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NEUROPROTECTIVE EFFECT OF CITICOLINE AND GLUCOCORTICOSTEROID COMBINATION UNDER CONDITIONS OF EXPERIMENTAL DEMYELINATING MODEL OF CENTRAL NERVOUS SYSTEM

SİTİKOLİN VE GLUKOKORTİKOSTEROİD KOMBİNASYONUNUN MERKEZİ SİNİR SİSTEMİNİN DENEYSEL DEMİYELİNİZAN MODELİ KOŞULLARINDAKİ SİNİR KORUYUCU ETKİSİ

Alexander A. Nefodov[1], Igor F.Belenichev[2], Elena A. Nefodova[1], Nina V. Bukhtiyarova[2], Sergii V. Levich[2*], Sergii N. Dronov[2]

Abstract

Multiple sclerosis is a multifactorial, autoimmune, chronic inflammatory demyelinating disease of the central nervous system. Recent studies do not give possibility to estimate the contribution of neurodegenerative changes in neurological deficit of individual patient, to predict the disease development and the effectiveness of therapy. The goal of our research was to investigate methylprednisolone and citicoline co-administration effect to the processes of energy providing of the mitochondria of the cerebral cortex neurons in experimental allergic encephalomyelitis. Experiments were carried out on rats of both sexes weighing 150-180 g. Experimental allergic encephalomyelitis was induced by a single subcutaneous inoculation of encephalitogenic mixture in complete Freund's adjuvant. As material, we used brains. We studied markers of mitochondrial dysfunction and content of adenine nucleotides, lactate, malate, isocitrate, aspartate, pyruvate. We also studied the state of neurons, their area, RNA-content and proportion of apoptotic cells. Formation of experimental allergic encephalomyelitis (EAE) led to permanent disturbance of energy metabolism of brain. The administrations of methylprednisolone did not have a significant effect. Co-administration of methylprednisolone and citicoline exerted significant influence on some parameters of mitochondrial dysfunction and brain energy metabolism. We also found neuronal damage of sensorimotor cortex of experimental animals and to the neuroapoptosis activation. Administration of methylprednisolone resulted in direct neuroprotective effect. Combination of citicoline and methylprednisolone limit activity of unproductive anaerobic glycolysis and increases aerobic ATP synthesis reaction. Thus, the combination of citicoline and methylprednisolone does not affect the activity of malate aspartatic shunt in EAE conditions.

Keywords: experimental allergic encephalomyelitis; citicoline; methylprednisolone; mitochondria; neurons

[1]SE, Dnipropetrovsk Medical Academy of Ministry of Health of Ukraine, Dnipro, Ukraine
[2]Zaporizhzhya State Medical University, Zaporizhia, Ukraine
[*]Corresponding Author: Department of Biochemistry and Laboratory Diagnostic, Zaporizhzhya State Medical University, Mayakovskiy avenue, 26, 69035, Zaporizhzhya, Ukraine. E-mail: ifb1914@mail.ru

Özet

Multipl skeleroz çok bileşenli, otoimmün ve kronik inflamatuvar demiyelizan bir merkezi sinir sistemi hastalığıdır. Yakın zamanda yapılan çalışmalar hasta bireylerdeki nörodejeneratif değişiklerin nörolojik bozukluklara katkısını, hastalığın gelişimi ve terapinin etkisinin nasıl olacağını tahmin etme olanağı vermiyor. Araştırmamızın amacı, deneysel alerjik ensafalomiyelitdeki metilprednizolon ve sitikolinin birlikte kullanılmasıyla oluşacak etkinin serebral korteks nöronlarının mitokondrilerine enerji sağlamasını incelemektir. Deneyler her iki cinsiyetteki 150-180 gram ağırlığındaki farelerle gerçekleştirilmiştir. Deneysel alerjik ensefalomiyelit, tam Freund adjuvanı içindeki ensefallojenik karışımının tek bir deri altından aşılaması ile indüklenmiştir. Materyal olarak beyni kullandık ve adenin nükleotidleri, laktat, malat, isositrat, aspartat, piruvat içerikleri ile mitokondriyel bozukluk belirteçleri üzerine çalıştık. Aynı zamanda nöronların durumunu, alanlarını, RNA içeriğini ve apoptotik hücrelerin oranını da inceledik. Deneysel alerjik ensefalomiyelit (DAE) oluşumu, beynin enerji metabolizmasının kalıcı olarak bozulmasına yol açmaktadır. Metilprednizolon uygulamalarının anlamlı bir etkisi bulunamamıştır. Metilprednizolon ve sitikolinin birlikte uygulanması, mitokondriyal bozukluk ve beyin enerjisi metabolizmasının bazı parametreleri üzerinde anlamlı bir etki göstermiştir. Ayrıca deney hayvanlarının sensorimotor korteksinde nöronal hasar ve nöroapoptoz aktivasyonu da bulunmuştur. Metilprednizolon uygulaması doğrudan sinir koruyucu etki ile sonuçlanmıştır. Sitikolin ve metilprednizolon kombinasyonu, işlevi olmayan anaerobik glikoz parçalanmasını sınırlamakta ve aerobik ATP sentez reaksiyonunu arttırmaktadır. Bu nedenle, sitikolin ve metilprednizolon kombinasyonu, DAE koşullarındaki malat aspartatik devrenin aktivitesini etkilememektedir.

Anahtar Kelimeler: *deneysel alerjik ensafalomiyelit; sitikolin; metilprednizolon; mitokondri; nöronlar*

1. Introduction

Multiple sclerosis (MS) is a multifactorial, autoimmune, chronic inflammatory demyelinating disease of the central nervous system (CNS). It is generally accepted to have a diffuse defeat of white and gray matter of the CNS in MS patients, leading to the development of the brain and spinal cord atrophy. Severity of neurological symptoms in MS is largely related to the overall brain atrophy, manifesting by a decrease in volume of brain parenchyma, increased ventricular and subarachnoid spaces. The average age of debut of the disease is 29 years; the ratio of female and male cases is usually close to 3:1 (Gusev et al., 2011). Disability in MS is primarily associated with impaired motor function, visual impairment, coordination of pelvic functions. MS - the second most common cause of disability among young people is not only socially but also economically significant disease (Boiko et al., 2013; Orton et al., 2006). Until not long ago, it was believed that violation of the conducting function of axons in MS occurs only as a result of multifocal lesions of the myelin sheath (Popescu & Lucchinetti, 2012). However, more recent studies have shown that neurodegeneration (inflammatory damage to gray and white matter of the brain) occurs in the early stages of MS, and plays a large role in the formation of irreversible neurological deficit (Bjartmar et al., 2001; Geurts & Barkhof, 2008; Lucchinetti et al., 2011). Moreover, a clear correlation detected degree of disability in MS data with degenerative brain changes, while the magnetic resonance imaging pattern inflammatory changes can significantly dissociate with the clinical picture (DeStefano et al., 2001). Data on the primary pathogenesis of neurodegeneration in MS are very few, remain unclear underlying causes and mechanisms of development. These studies, available in clinical practice, do not give possibility to estimate the contribution of neurodegenerative changes in neurological deficit of individual patient, to predict the disease development and the effectiveness of therapy.

The goal of our research was to investigate the effect of methylprednisolone and citicoline co-administration to the processes of energy providing of the mitochondria of the cerebral cortex neurons and histomorphometric parameters of its formation in experimental allergic encephalomyelitis.

2. Materials and Methods

2.1. Animals

Experiments were carried out on 40 rats of both sexes weighing 150-180 g. Prior to the implementation of upcoming research protocol of the work was approved by the bioethics Commission of Zaporizhzhya State Medical University. According to the requirements of GLP and the European Convention for the Protection of Vertebrate Animals used for experimental and other purposes agreed by all the procedures related to the maintenance of the animals, the humane treatment of them and their use in experiments.

2.2. Experimental procedure

The experimental animals were kept in standard conditions with a light regime of day - night 12 hour / 12 hours at an air temperature of 20 - 22°C with free access to food and water. Experimental allergic encephalomyelitis (EAE) was induced by a single subcutaneous inoculation of encephalitogenic mixture (EGM) in complete Freund's adjuvant (CFA) at the rate of 100 mg of spinal cord homogenate homologous; 0.2 ml CFA (Mycobacterium killed content of 5 mg / ml) and 0.2 ml saline per animal. EGM was injected into the base of the tail under light ether anesthesia in a volume of 0.4 ml (Degano & Roth, 2000).

2.3. Biochemical analysis

Biochemical studies carried out on brain, for this purpose the animals were decapitated under anesthesia using Thiopental (30 mg/kg, intraperitoneally).

2.3.1. Material

Blood was rapidly removed from the brain; investigated pieces were separated from the meninges and placed

in liquid nitrogen. Then grounded in liquid nitrogen to a powder and homogenized in 10 times volume of the medium at (2 °C) containing (in mmol): sucrose - 250-HCl-Tris buffer - 20-1 EDTA (pH 7.4). Mitochondrial fraction was isolated by differential centrifugation in refrigerated centrifuge at the temperature of + 4 °C. To clean the mitochondrial fraction from large cell fragments it was previously centrifuged for 7 minutes at 1000 g. The supernatant was then re-centrifuged for 20 minutes at 17000 g. The supernatant was decanted and stored at -800 S. Mitochondrial pellet was resuspended in isolation medium containing bovine serum albumin (0.5 mg / ml) and again precipitated for 10 minutes at 17,000 g. The mitochondria were suspended in isolation medium suspension contained 40-60 mg protein / ml. For long-term storage mitochondria is frozen at -80 °C. To determine the speed potential of the inner mitochondrial membrane and opening of mitochondrial pores used suspension 0.5-1.0 mg protein / ml.

2.3.2. Enzymatic activity

Total activity of creatine phosphokinase (CK) and the cerebral fraction of the cytocholic isoenzyme of creatine phosphokinase (BB-CK) was determined in serum with Cormay Prestige 24i chemistry analyzer.

2.3.3. Energy metabolism

The state of energy metabolism was determined by the level of the most important intermediates – ATP, ADP, AMP, lactate, pyruvate and malate. The quantity of malate was detected according to method of Hohorst (Hohorst, 1970). The creation of renovated form of NAD+ is equal to the quantity of oxidized malate, the growth of which is indicated at 340 nm. Adenylate nucleotides were determined by thin layer chromatography (Prohorova, 1982). The content of pyruvate was determined by method of Zoh-Lompreht (Prohorova, 1982). The content of lactate was determined by the method of Hohorst (Hohorst, 1970). The creation of new form of NAD+ is equal to the quantity of oxidized lactate, the quantity growth of which is indicated at 340 nm.

2.3.4. Mitochondrial fraction

The development of experimental allergic encephalomyelitis was characterized by the magnitude of the mitochondrial inner membrane potential of mitochondria and the degree of mitochondria swelling.

For this purpose, brain of animals were washed with chilled 0.15 M KCl solution at 4°C. Then neuronal tissue was thoroughly comminuted and homogenized in 1000 % w/vol of the medium consisting of: sucrose – 250 mM, Tris-HCl-buffer – 20mM (pH 7.4) and EDTA – 1 mM. Mitochondria were isolated at 4°C by differential centrifugation in the refrigerated centrifuge Sigma 3-30k (Germany). For cleaning the mitochondrial fraction from large cell fragments primary centrifugation was conducted for 7 minutes at 1000 g, and then supernatant was centrifuged for 20 minutes at 17000g. The supernatant was decanted and stored at -80°C. The pellet of mitochondria was resuspended in the medium, containing bovine serum albumin (0.5 mg/ml) and then precipitated by centrifugation for 10 minutes at 17,000 g. The mitochondria were suspended in the isolation medium, suspension contained 40-60 mg protein/ml. To record the opening of MO, to incubation mixture, which consisted of 120 μM of KCl, 0.5 mM of KH2PO4, 2 mM of glutamate, 1 mM of malate and 20 mM of Tris-HCl-Molecular and biochemical mechanisms buffer (pH 7.4) was added 1 mg of mitochondria suspension.

Mitochondrial membranes barrier function changes were determined spectrophotometrically as a decrease in absorbance at 540 nm caused by mitochondria swelling. The process was induced by introduction of 50 μM of Ca2+ into non-mitochondrial medium after Ca2+ - recharge (ΔE) in the study samples, which characterized the intensity of the process.

The potential generated at the inner mitochondrial membrane, was recorded on a spectrophotometer, a two-wave mode (511 - 533 nm) with Safranin O. as a voltage-dependent probe (18 uM). Measurements were performed in 10x10 mm glass cell with a working volume of 2 ml. Measurement was carried out in 0.62 mM KCl; 40 mM Caps (3- [cyclohexylamino] -1-propanesulfonic acid) -KOH (pH = 10); protonophore-uncoupler FCCP and monensin antiporter were used to dissipate potential.

2.3.5. Morphometric analysis

Brain was fixed in 10% Bouin's fluid (24 hours) and was embedded in paraffin blocks. These blocks we used for preparation of 5-micron frontal histological sections of the postcentral gyrus (somatosensory bark). To study the morphological and functional state of neurons of IV-V cortical layers and for specific detection of RNA, histological sections were deparaffinized and then were stained by gallocyanin - chrome alum (Einarson method). Images of the cerebral cortex were obtained by microscope Axioskop (Zeiss, Germany), using an 8-bit CCD-camera COHU-4922 (COHU Inc., USA) and then were processed by computer image analysis system VIDAS-386 (Kontron Elektronik, Germany). Morphometric analysis of brain cells was performed in automatic mode using the VIDAS-2.5 software (Kontron Elektronik, Germany).

The following parameters were defined:

- density of neurons, glial cells, apoptotic and destructed neurons (number of cells per 1 mm2 of area of cerebral cortex section);

- area of bodies of normal, apoptotic and destructed neurons (μm2);

- RNA concentration in normal, apoptotic and destructed neurons (absorbance units, EOD), which was calculated as the logarithm of the ratio of the optical density of the cell body to the optical density of the intercellular substance.

Degenerating neurons were considered those showing signs of disease or cytolysis. Density of degenerating and surviving neurons, the ratio of intact neurons to perishing (neurodegeneration index) and ratio of the surviving neurons in the use of medication to intact neuronal density in control group (survival index improvement) were measured by software. The rate of the neurodegeneration index less than one unit testified the predominance of

the dying neurons number to surviving. The index of the survival improvement and activity of microglia more than one unit showed a positive pharmacological effect of the drug, less than one - negative. The functional state of the surviving neurons was evaluated on the basis of changes in the area of nuclei and nucleoli of nucleic acids neuronal content, nuclear-cytoplasmic ratio and the number of multinuclear cells (Chekman et al., 2016).

2.3.6. Statistical analysis

The results of the investigation were calculated using the standard analysis package of computer program «STATISTICA® for Windows 6.0» (StatSoftInc., №AXXR712D833214FAN5), as well as «SPSS 16.0», «Microsoft Office Excell 2003».Verification of normality was performed using the Shapiro-Wilk test. Data are presented as the sample mean. Accuracy of differences between sample means was assessed using Student t-test under normal distribution. The Mann-Whitney U test was used in the case of nonnormal distribution or analysis of ordinal variables. The analyses of variance (ANOVA) under normal distribution or Kruskal-Wallis test for nonnormal distribution were used for comparison of the independent variables in more than two samples. The difference p <0.05 (95%)

was considered statistical significant for all analyses (Zaycev et al., 2006).

3. Results

Formation of experimental allergic encephalomyelitis led to activating anaerobic glycolysis (increased lactate / pyruvate ratio), inhibition of oxidation in the Krebs cycle (reduction of malate by 51% and isocitrate 45 %) and power shortage (a decrease of 42% of ATP, ADP, 43% AMP against increase of 82%), malate-aspartate shunt inhibition (lowering of malate level by 51%, and aspartate by 40% in comparison with the intact group) (Table 1, 2).

Formation of EAE also led to the increasing of mitochondrial pore opening rate at 9.1 times and fall of the inner mitochondrial membrane potential by 78% (Table 3).

Co-administration of methylprednisolone and citicoline exerted significant influence on some parameters of mitochondrial dysfunction and brain energy metabolism. Thus, the introduction of this combination resulted in a significant reduction of mitochondrial pore opening speed by 66% and increase of the mitochondria inner membrane charge by 69%.

Multiple sclerosis experimental equivalent leads to neuronal damage of sensorimotor cortex of experimental animals. Thus, in a group of untreated animals with EAE neurons density decrease was observed in 19%, indicating the cell death, increasing their surface by 10%, indicating edema. It has also been found to decrease transcriptional processes in neurons of the sensorimotor cortex in the simulation of EAE, as evidenced by RNA decline by 21%. It was also observed neuroapoptosis activation. So, in animals with EAE increase in the density of apoptotic and destructive cells of the sensorimotor area of the cortex by 150% was observed (Tables 4-5). Percentage of apoptotic cells in the brain structure in animals with EAE increased from 3.4 to 15%, 7%, i.e. nearly 5 times. Administration of methylprednisolone to animals with EAE resulted in a significant increase in the cortical sensoromotor neurons density by 3% and reducing their size by 8%.

Tablo 1. Adenile nucleotides in the brain of animals with experimental allergic encephalomyelitis

Group of animals	ATP, umol/g of tissue	ADP, umol/g of tissue	AMP, umol/g of tissue
Intact	2,80 ± 0,15	0,27 ± 0,013	0,114 ± 0,008
Experimental allergic encephalomyelitis	1,60 ± 0,08 (-42%)	0,153 ± 0,010 (-43%)	0,208 ± 0,021 (+82%)
Experimental allergic encephalomyelitis + methylprednisolone	1,67 ± 0,13	0,148 ± 0,025	0,202 ± 0,016
Experimental allergic encephalomyelitis + methylprednisolone + citicoline	1,81 ± 0,11* (+13%)	0,178 ± 0,011* (+16%)	0,166 ± 0,011 (-20%)

Tablo 2. Markers of energetic metabolism in the brain of animals with experimental allergic encephalomyelitis

Group of animals	Lactate	Malate	Pyruvate	Isocitrate	Aspartate
	mcmol/ g of tissue				
Intact	2,41 ± 0,09 4,86	0,45 ± 0,02 0,22	0,498 ± 0,026 0,25	0,288 ± 0,018 0,158	11,7 ± 0,80 6,95
Experimental allergic encephalomyelitis (EAE)	± 0,20 (+101%)	± 0,03 (-51%)	± 0,026 (-49%)	± 0,010 (-45%)	± 0,64 (-40%)
Experimental allergic encephalomyelitis + methylprednisolone	4,87 ± 0,21 (+0,2%)	0,24 ± 0,04 (+9%)	0,246 ± 0,050 (-1,6%)	0,164 ± 0,030 (+4%)	7,60 ± 0,50 (+9%)
Experimental allergic encephalomyelitis + methylprednisolone + citicoline	3,52 ± 0,31 (-27%)	0,34 ± 0,05 (+54%)	0,338 ± 0,030 (+35%)	0,286 ± 0,018 (+81%)	8,00 ± 0,66 (+15%)

Tablo 3. Dysfunction indicators of brain mitochondria in experimental allergic encephalomyelitis

Group of animals	Opened mitochondrial pores, Δ E (540nM)	Mitochondrial membrane potential (Safranin-O)
Intact	0,019 ± 0,001	50,9 ± 2,05
Experimental allergic encephalomyelitis (EAE)	0,193 ± 0,013 (+915%)	10,9 ± 1,21 (-78%)
Experimental allergic encephalomyelitis + methylprednisolone	0,186 ± 0,015	13,0 ± 1,21
Experimental allergic encephalomyelitis + methylprednisolone + citicoline	0,065 ± 0,005* (-66%)	18,5 ± 1,8* (+69%)

Tablo 4. Morphological and functional indicators of neurons of the sensorimotor cortex brain of animals with experimental allergic encephalomyelitis

Group of animals	The density of neurons (neuron /mm^2)	Area neurons (um^2)	RNA content (Eon)
Intact	1250,2 ± 25,5	83,0± 3,86	9,52± 0,33
Experimental allergic encephalomyelitis	1006,7 ± 10,7 (-19%)	91,5± 3,93 (+10%)	7,45± 0,62 (-21%)
Experimental allergic encephalomyelitis + methylprednisolone	1037,4 ± 6,8* (+3%)	84,2± 2,73* (-8%)	7,33± 0,44 (-2%)
Experimental allergic encephalomyelitis + methylprednisolone + citicoline	1101,4 ± 7,4* (+9,4%)	84,2± 2,11* (-8%)	8,10± 0,42 +8,7%

In animals with EAE treated with a combination of methylprednisolone and citicoline neuronal density increased by 9.4%, their area has reached values of intact animals, RNA analysis of increased by 8.7%. The combination of methylprednisolone and citicoline slowed the sensoromotor cortex neurons neuroapoptosis in conditions of EAE. Thus, the density of apoptotic cells and destructive decreased by 19.5%, while the proportion of apoptotic cells decreased from 15.7% in the control of up to 9% in the group receiving methylprednisolone with citicoline (Table 5).

Tablo 5. Dysfunction indicators of brain mitochondria in experimental allergic encephalomyelitis

Group of animals	The density of optical and destructive cells for 1 mm^2	The proportion of apoptotic cells, %
Intact	59,4±6,89	3,4±0,96
Experimental allergic encephalomyelitis	148,0±16,4 (+149%)	15,7±1,7 (+361%)
Experimental allergic encephalomyelitis + methylprednisolone	141,6±11,0 (-4%)	15,0±1,0 (-4%)
Experimental allergic encephalomyelitis + methylprednisolone + citicoline	119,2±9,68* (-19,5%)	9,0±1,0* (-42%)

4. Discussion

Formation of experimental equivalent of multiple sclerosis - experimental allergic encephalomyelitis (Nefedov et al., 2014) led to permanent disturbance of energy metabolism of brain tissue (Table 1, 2). It led to inhibition of mitochondria-cytosolic compensatory shunts energy production, in particular malate aspartate shunt. Malate-aspartate shuttle transports the recovered equivalents generated in the cytoplasm during glycolysis in mitochondria in ischemia. Formed in the cytoplasm under conditions of low oxygen, NADH+ is used to convert oxaloacetic acid to malate, which penetrates into the mitochondria and takes part in the export of α-ketoglutarate. This mitochondrial malate is converted to oxaloacetic acid to form the NADH available for electronic transport chain (3 molecules of ATP are formed from 2 protons). Oxaloacetic acid formed from malate is converted to α-ketoglutarate and aspartate. α-ketoglutarate comes from mitochondria in exchange for malate, and aspartate is exchanged to glutamate. The transfer is due to the gradient of glutamate and high intramitochondrial relationship of glutamate / aspartate. The ratio of NADH / NAD + and malate / acetic acid are regulated by malate-dehydrogenase (MDH). In modeling the EAE malate-aspartate shunt inhibition was observed. These changes appear to be consequences of a secondary mitochondrial dysfunction. Studies of an isolated from the brain neurons mitochondria functional activity confirmed of this hypothesis.

The administrations of methylprednisolone to the experimental rats with EAE did not have a significant effect on the studied parameters of energy metabolism and mitochondrial dysfunction (Tables 1-3). In the same time methylprednisolone and citicoline co-administration exerted significant influence.

Multiple sclerosis experimental equivalent leads to neuronal damage of sensorimotor cortex caused by neuroapoptosis activation. Administration of methylprednisolone to animals with resulted in a significant increase in the cortical sensoromotor neurons density, indicating a direct neuroprotective effect of hormone therapy. However, the administration of methylprednisolone alone had no effect on the functional characteristics of neurons (RNA levels did not change) and had no effect on neuroapoptosis indicators. Administration to animals with EAE combination therapy of methylprednisolone and citicoline neuroprotection increased efficiency (Nefedov, 2015; Nefedov & Mamchur, 2015a; Nefedov & Mamchur, 2015b).

Neuroprotective agent Citicoline (Ceraxon) does not show a direct energy tropic action. The drug has a strong mitoprotective effect. Citicoline can maintain the integrity of the inner membrane of the mitochondria, as evidenced by the recovery of its capacity (Belenichev et al., 2015). This mechanism is associated with reduction of cardiolipin level in the inner mitochondrial membrane. It is also found that citicoline, indirectly, through increasing the activity of glutathione-related enzymes (glutathione-reductase and glutathione-transferase) regulates the level of reduced glutathione. Reduced glutathione, especially mitochondrial, inhibits oxidative degradation Red-Oxi - sensitive areas of the mitochondrial membrane and the formation of persistent mitochondrial dysfunction (Belenichev et al., 2014). Iincreasing of the reduced glutathione level can reduce Ceraxon nitrosating stress reaction and inhibit NO-dependent mechanisms of neuroapoptosis. Safety of recovered glutathione equivalents helps to limit the cytotoxic effects of NO and prevent nitrotyrosine accumulation. Balance of pro- and anti-apoptotic mechanisms in nitrosating stress is associated with the level of NO. In conditions of excessive active forms of oxygen level (primarily peroxynitrite and a hydroxyl radical) anti-apoptotic proteins are gone under the oxidative modification (bcl-2 and others), and the excess of NO-radical amid increased activity iNOS enhances the synthesis of pro-apoptotic proteins (FAS and APO-1) in neurodegenerative pathologies. When neurodegeneration, including EAE, proinflammatory cytokines expression is enhanced (IL-1, TNF-α, HIF-1) and the factors responsible for the transcription of NF-κB, AP-1, JNK as well, which indirectly enhance further formation of NO cytotoxic derivatives, leading to increased molecular reactions of mitochondrial dysfunction and neuroapoptosis. Strengthening of the methylprednisolone with Ceraxon (co-administration) neuroprotective effect can be explained by the prism of NO-dependent mechanisms of neuroapoptosis and mitochondrial dysfunction (Belenichev & Bukhtiyarova, 2014; Sukumaran et al., 2012).

Thus, on the basis of the foregoing, it can be concluded that the combination of citicoline and methylprednisolone limit activity of unproductive anaerobic glycolysis and increases aerobic ATP synthesis reaction by activation of oxidation in the Krebs cycle at tricarboxylic portion (isocitrate increase). Thus, the combination of citicoline and methylprednisolone does not affect the activity of malate aspartatic shunt in EAE conditions. Apparently, the effects of citicoline and methylprednisolone in EAE focus

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on mutual suppression of iNOS expression and activity.

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