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# Oxidative Processes and Histopathology of Madopar-Induced Neurodegeneration and its Experimental Therapy

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**Abstract**

Nurodegenerative diseases (NDD) such as Alzheimer's disease (AD), Parkinson's disease (PD) are the third most common cause of mortality after cardiovascular and oncological diseases. In PD, most of the dopaminergic neurons die in a special section of the brain called the nigrostriatal system. People have impaired motor function: there is trembling, stiffness of movements. In AD, cholinergic neurons die in the hippocampus and in the cortex, which are responsible for memory and learning. These functions are also affected in humans.

**Keywords:** Madopar neurodegeneration; Experimental therapy; Histopathology; Oxidative processes

## Introduction

Nurodegenerative diseases (NDD) such as Parkinson's disease (PD) are the third most common cause of mortality after cardiovascular and oncological diseases. In PD, most of the dopaminergic neurons die in a special section of the brain called the nigrostriatal system. People have impaired motor function: there is trembling, stiffness of movements.

The etiology and pathogenesis of NDD are far from being elucidated. In the mechanisms of their development, great importance is attached to the imbalance of neurotransmitter systems. Dyskinesias, depression, pathological aggressiveness are associated with violation of the dopaminergic system.

Oxidative stress is the most significant mechanisms leading to neuronal death in PD [1-3]. Bayer TA et al. reported a decrease in the activity of the antioxidant system is noted in PD. It disturbs the equilibrium between prooxidant and antioxidant processes [4]. Excess generation of the active form of oxygen (AFO) takes cascade character, which leads to lipid and protein disorders in the structure of cell membranes, changes in the microviscosity of the lipid bilayer, conformation of membrane proteins, which affects the functioning of ion channels and affinity of receptors with ligands.

Pathognomonic structural changes in parkinsonism are the degeneration and decay of dopaminergic neurons of a compact zone of a black substance containing neuromelanin. These changes are specific only for parkinsonism [5]. 75-90% of neurons undergo degeneration. The decomposed neurons are engulfed by glial cells. So-called necrosis fields are formed, which are the "shadows" of cells ("phantoms") and neuromelanin, located extracellularly and undergoing phagocytosis with the formation of phagolysosome.

In addition to the degeneration of nigostriate neurons, structural changes occur in the cytoplasm of neurons in certain parts of the brain (hypothalamus, cortex) like neurofibrillar degenerations. As a result Levi bodies are formated-synuclein accumulats there [4], which is a pro-apoptotic factor.

To find therapeutic agents among drugs that have antioxidant properties are actually. From a practical point of view, it is purely empirical (experimental-therapeutic) interest to find ways to alleviate the suffering of antioxidants. In previous studies, the effect of the Mkrtchyan Embryonic Antitumor Modulator (Patent of Russian Federation No. 2240810, Jan. 2004) on β-amyloid and AlCl3-induced neurodegeneration, which besides antioxidant and a number of other sanitizing abilities was studied [6,7].

The purpose of this work was to study oxidative processes and structural changes occurring in different systems of neurons and cells of the neuronal cortex and cerebellum on the model of hyperfunction of the dopamine system and in experimental therapy. As a therapeutic drug, our choice fell on the Mkrtchyan Embryonic Antitumor Modulator (EATM-LM) [8,9]. EATM is composed of a wide pool of fetal proteins and proteoglycans isolated exceptionally from normal embryonic substances [10]. It should be noted that EATM is deprived of the etiotropic action; it is effective in several pathological conditions such as cancer [11] and neurodegenerative diseases [6,7].

## Materials and Methods

The model of the present study was Madopar-induced neurodegeneration [12]. The studies were performed on old (18-24 months) Wistar rats of both sexes, who received intraperitoneal injection of medication "Madopar" at a dose of 50 mg per 1 kg of animal weight, which corresponds to 100 mg/kg of L-DOPA (dioxyphenylethylamine). All treated animals receive intraperitoneal injection of EATM-LM in dose 2 ml/animal

The animals were divided into the following groups:

1. Intact (n=7) no treatment

2. Animals receiving L-DOPA (7, 14 days) (n=7)

3. Animals receiving L-DOPA received a dose of 2 ml EATM-LM (n=7) on the 7th and 14th days,

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4. Animals, twice (on the 1st and 7th day) receiving 2 ml EATM-LM, received a madopar 14 days (n=7)

The activity of lipid peroxidation (ALP) was judged by the amount of malonic dialdehyde formed (MDA). Malonic dialdehyde was determined by reaction with thiobarbituric acid [13]. The amount of protein was determined by Lowry [14].

The extracted whole brain was fixed in neutral formalin. (To prepare the reaction of Ca2+ -dependent alkaline phosphatase, freshly frozen sections with a thickness of 40 microns were prepared). Paraffin sections were stained with modified by the Picro-Mallory method and according to Nissl.

## Results and Discussion

Injection of L-DOPA increased MDA content in the total brain homogenate in the study of the ALP process (Table 1).

Content of MDA in intact rats was 5.18 ± 0.36. The injection of L-DOPA leads to an increase in MDA content of 8.85 ± 0.27.

The high content of easily oxidizable substrates such as polyunsaturated fatty acids, catecholamines, the relatively low level of antioxidants-glutathione and vitamin E, the superoxide dismutase enzyme, and the presence of non-heminic iron, which is an activator of ALP, contribute to the increase in the peroxide content in the brain [15].

The results of the study showed that intraperitoneal injection of EATM-LM to animals under conditions of experimentally induced PD leads to a normalization of the amount of peroxides, bringing them closer to the parameters of control animals. Those animals that received a single dose of EATM-LM on the 1st day of the experiment, after 7-day administration of madopar, the content of peroxides slightly decreased, which was statistically not reliable. With the introduction of a longer period (14 days), the peroxide amounts were statistically significant.

In the histological study of the cerebral cortex of rats receiving a placebo, cytoarchitectonics, especially of medium and large pyramidal cells, is clearly seen. The polarity of the latter is monitored in all fields of vision, and the truncular part of the dendrite is clearly contoured. It is rarely an axon. Such a pattern is inherent not only in preparations stained according to Nissl and impregnated with silver, but also in the reaction to $Ca^{2+}$ -dependent alkaline phosphatase, which reveals the intracellular tubular system (Figure 1).

Stained according to Nissl in the body of neurons, grains and lumps of basophilic substance are revealed (Figure 2). This chromatophilic substance is caused by flattened cisterns of the granular endoplasmic reticulum. In some places, the transition of neurofibrils from the body of neurons to the base of the dendritic processes is distinct. The nuclei of the neurocyte, especially the large pyramidal cells are rounded and have a central location.

Under the influence of madopar in the brain of old rats, gross structural changes and tinctorial abnormalities occur, indicating its pronounced alterating effect on the nervous tissue.

| Control | Injection L-DOPA[1] | Experimental treatment EATM-LM[2] | Inoculation of EATM-LM |
|---|---|---|---|
| 5.18 ± 0.36 | 8.85± 0.27<br>P<0.001 | 6.32 ± 0.25<br>P<0.001 | 8.26 ± 0.34 |

**Note:**

1. Reliability of differences in comparison with the control

2. Reliability of differences from the data obtained in the study of animals with DOPA, who did not receive the drug

**Table 1:** The content of LPO products in the brain homogenate.

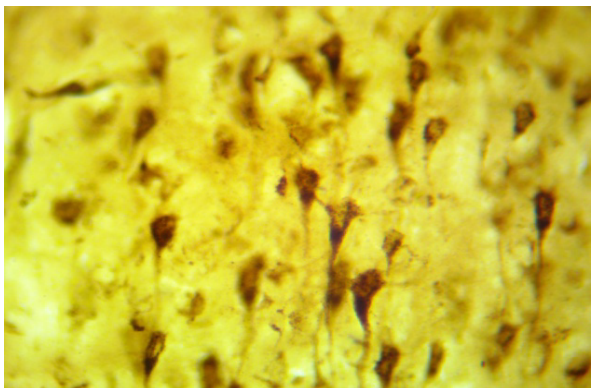

**Figure 1:** Positively stained intracellular tubular system in a layer of pyramidal cells with well-preserved polarity (arrowed). Ca2+ dependent phosphatase, x400.

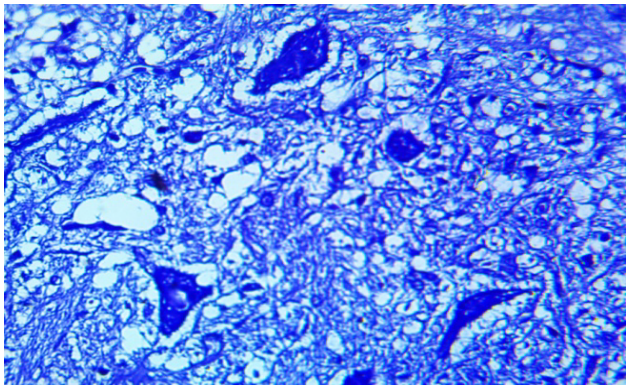

**Figure 2:** In some parts of the cortex in the layer of large pyramidal cells polymorphous giant cells with a pronounced chromatophilic substance are found (arrowed). Stained according to Nissl, x400.

First of all, attention is drawn to the violation of cytoarchitectonics in the layer of large pyramidal cells (Figure 3). In separate fields of vision there is a violation of the polarity of the pyramidal cells. There are neurocytes in which outgrowths from diametrically opposite parts depart, and it is not possible to distinguish the base of the nerve cell from its apex in view of the violation of the configuration of the polarity of the neurons. In other words, it is rather difficult to differentiate axons from dendrites. Some of the processes are not rectilinear, they have a tortuous course. In view of the presence in them of neurofibrils originating from the body of the cell, one can only assume the dendritic nature of the processes. Stained according to Picro-Mallory neurofibrils in gray matter in places acquire light purple hues. Changes in the nuclei of neurons are very diverse - from their enlightenment and pycnotic to complete lysis (Figure 4).

Pericellular edema is found with a greater quantitative stability with Madopar intoxication. Various stages of the shrinking of neurons and the expression of pericellular edema are traced, which, as is known, reaches its apogee in spongiform encephalopathy with the formation of voids in the gray matter.

In the zone of gross cytopathological changes of the cortex, capillaries with agglutination of erythrocytes occur, indicating a gross violation of microcirculation by stasis. In larger venular vessels, there is a plethora with erythrodiapedesis.

Particularly distinct the changes in Purkinje cells stained by Picro-

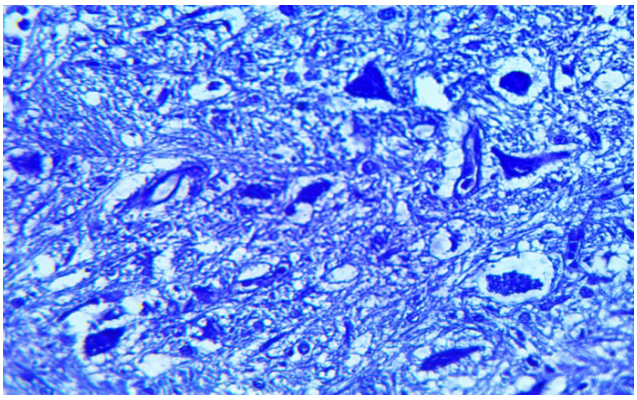

**Figure 3:** Cytoarchitectonics and polarity of pyramidal cells are disrupted. Pronounced pericellular edema (arrowed). Stained according to Nissl, x400.

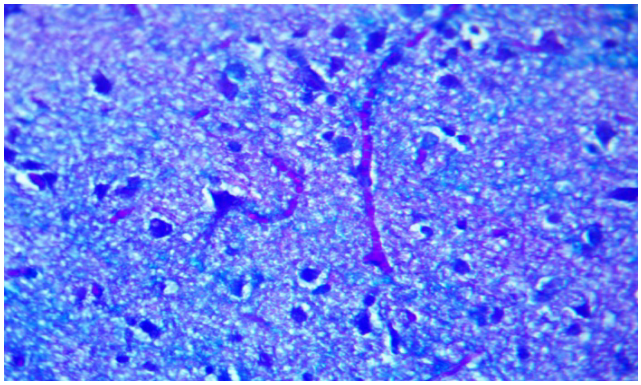

**Figure 4:** Cortex of the cerebral hemispheres. Stasis in the capillaries (arrowed). Stained by Picro-Mallory, x400.

Mallory. Against the background of the preserved histoarchitectonics of the cerebellum, the structural-tinctorial mosaic of these pear-shaped neurons is revealed. In the overwhelming majority of their processes completely shortened, cells are rounded, get the wrong shape. The nuclei of these cells in places intensively perceive the dye, while in the neighboring fields of vision they are enlightened and completely lysed. The described mosaic of changes in Purkinje cells should be classified as one of the most characteristic histomorphological manifestations of Madopar neurodegeneration (Figures 5 and 6).

Experimental therapy of madopar induced neurodegeneration through the Embryonic Antitumor Modulator (EATM-LM) included two options - preventive and curative. The therapeutic option was intended to clarify the effect of EATM-LM on the already developed neurodegeneration.

In histological studies of brain sections (Nissl, Picro-Mallory, Ca + 2-dependent alkaline phosphatase), in both variants of experimental therapy, various stages of dystrophic and necrobiotic changes of the actual nerve cells (Figures 7 and 8).

In the most pronounced form this manifested itself in the III and IV layers of the cerebral cortex, especially in large pyramidal cells. Their death leads to the formation of voids, often with a centrally located shrunken detrital mass (Figure 9). The formation of voids in the place of necrotic neurocytes is preceded, in our opinion, by pericellular edema (Figure 10). Such "devastation" in the brain in the most vivid form manifests itself in spongy encephalopathy, which is the cardinal morphological criterion of prion pathology [16]. In terms of its intensity, the spongiosis of gray matter in the cerebral cortex in the preventive series of experiments is significantly inferior to the therapeutic one, which indicates a relatively greater effectiveness of the preventive administration of EATM-LM.

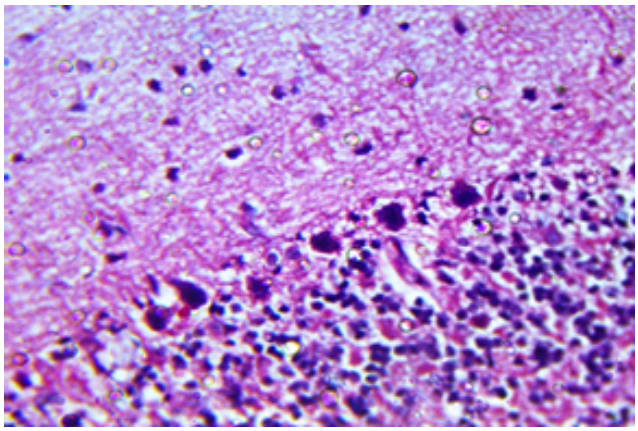

**Figure 5:** The marked pycnotic and dystrophic changes (arrowed dark) in Purkinje cells, complete atresia of the processes. The free fuchsin positive clumps in the granular layer (arrowed light). Picro-Mallory, x400.

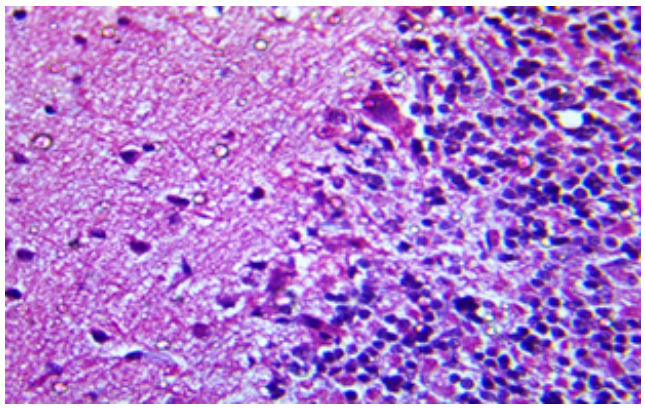

**Figure 6:** The marked pycnotic and dystrophic changes (arrowed dark) in Purkinje cells, complete atresia of the processes. The free fuchsin positive clumps in the granular layer (arrowed light). Picro-Mallory, x400.

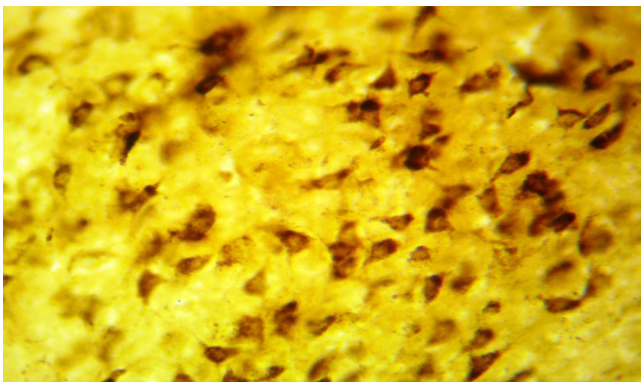

**Figure 7:** Necrobiotic changes, cytoarchitectonics disorder and polar orientation of cortical cells (arrowed). Ca2+ dependent phosphatase, x400.

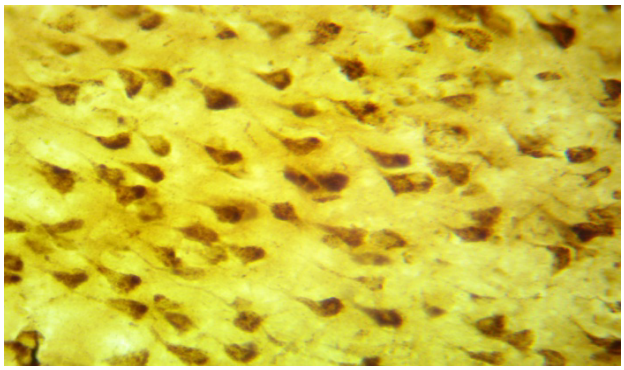

**Figure 8:** Protection of cytoarchitectonics (arrowed) in the preventive variant of experimental therapy. Ca2+ dependent phosphatase, x400.

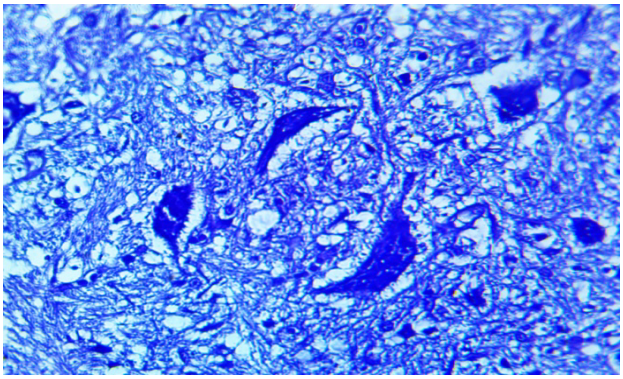

**Figure 9:** Hypertrophy, disturbance of the polarity of large pyramidal cells (arrowed). Stained according to Nissl, x400.

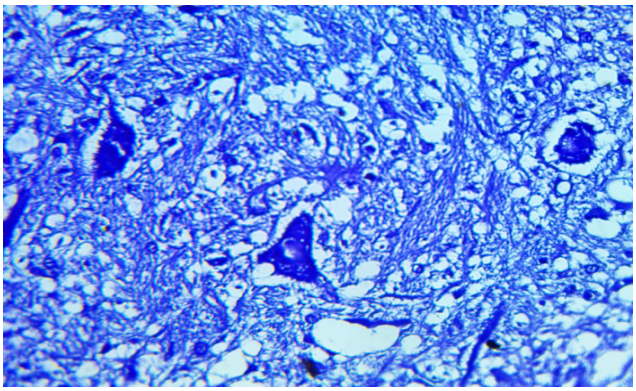

**Figure 10:** Moderate hypertrophy of the standing pyramidal cells (arrowed). Satelitosis with lysis (below). Stained according to Nissl, x400.

## Conclusion

As a result of the study, intra-peritoneal administration of EATM-LM with experimentally induced PD leads to normalization of the number of peroxides, bringing them closer to that of the control animals. Preventive single administration of EATM-LM after administration of madopar for 7 days slightly reduced peroxide content that was not statistically significant. With longer administration (for 14 days), the amount of peroxide indicators became statistically significant. Thus, administration of EATM-LM has a protective effect, which is a proof of the ability of the drug as an anti-oxidant to have a therapeutic effect.

Madopar in the studied dose causes gross histopathological changes in the brain of old rats. The first of all they concern the neurocytes of different brain structures and manifest themselves in the form of dystrophic changes of the proper nerve cells up to their necrosis. Reducing the number of neurocytes, especially pyramidal cells, leads to the phenomenon of "devastation" of the nervous tissue. In the convolutions of the cerebellum, degeneration and necrobiosis is very clearly manifested in Purkinje cells: wrinkling, carious disease, complete atresia of dendrites, formation of shadow cells, a wide range of structural-tinctorial shifts in the stained by Picro-Mallory.

Experimental therapy with EATM -LM documents a noticeable moderation in the degree of dystrophy and lysis of neurons and the weakening of various manifestations of circulatory disorders. There is a partial restoration of neurons, including pear-shaped cerebellar neurocytes. An important manifestation of the sanitizing effect of EATM -LM is the preservation of nerve processes in pear-shaped neurocytes and pyramidal cells of the cortex.

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