Oligodendrogenesis and neurogenesis in remyelination in the cuprizone model of multiple sclerosis: correlation with the degree of lesion

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Abstract. In this research, a cuprizone model of multiple sclerosis (MS) was used to study oligodendrogenesis and neurogenesis in remyelination. It has been shown that, with the administration of cuprizone, the amount of myelin in a number of structures of white and gray matter and the level of neurogenesis decrease, while the level of oligodendrogenesis increases. The withdrawal of cuprizone leads to the restoration of myelin content, the reduction of the excessive production of oligodendrocytes and to the restoration of the number of neurons to control values. The negative correlation between the number of oligodendrocyte precursors (OPCs) and the degree of demyelination of the corpus callosum indicates migration of OLG precursors from the subventricular zone (SVZ) to the structure during demyelination.

1. Introduction

One of the most significant demyelinating diseases, the development mechanisms of which still remain unclear, is multiple sclerosis (MS), which occurs mainly in young and middle-aged people and leads to a severe disability of patients. MS is characterized by a wave-like clinical course, during which exacerbations are replaced by remissions; therefore, the study of regeneration processes is extremely important for the disease prognosis, as well as for the development of new methods of the therapy.

In our study we resorted to a widely used cuprizone-induced model of MS, which allows studying the demyelination of brain nerve fibers that can be identified by both modern MRI methods and histology [1-3]. Administered orally for several weeks, the cuprizone toxin, being a copper chelator and an inhibitor of copper-dependent enzymes, leads to a selective death of oligodendrocytes (OLGs) and to the demyelination of the brain [4]. During several weeks following the withdrawal of cuprizone, an almost complete recovery of myelin is observed [5]. OPCs are the main population of cells involved in the recovery of myelin under various pathological conditions [6]. It is known that OPCs, at least in part, are formed in the SVZ, the so-called neurogenic niche, which is also one of the two known brain regions that generate neuronal precursors (NPCs) in adult age [7]. In a healthy brain, the neurons that form in the SVZ
migrate to the olfactory bulb, where they turn into adult interneurons [7,8]. OPCs formed in the SVZ normally migrate to the corpus callosum (CC) and cortex [6,8]. Neurogenesis also undergoes changes in the cuprizone model of demyelination [9]. We assumed that, during demyelination/remyelination, neurogenesis and oligodendrogenesis are inversely related, and the differentiation of stem cells shifts towards OPCs.

The aim of the work was to study the changes in neurogenesis and oligodendrogenesis during demyelination and remyelination, as well as studying their dependence on the extent of damage in the cuprizone model of MS.

2. Methods
Demyelination was induced in 4 male CD-1 mice by administering 0.5% cuprizone orally (mixed with standard food) for 10 weeks. To study remyelination, mice (N=4) were fed 0.5% cuprizone mixed with standard food for 5 weeks, after which they were returned to normal food. The control group of mice (N=4) received standard food. On the last day of the experiment, all the animals were subjected to an MRI imaging study, using a scanner for small laboratory animals Bruker "BioSpec 117/16USR" 11.7T under gas anesthesia with 1.5-2% isoflurane. The scanning was carried out in accordance with the protocol for obtaining T2-weighted images (100x100 μm resolution, 0.5 mm section thickness). Then the animals underwent a transcardial perfusion with 4% paraformaldehyde under a light ether anesthesia, and the brain was removed and frozen for a further immunohistochemical examination. Image processing was carried out in ImageJ software application. The T2-weighted images were used to assess the degree of lesion by checking the size of the corpus callosum, which was manually contoured in three consecutive sections (-0.82 ÷ -0.94 from bregma according to the atlas [10] (Figure 1). The sections were stained with the following markers: 1) neuro-glial antigen 2 (NG2) – a marker of young OLG precursors, 2) doublecortin (DCX) – a marker of immature neurons, and 3) a marker of the myelin basic protein (MBP). The mean intensity of MBP staining was measured in the following structures: corpus callosum, commissura anterior, capsula interna, thalami, caudoputamen, hippocampus, and cerebral cortex. Oligodendrogenesis was assessed in the same structures. Neurogenesis was assessed in typical neurogenic zones – lateral ventricles (SVZ) and dentate gyrus (DG) – by means of counting DCX-positive cells. The groups were compared using an independent Student test, and the correlation relationships were examined with the use of the Pearson correlation coefficient.

![Figure 1](image-url)  
**Figure 1.** A decrease of the size of the corpus callosum according to MRI scans. A – An example of T2 image processing with a delineated corpus callosum. B-D – T2-weighted images of mice’s brain from different groups: B – the control group, C – the cuprizone-treated group, D – the remyelination group. The arrows indicate the decreased corpus callosum in a mouse from the demyelination group and the normal size of the corpus callosum in mice from the control and remyelination groups. The significant decrease of the size of the corpus callosum in cuprizone-treated mice compared to the control group. **p < 0.01, T-test.**
3. Results

The resulting T2 images clearly visualize differences between the groups in the degree of myelination in the brain structures of the mice. The changes in the corpus callosum are particularly noticeable: when cuprizone is introduced, the size of the corpus callosum becomes reduced by 19.5% as compared to the control group (p<0.01), while a recovery of the volume to the control level is observed at remyelination (Figure 1). The intensity of MBP staining in the demyelination group is lower than that in the control group in all brain structures (p<0.05) with the exception of the corpus callosum, front commissura and internal capsula (Figure 2).

![Figure 2](image)

**Figure 2.** The significant decrease of myelin basic protein (MBP) in CPZ-treated mice compared to the control and remyelination groups. * – p<0.05, T-test.

At remyelination, the level of fluorescence signal on the stained MBP sections is restored to the control values. Thus, a noninvasive assessment of demyelination by T2-weighted images is confirmed by immunohistochemical staining data of the myelin basic protein. The DCX-staining revealed a significant reduction in neurogenesis in the demyelination group (p<0.001), whereas in the remyelination group the values did not differ from the control ones (Figure 3).

![Figure 3](image)

**Figure 3.** The cuprizone-induced significant decrease in DCX+ cells (newborn neurons) in the dentate gyrus (DG) and the subventricular zone (SVZ) was restored in the remyelination group. Significant differences compared with the control group: ** – p<0.001, T-test.
The NG2-staining, on the contrary, showed an increase in the level of oligodendrogenesis in the demyelination group for all investigated structures as compared to the control group (p<0.001), while there were no significant differences between the control group and the group of mice with remyelination (Figure 4). Moreover, a negative correlation was found between the number of NG2+ cells in the structure and the size of the corpus callosum in the resulting T2-weighted image (r = -0.70, p<0.05).

Figure 4. The cuprizone-induced significant increase in NG2+ cells (immature oligodendrocytes). The number of NG2+ cells is restored up to the control level in the remyelination group in the corpus callosum and striatum. The significant differences between groups: ** – p<0.01, * – p<0.05, T-test.

4. Discussion
It is known that cuprizone is a chelator of copper, and it leads to the inhibition of copper-dependent mitochondrial enzymes of cytochrome oxidase and monoamine oxidase. The administration of cuprizone causes the death of mature oligodendrocytes and demyelination, followed by remyelination upon the withdrawal of cuprizone [5,6]. The results of our experiments also indicate the loss of myelin during demyelination and its recovery during remyelination, which is shown by both MRI studies and immunohistochemical staining of the myelin basic protein.

As shown by the experimental data, oligodendrogenesis increases with demyelination and decreases with remyelination, which is consistent with the data of other researchers on the early OPC reaction after demyelination, where proliferation and colonization of the lesion area by OLG precursors was demonstrated [11-14]. This is due to the presence of OPCs, which are preserved in the process of cuprizone intoxication during demyelination since their metabolism is still not very active [15,16]. A part of OPCs migrates radially from the SVZ zone in the direction of the demyelinated corpus callosum. Next, OPCs generate OLGs. The newly formed OPCs and mature OLGs are much more exposed to the strong oxidative stress and the stress of the endoplasmic reticulum (ER), which leads to the death of OLGs [15-17]. The negative correlation between the degree of demyelination and the number of OPCs in the structure of the corpus callosum can indicate that the OPC cells migrating from the SVZ zone make up the majority of the OLG precursors at the time of demyelination since the migration path of these cells lies exactly in this direction. At the same time, the negative correlation between the number of OPC and the degree of lesion, as assessed by the size of the corpus callosum, indicates an intensification of the
regeneration processes with a more pronounced intoxication with cuprizone. The destruction of the OLG perikaryon and of the myelin sheath causes apoptosis of OLGs after several days of the exposure to cuprizone, and on the fourth week there is a massive apoptosis of OLGs under the influence of the immune system. By the sixth week, a process of remyelination is observed, with OPCs actively differentiating into OLGs [18-24].

According to our results, during the process of demyelination, the level of neurogenesis in both neurogenic zones decreases. According to publications, neurodegeneration at cuprizone intoxication can occur as a result of the disruption of the metabolic connection between neurons and astrocytes [25]. At that, the destruction and extensive degeneration of the perikaryon of the neuron in the hippocampus area can reach up to 50%, but they can quickly recover afterwards, during the subsequent 5-week remyelination [26]. Neurodegeneration should lead to more intensive recovery processes, i.e. to the enhancement of neurogenesis in the proliferative zones; however, we observed a reverse picture in our experiment. Since the administration of cuprizone leads primarily to a selective death of OLGs, and a significant part of OPCs originates from stem cells in neurogenic niches, it is likely that the reduction of neurogenesis during demyelination and its recovery during remyelination is associated with a shift in the differentiation of stem cells towards OPCs.

5. Conclusion
This study has shown that, with the administration of cuprizone, the amount of myelin in a range of structures of white and gray matter and the level of neurogenesis decrease, while the level of oligodendrogenesis increases. The withdrawal of cuprizone leads to the restoration of myelin content, to the reduction of the excessive production of oligodendrocytes, and to the restoration of the number of neurons to control values. The negative correlation between the number of OPCs and the degree of demyelination of the corpus callosum indicates migration of OPCs from the subventricular zone (SVZ) to the demyelinating structures.

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