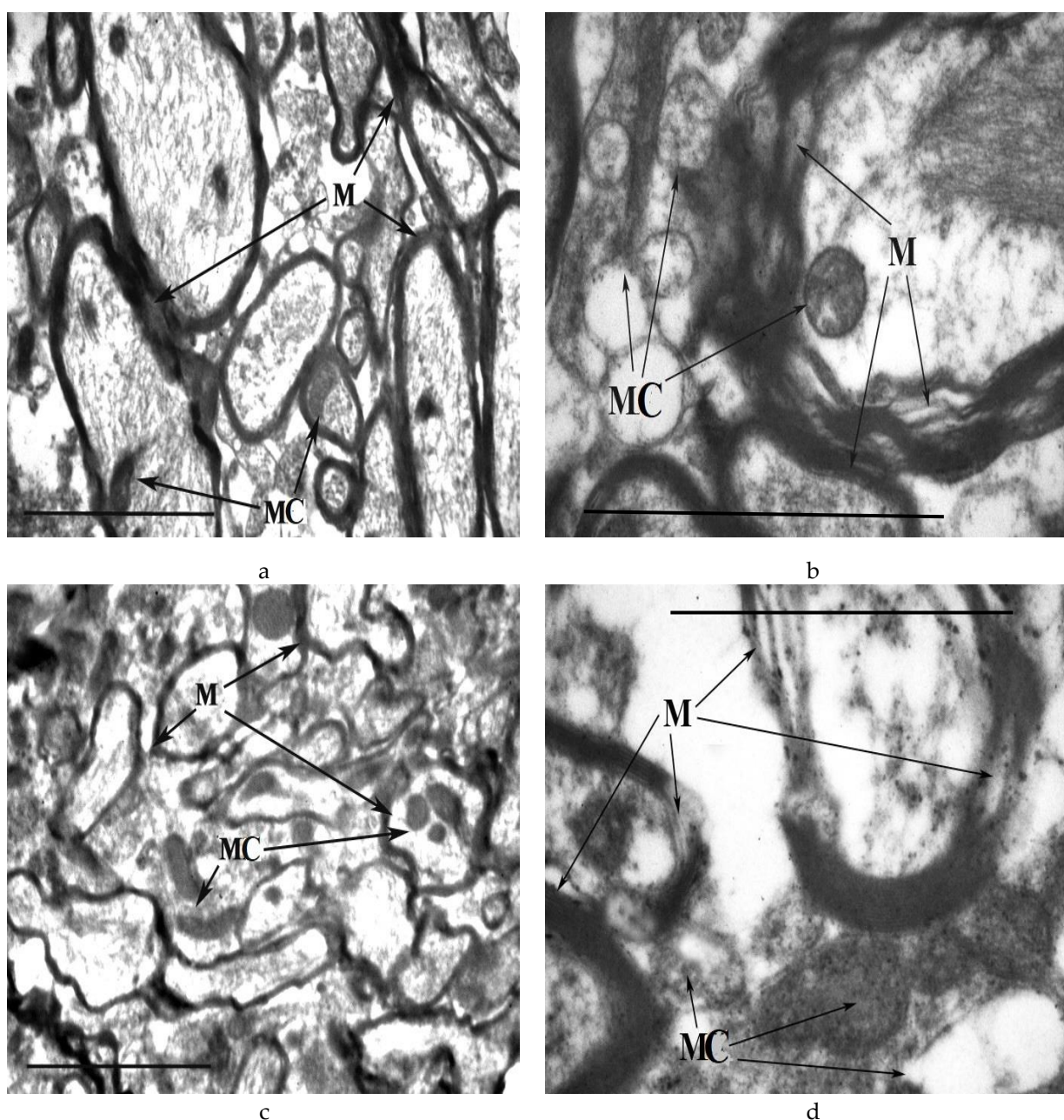


Figure 1. Ultrastructural organization of myelin in the medulla oblongata (a) and striatum (c) in control animals and under experimental Parkinsonism (b and d, respectively). M - myelin; MC - mitochondria. Scale 1 μ m.

In the pathogenesis of hyperhydration, a significant role, apparently, is played by vasomotor-trophic disorders occurring in the brain tissue [37]. Trophic disorders can rightfully include changes in oxygen consumption by tissues, and the emergence of secondary tissue hypoxia, which occurs largely due to structural damage to mitochondria.

We have shown that EP was accompanied by significant changes in the mitochondrial apparatus of brain tissues (Tab.).

Table

Some morphometric characteristics of mitochondria in the medulla oblongata and striatum (M ± m)

Conditions of experiment	Total amount of mitochondria, units/10 μm ²		Quantity of structurally damaged mitochondria, %	
	Medulla oblongata	Striatum	Medulla oblongata	Striatum
1. Control (a = 145)	14,3±2,5	15,9±1,8	7,6±1,8	6,4±1,2
2. Experimental Parkinsonism (a = 150)	9,4±1,1 *	14,0±1,6	47,3±6,4 **	31,8±8,4 **
3. Experimental Parkinsonism with cerebrolysin application (a = 135)	16,2±1,3 #	13,3±1,7	23,2±5,4 *#	18,8±4,6 *#

Notes: * - p <0.05 relative to control; ** - p <0.01 relative to control, # - p <0.05 relative to experimental Parkinsonism; a - is the number of studied fields

As evidenced by our results, under EP in the medulla oblongata tissue, there is a significant decrease in the total amount of MC (by 34.3%); in the striatum, no such changes are observed. Along with this, in both tissues, there is a sharp increase in the number of structurally damaged organelles that are not capable of efficient ATP synthesis: 6.2 times in the medulla oblongata and 5 times in the striatum (the total number of structurally impaired MC was 43% and 29%, respectively). Therefore, it can be assumed that EP is accompanied by the development of mitochondrial dysfunction in the studied structures, which leads to tissue hypoxia with the possible development of destructive processes in them. The latter includes the identified violations of the integrity of myelin.

The performed morphometric determinations, as noted above, showed that both in the medulla oblongata and in the striatum, 50-70% of myelin (the area of damage relative to the total area of myelin in the field of view) was destructured and/or hyper hydrated (Fig. 2 a, b). This process was somewhat more pronounced in the tissue of the medulla oblongata. Such changes are considered to be an indication of the formation of convulsive readiness in the organism due to impaired conduction of potentials along myelinated nerve fibers owing to damage in myelin structures [25]. It should be assumed that the preservation of the integrity of myelin and the remyelination of axons can increase the survival of neurons, which is extremely important under Parkinsonism.

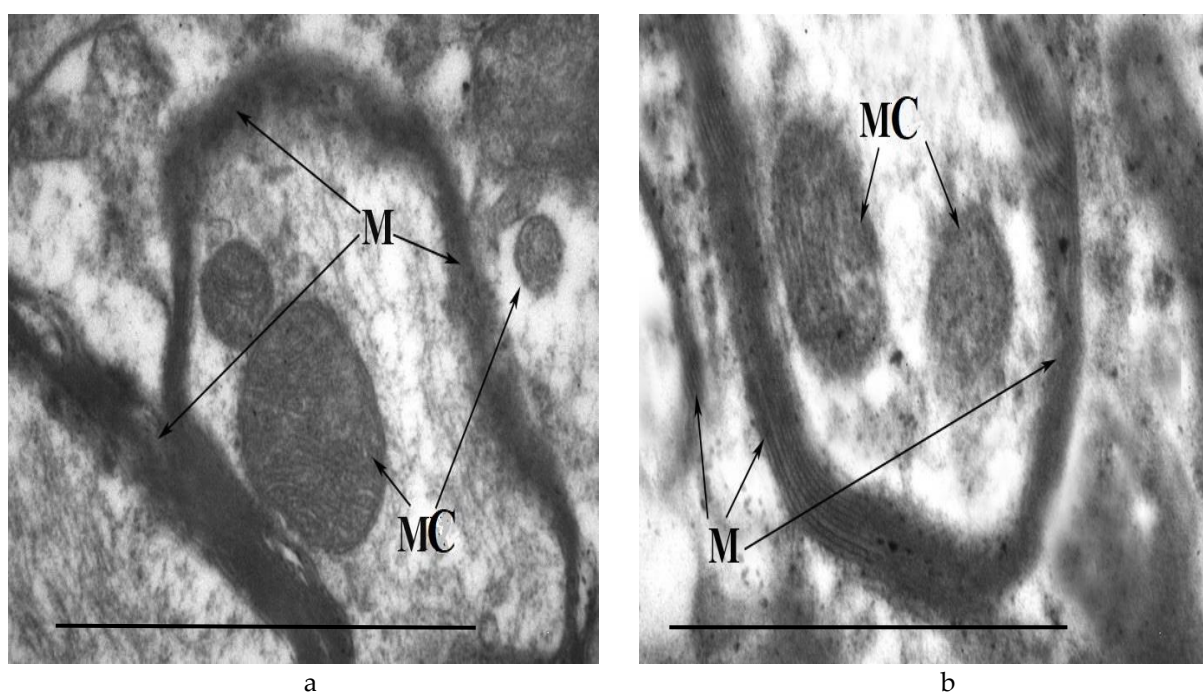


Figure 2. Ultrastructural organization of myelin in the medulla oblongata (a) and striatum (b) in experimental Parkinsonism and at the administration of Cer, M - myelin; MC - mitochondria. Scale 1 μm .

It is now believed that when nerves are damaged, so-called antisense RNA (AS-RNA), which regulates the repair of the myelin sheath, is expressed. Substrates and energy sources for AS-RNA biosynthesis are macroergs, including ATP. Consequently, a significant role in myelin damage must play mitochondrial dysfunction in brain tissues [28], the presence of which was revealed by us under EP. Therefore, mitochondrial dysfunction in brain tissues, the presence of which was revealed by us under EP, can play a significant role in myelin damage.

Administration of Cer to animals during EP was accompanied by a significant decrease in the severity of the structural component of mitochondrial dysfunction, which testified to an increase in the total amount of MC in the medulla oblongata and a significant decrease in the quantity of structurally damaged organelles in both tissues compared with the values detected during EP (see tab. 1). At the same time, a significant improvement in myelin ultrastructure was also observed: in the medulla oblongata, structurally impaired myelin remained 20-30% (decrease exceeded 2 times), and in the striatum was observed less than 10% of damaged myelin (decrease exceeded 4 times) (see fig. 2).

Thus, the formation of MD and the disruption of myelin ultrastructure are parallel processes. Moreover, in our studies this has been shown experimentally; however, in clinical practice, conduction disturbances also occur in patients with PD, and such conduction disturbances, most likely, are a violation of myelin and, consequently, the development of structural disorders in the mitochondrial apparatus, which is observed in such patients [4, 29].

The positive effect of Cer at EP, apparently, is based on its antihypoxic effect and the ability to influence transmembrane conductivity, including directly on the transmembrane conductivity of mitochondria. The latter is considered one of the main neuroprotective effects of the drug [30].

The presented data of experimental studies are in good agreement with the results of the clinical and electromyographic evaluation of the Cer effectiveness in patients with PD during course treatment with the drug. Thus, a significant decrease in the threshold for the occurrence of a monosynaptic H-reflex was found (Fig. 3), which indicates an improvement in the excitability of spinal cord motoneurons.

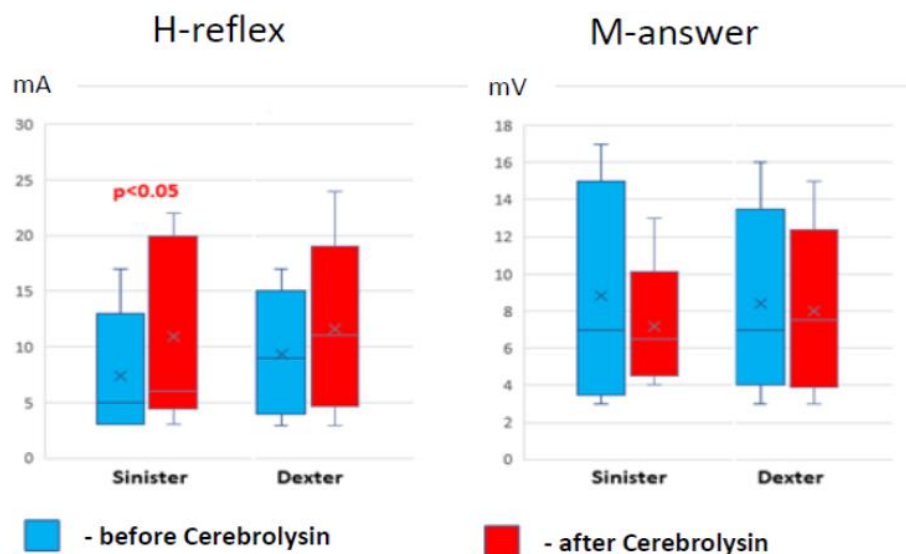


Figure 3. Thresholds of H-reflex and M-response occurrence before and after the course of Cer. Sinidyer - m. gastrocnemius sinister, Dexter - m. gastrocnemius dexter.

It should be assumed that the effect of Cer on the process of descending supraspinal control of the level of segmental excitability \bar{o} in particular, in the regulation of α -motor neuron activity, reflects the improvement in the structural organization of the myelin sheath in parkinsonism, shown in our experimental studies.

Conclusion.

Studies have shown that one of the mechanisms associated with myelin damage in Parkinsonism is the development of mitochondrial dysfunction, in any case, its ultrastructural component. The use of Cer significantly eliminates the manifestations of mitochondrial dysfunction and myelin damage. In this case, it can be assumed that the positive effect of the drug is based on the antioxidant effect, which, in turn, can affect transmembrane conductivity, and should be considered as one of the neuroprotective effects of the drug.

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Conflicts of Interest: The authors declare no conflict of interest.

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