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CONTENTS

EFFICIENCY OF NEUROPROTECTIONS AT EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS ON THE BACKGROUND OF THERAPY BY METHYLPREDNISOLONE

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Annotation. *A comparative analysis of the neuroprotective effect of Citicoline, Neurovitan and α-lipoic acid on the model of experimental equivalent of multiple sclerosis in rats under baseline hormone therapy with methylprednisolone (Solu-Medrol) was conducted. Experimental allergic encephalomyelitis (EAE) was developed for 9 - 11 days after inoculation of encephalitogenic mixture in 92% of the rats of the control group. Injection of Solu-Medrol (SM, 3.4 mg / kg intravenously over a week) prevented the development of EAE in 20% of animals from infected rodents and significantly reduced the severity and duration of neurological disorders, in 2 - 3 times (compared with control) reducing the average cumulative index and duration of the disease. The animals treated with Citicoline (500 mg / kg) in a course of hormone therapy SM EAE developed only after completion of drug administration (19 - day 20), leaking momentarily (average 5 days) and mild (average cumulative index 6.6). High efficiency of Citicoline with EAE mediated likely to continue internal and external (cytoplasmic and mitochondrial) neuronal membranes due, on the one hand, the weakening of the activity of phospholipase A2 activation and neuronal mitochondrial cytochrome oxidase, and the other - the inhibition of glutamate-induced apoptosis.*

Key words: *multiple sclerosis, experimental allergic encephalomyelitis, methylprednisolone, neuroprotection, Citicoline, Berlitione, Neurovitan.*

Formulation of the problem. Multiple sclerosis (MS) is a severe demyepinizing disease of the central nervous system that occurs as a result of the development of autoimmune reactions to myelin proteins with subsequent axonal damage to neurons of varying degrees [1]. MS is one of the socially significant problems of modern neurology, which is determined by its prevalence, the unpredictability of the course, the defeat mainly of young people of working age and frequent disability [2].

The generally accepted model of MS in laboratory animals is experimental allergic encephalomyelitis (EAE), which has clinical manifestations and pathogenetic mechanisms similar to multiple sclerosis [3].

The main standard method of treating MS is the use of intravenous corticosteroid regimens in pulse doses: the appointment of pulse therapy with methylprednisolone is considered to be recognized. Methylprednisolone (solu-medrol) usualy injects in a dose of 500-1000 mg in 200-400 ml of solution NaCL 0,9% intravenously drip of 25-30 drops

per minute 1 time per day. Duration of the course is 3-7 days [4].

However, despite extensive research, the PC continues to be a problem with many unresolved issues. This applies not only to the origin and essence of the disease, but also to its clinic, which, it would seem, has been adequately studied [5].

The desire to increase the effectiveness of the treatment of multiple sclerosis has led to the development and application of new universal therapeutic approaches to the treatment of MS, one of which is neuroprotection aimed at regulating the balance of immune and neurotrophic factors, remyelination processes. The feasibility of its appointment is determined by the need to maintain axon function and prevent the development of axonopathy. The need for neuroprotection is due to damage to both myelin sheaths of nerve fibers and the neurons themselves (this leads to the atrophy of brain matter), as well as oligodendrocytes (glial cells that support the maintenance and reproduction of myelin) [6].

Given the current level of development of knowledge about neuroprotection in MS, the optimal neuroprotective therapy in the treatment of this disease from the standpoint of practical neurology is carried out by using the following neuroprotective complex [7]:

- anti-inflammatory therapy: TNFa antagonists (trental);
- trophic factors (cerebrolysin);
- inhibition of glutamate excitotoxicity (amantadine - PC-Merz);
- antioxidant therapy: α-lipoic acid, cerebrolysin;
- remyelinating therapy: immunoglobulins, "B" -vitamin complexes.

The use of components of the neuroprotective antioxidant complex inhibits the progression of MS, slowing the rate of apoptosis of neurons and oligodendrocytes, as well as reducing the intensity of damage to myelin by free radicals, antibodies, and inflammatory cytokines [6; 7].

To evaluate the effectiveness of neuroprotective therapy of experimental allergic encephalomyelitis in the conditions of basic therapy with solu-medrol. For this, a comparative analysis of the protective effect of citicoline, neurovitan, and α-lipoic acid was carried out on an EAE model in rats with methylprednisolone.

Materials an Methods. Prior to commencement of work, the bioethics commission approved a protocol for upcoming studies. According to the requirements of GLP and the European Convention for the Protection of Vertebrate Animals, which are used for experimental and other purposes, all procedures related to keeping animals, humane handling and their use in an experiment are agreed.

The animals were kept under standard conditions with a day-night light regime of 12 hours / 12 hours at an air temperature of 20 - 220 C with free access to water and food. EAE was induced by a single subcutaneous injection of an encephalitogenic mixture (EGM) in complete Freund's adjuvant (CFA) based on 100 mg of homologous spinal cord homogenate; 0.2 ml of CFA (the content of killed mycobacteria 5 mg / ml) and 0.2 ml of physiological saline per animal. EGM was introduced into the base of the tail under light ether anesthesia in a volume of 0.4 ml [8]. Immunized animals were divided into 5 groups: I - animals with EAE (control), n = 12; II - EAE + solu-medrol (SM: 3.4 mg / kg), n = 10;

III - EAE + SM + neurovitan (25 mg / kg in terms of octothiamine), n = 8; IV - EAE + SM + citicoline (500 mg / kg), n = 8; V - EAE + SM + berlition (50 mg / kg α-lipoic acid), n = 8.

Solu-medrol was injected to animals of groups II – VI according to the clinical algorithm for the use of the drug [4] at the rate of 3.4 mg / kg into a vein dropwise in the volume of physiological saline equal to 1/10 of BCC [9] for a week. In rodents of the III – VI groups, additionally, against the background of basic hormone therapy, the test substances were administered intragastrically once a day from the second to the 16th day after the induction of EAE (latent phase + clinical phase until the end of the peak of the disease). The control group consisted of animals with induced EAE (group I), which received distilled water intragastrically for 16 days.

Daily for a month (the average duration of EAE), animals were weighed and their neurological status was assessed: the time of onset of the disease, its duration and severity of neurological disorders were recorded, which was evaluated in points by the clinical index. The clinical index (Clin-I) was determined on a scale: muscle weakness of one limb - ½ point, paresis - 1 point, paralysis - 1 ½ point. When several extremities were involved in the process, the points were summarized. Absence of violations was taken as 0 points, fatal outcome - 6 points. Animals with a clinical index of ½ - 2 ½ points were considered easily ill; 3 - 6 points corresponded to the severe course of EAE. For an integrative assessment of EAE severity for each animal, a cumulative index (Cumul-I) was calculated - the sum of individual clinical indices for the period of the disease [10]

To assess the effectiveness of the protective effect of the studied drugs on the EAE model for each group of rats, we calculated: 1) the duration of the latent period; 2) the total number of sick and seriously ill rats (in% of the number in the group); 3) the average clinical index at the peak of the disease; 4) the average cumulative index of the disease; 5) the average duration of the disease. All indicators were compared with the corresponding animals of the control group and the group receiving basic therapy with solu-medrol.

Digital experimental data were processed by the method of variation statistics using personal computer equipment - Intel Pentium-IV and the statistical analysis program AnalystSoft, StatPlus. Version 2006 [11]. The mathematical processing of the obtained data included the calculation of arithmetic mean values (M), their errors (± m).

A comparative analysis of the clinical and cumulative index was carried out using the non-parametric Mann-Whitney test. To assess the significance of differences in the shares of patients and seriously ill rats compared with the control, a more accurate Fisher test was used, and for analysis of the latent period and EAE duration, the Student’s criterion with Bonferroni correction was used for multiple comparisons.

Main results of the study. The results of the studies indicate that under conditions of a single subcutaneous inoculation of encephalitogenic mixture (EGM)in Freund's complete adjuvant in animals of the control group, the development of neurological disorders of varying severity was recorded; a fatal outcome of the disease was observed in one of 12 rodents (8.3%). In the area of inoculation, manifestations of inflammation were noted that persisted for more than 20 days.

After EAE induction in rats of the control group, the first neurological symptoms of the disease were recorded on days 9–11. The peak of clinical manifestations of encephalomyelitis in most animals developed on days 12-14 and lasted an average of 4 days; the duration of EAE was 16.4 + 1.8 days with an average cumulative index of 27.2 points.

At the peak of the clinical manifestations of EAE, the number of animals with a clinical index of ½ - 2 ½ points was 41.7% of rats, which corresponded to a mild disease, and severe EAE was observed in 58.3% of rodents (clinical index 3 - 6 points).

An analysis of the obtained experimental data established that the dynamics and severity of the development of neurological disorders corresponded to the manifestations of EAE described earlier by us or other authors [12; thirteen].

The injection of solu-medrol (3.4 mg / kg into a vein drip during the week) eliminated lethal outcomes, completely prevented the development of neurological disorders in 20% of animals, and also reduced the number of rodents with severe EAE to 30%.

The course application of neurovitan (25 mg / kg in terms of octothiamine) from the second to the 16th day after inoculation of EGM with solu-medrol therapy prevented the development of EAE in only 25% of animals with a mild course of the disease, without significantly changing the duration of the latent period of EAE compared with SM group.

At the same time, neurovitan reduced, on average, 1.1-1.2 times the clinical index at the peak of pathology, as well as the cumulative index and duration of the disease compared with the group receiving basic hormone therapy.

The therapeutic effect of neurovitan in relation to the manifestations of experimental allergic encephalomyelitis in the conditions of basic therapy with solu-medrol is mediated by a high-dose complex of B vitamins in its composition. This ensures the development of neuroprotective, antioxidant and trophic, promoting remyelination effects. The property of this combination is based on the pharmacological effects characteristic of high doses of these vitamins, and does not depend on their deficiency. Obviously, octothiamine, by enhancing energy supply in the form of ATP, supports axoplasmic transport, while pyridoxine is involved in the synthesis of transport proteins, and cyanocobalamin provides the delivery of fatty acids for cell membranes and myelin sheath [14].

The combined use of the antioxidant berlition (50 mg / kg α-lipoic acid) and methylprednisolone completely prevented the development of EAE in 25% of animals with mild disease. In other rodents, administration of the drug against the background of basic hormone therapy moderately weakened the severity and duration of EAE: the clinical index at the peak of pathology decreased by 19%, the cumulative index by 17%, and the duration of EAE by 1.3 times compared with the group treated with solu-medrol.

Compared with mono-injection of glucocorticoid, the combined use of citicoline and methylprednisolone more strongly reduced the severity of neurological disorders. In particular, in this series of studies, the clinical index at the peak of EAE decreased by 38%, the cumulative index of the disease was reduced by 30%, and the duration of EAE was shortened from 8.4 to 5 days compared with the group receiving basic hormone therapy.

Exogenous citicoline, being a neuroprotective agent, is involved in the biosynthesis of membrane phospholipids of neurons, primarily phosphatidylcholine (lecithin). Phospholipids form the structural and functional basis of neural membranes that support the activity of nerve cells and the brain as a whole (maintaining the ionic balance and activity of membrane-bound enzymes, providing a nerve impulse, etc.). When exogenously administered, citicoline is rapidly hydrolyzed in the body to circulating cytidine and choline, of which, after absorption into the systemic circulation, CDP-choline is synthesized. The main mechanism of action of citicoline, which determines its neuroprotective properties, is the preservation of the external and internal (cytoplasmic and mitochondrial) neuronal membranes, primarily by attenuating the activity of phospholipase A2, activation of neuronal mitochondrial cytochrome oxidases and inhibition of glutamate-induced apoptosis [17; 18].

Thus, citicoline, which most effectively prevents the development of neurological disorders and is statistically significant, reduces by 3.3 to 4 times ($p < 0.05$) compared with the control group, the most optimal neuroprotection for EAE under conditions of therapy with solu-medrol and the duration of the experimental equivalent of multiple sclerosis in animals. The moderate severity of the therapeutic effect of berlition and neurovitan in our experimental conditions, obviously, indicates the need for a longer course of application of these components of antioxidant and remyelinating therapy.

Conclusions:

- inoculation of the encephalitogenic mixture on days 9–11 in 91.7% of the animals of the control group causes the development of EAE, characterized by a severe and prolonged course;
- course use of citicoline, neurovitan and α-lipoic acid in EAE under the conditions of basic therapy with solu-medrol to various degrees prevents the development of the disease; however, in diseased animals, EAE occurs briefly and mainly in mild form;
- the ability to prevent the development of neurological disorders and reduce the severity and duration of the experimental equivalent of multiple sclerosis decreases in the series citicoline (500 mg / kg)> berlition (50 mg / kg)> neurovitan (25 mg / kg in terms of octothiamine).

References:

1. MakDonald V.Ia., Fazekas F., Tompson A.D. (2003). Diagnosis of multiple sclerosis: Journal of Neurology and Psychiatry named after S.S. Korsakova. Multiple sclerosis: an appendix to the journal, vol.2, 4–9.

2. Owens T. (2003). The enigma of multiple sclerosis: inflammation and neurodegeneration cause heterogeneous dysfunction and damage: Curr. Opin. Neurol., Vol. 16, N3. 259 – 265.

3. Nefodov O.O., Mamchur V.I., Dronov S.N., Kryvoshei V.V. (2015). Multiple sclerosis: pathogenetic mechanisms of development and features of the experiment: Proceedings of the Third International Scientific and Practical Conference "Economic

Development: Theory, Methodology, Management", Budapest-Prague-Kiyv, 159-166.

4. Nefodov O.O., Mamchur V.I. (2015). Possibilities of pharmacological correction of cognitive disorders in conditions of experimental equivalent of multiple sclerosis: Medicni perspektivi, Vol. XX, N2, 4-11.

5. Nefodov O.O., Mamchur V.I. (2018). Pharmacological analysis of neuroprotection under multiple sclerosis: LAP Lambert Academic Publishing RU, 286 p.

6. Evtushenko S.K., Derevianko Y.N. (2015). Modern approaches to the treatment of multiple sclerosis (II post): International Neurological Journal, Vol. 2(6), 70 – 85.

7. Nefodov O.O., Mamchur V.I. (2015). Pharmacotherapy for multiple sclerosis - current standards: Pharmacology and drug toxicology, Vol.3 (144), 10-16.

8. Nefedov A.A., Mamchur V.I. (2015). Pharmacological Correction of Neurological Disorders in Case of Multiple Sclerosis: Galician Medical Journal, Vol. 22, N 4, part 2, 39-41.

9. Stefanov A.V. (2002). Preclinical Drug Studies, Kiev: Avicenna, 568 p.

10. Serebryanaya N.B., Karpenko N.M., Zhitnukhin Yu.L. (2010). Study of the protective effect of the drug ferrovir in acute experimental allergic encephalomyelitis: Cytokines and inflammation, Vol. IX, N1, 33 – 38.

11. The program of statistical analysis [Electronic resource]: Access mode – www. analystsoft.com/ru/

12. Nefodov O.O., Mamchur V.I. (2014). Modeling and evaluation of experimental allergic encephalomyelitis: Bulletin of problems of biology and medicine, Vol. IV, N2 (114), 205 – 208.

13. Nefodov O.O., Mamchur V.I., Tverdokhleb Y.V. (2016). Features of the ultrastructure of the frontal cortex and hippocampus of rats in experimental allergic encephalomyelitis: Morphologia, Vol.10, N1, 54-61.

14. Mamchur V.Y., Dronov S.N. (2009). Clinical and pharmacological aspects of the use of complexes of B vitamins in the treatment of vertebral neurological pain syndromes: Health of Ukraine, N9, 60 – 61.

15. Nefodov O.O., Mamchur V.I. (2016). The use of citicoline for the correction of ultrastructural changes in the central nervous system induced by experimental allergic encephalomyelitis: Bulletin of problems of biology and medicine, Vol.2 (129), 235-240.

16. Suzuki Y.J., Tsuchiya M., Packer L. (1991). Thioctic acid and dihydrolipoic acid are novel antioxidants which interact with reactive oxygen species: Free Radic Res Commun, Vol. 15, N5, 255 – 263.

17. Nefedov Alexander A., Mamchur Vitaly I., Abramov Andrey V., Bukhtiyarova Nina V. (2016). Pharmacological Analisis of Neuroprotective Action of Methylprednisolone with Citicoline in Conditions of Experimental Allergic Encephalomyelitis: Biological Markers and Guided Therapy, Vol. 3, N 1, 115-124.

18. Nefedov A.A., Mamchur V.I. (2015). Pharmacological Correction of Neurological Disorders in Case of Multiple Sclerosis: Galician Medical Journal, Vol. 22, N 4, part 2, 39-41.