

Effects of insulin-like growth factor 1 on pathologic processes in the cuprizone model of multiple sclerosis

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Abstract. The study aims to evaluate the effect of insulin-like growth factor 1 (IGF-1) on the demyelination and astrogliosis using the cuprizone murine model. Demyelination was induced in 14 adult male mice by 0.3% cuprizone in drinking water. Five animals from the cuprizone-treated group received subcutaneous injections of IGF-1. Seven animals were used as a control group. The extent of demyelination was evaluated as a decrease in the size of the corpus callosum on T2-weighted images that were received using an 11.7T animal MRI scanner. Brain sections were immunohistochemically stained for glial fibrillary acidic protein (GFAP), a marker of astrocytes. It was revealed that the cuprizone caused extensive demyelination and astrogliosis. IGF-1 treatment restored the size of the corpus callosum and the number of astrocytes in the corpus callosum and the anterior commissure to the control level.

1. Introduction

Multiple sclerosis (MS) is one of the most common demyelinating diseases. Drugs whose action is aimed to decrease demyelination and stimulate remyelination processes still remain mostly at the stage of experimental testing [1,2]. The cuprizone administration induces extensive myelin damage both in white and gray matter [3-5], which can be evaluated by MRI [6-8] and histologically [3-7]. Oligodendrocytes are a primary type of cells affected by cuprizone [3-5]. However, the other types of neural cells including neurons [2,9,10] and astrocytes [3,11,12] are also affected by cuprizone administration even in the absence of autoimmune processes, though to a lesser extent. Insulin-like growth factor 1 (IGF-1) is a perspective agent which can stimulate remyelination due to its influence on cell differentiation into oligodendrocytes [13]. The purpose of the research was to study the prospects of using IGF-1 for the reduction of the severity of pathological processes in the brain inflicted by the administration of cuprizone.

2. Materials and methods

The study involved three groups of male mice of C57Bl/6j line, 8 weeks of age, obtained from N.N. Vorozhtsov Novosibirsk Institute of Organic Chemistry (Novosibirsk): "Control" (N=7),



"Demyelination" (N=5) and "IGF-1" (N=7). Demyelination in the groups "Demyelination" and "IGF-1" was caused by a chronic oral administration of 0.3% aqueous solution of cuprizone for 53 days. The animals from the "IGF-1" group were injected with IGF-1 subcutaneously twice a week, and the animals from the other groups received injections of saline. On day 53, being under 1.5-2% isoflurane anesthesia, the animals underwent MRI scanning of the brain with the use of a BioSpec 117/16 USR ultra-high-angle tomograph (Bruker, Germany), which allowed obtaining T2-weighted images. The animals were then euthanized under ether anesthesia by transcardial perfusion with 4% paraformaldehyde. The brain was removed, and cryoprotection was performed in 10% and 20% solutions of sucrose, followed with freezing in liquid nitrogen vapor. For the assessment of astrogliosis, 10- μ m-thick sections of the brain were obtained on the cryotome HM525 (TermoScientific, Germany) in the area of +0.38 mm from bregma, and then they were stained immunohistochemically with antibodies with an affinity for glial fibrillary acid protein (GFAP) – an astrocyte marker. Photomicrographs of sections taken with the Axiolmager Z2 microscope (Carl Zeiss, Germany) and T2-weighted images were processed with ImageJ software. The corpus callosum was manually contoured on the T2-weighted images and its area was counted. In order to calculate the number of astrocytes, the areas of the corpus callosum of 200x200 μ m were selected, as well as the front commissures within the natural boundaries. Statistical processing was carried out using Statistica 8.0 software with the help of variance analysis and the Mann-Whitney test.

3. Results

The T2-weighted images showed a significant decrease in the corpus callosum size in the "Demyelination" group as compared to the control group. Statistical analysis revealed a significant decrease in the size of the corpus callosum caused by the administration of cuprizone as compared to the control group ($p < 0.001$). The administration of IGF-1 on the background of cuprizone intoxication restored the size of the corpus callosum to the control level ($p > 0.05$).

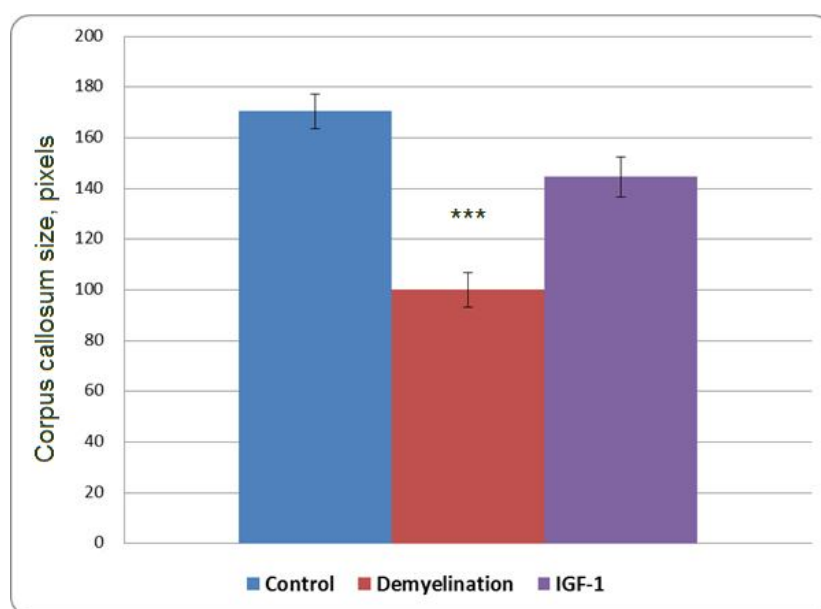


Figure 1. The size of the corpus callosum in the groups "Control", "Demyelination" and "IGF-1". The columns reflect the mean \pm standard error of the mean. The significant differences compared to the "Control" group: *** – $p < 0.001$.

In the studied structures of the brain of the animals from the "Demyelination" group, a large number of hypertrophied astrocytes with a large number of processes was found, which indicates a

pronounced intoxication with cuprizone. Fewer hypertrophied astrocytes were observed in the "IGF-1" group than in the "Demyelination" group. Statistical analysis revealed a significant increase in the number of astrocytes in the "Demyelination" group as compared to the control group both in commissures ($p < 0.01$) and in the corpus callosum ($p < 0.01$). At the same time, this indicator did not differ from the control level for the "IGF-1" group ($p > 0.05$).

It is known that IGF-1 has anti-inflammatory properties, and can increase the survival and proliferation of oligodendrocytes, contributing to remyelination [2]. Presumably, a decrease in the number of active astrocytes on the background of the administration of cuprizone, which causes astrogliosis, is associated with the neuroprotective and remyelinating effect of IGF-1.

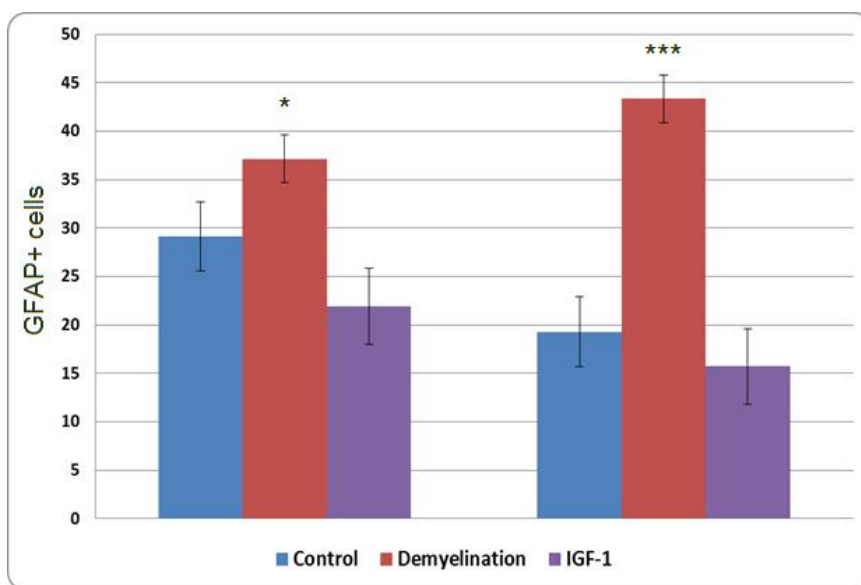


Figure 2. Differences in the number of astrocytes in the corpus callosum in the groups "Control", "Demyelination" and "IGF-1". The columns reflect the mean \pm standard error of the mean. The significance of differences in relation to the "Control" group is * – $p < 0.05$; *** – $p < 0.001$.

4. Conclusion

A chronic cuprizone intoxication caused demyelination in mice, which resulted in a decrease in the size of the corpus callosum, an increase in the number of astrocytes in the corpus callosum and anterior commissure, and a change in their morphology. The administration of IGF-1 on the background of cuprizone intoxication had a positive effect on demyelination, increasing the size of the corpus callosum to control values and reducing the astrogliosis caused by cuprizone in the corpus callosum and in anterior commissures.

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References

- [1] Virley D. 2005 Developing Therapeutics for the Treatment of Multiple Sclerosis *NeuroRx* **2**(4) 638–649
- [2] Khodanovich M, Glazacheva V, Pan E, Akulov A, Krutenkova E, Trusov V and Yarnykh V.. 2015 MRI study of the cuprizone-induced mouse model of multiple sclerosis: demyelination is not found after co-treatment with polyprenols (long-chain isoprenoid alcohols). *Journal of Physics Conference Series* **677**(2) UNSP 012007.
- [3] Kipp M, Clarner T, Dang J and Beyer C 2009 The cuprizone animal model: new insights into an old story *Acta Neuropathologica*. **6** 723
- [4] Torkildsen O, Brunborg LA, Myhr K-M and Bo L 2008 The cuprizone model for demyelination *Acta Neurol Scand* **11** 72.
- [5] Acs P, Kalman B 2012 Pathogenesis of multiple sclerosis: what can we learn from the cuprizone model. *Methods Mol Biol* **900** 403–431.
- [6] Wood T, Simmons C, Hurley S., Vernon A, Torres J, Dell'Acqua F., Williams S and Cash D. 2016 Whole-brain *ex-vivo* quantitative MRI of the cuprizone mouse. *PeerJ* **4** e2632.
- [7] Khodanovich M, Sorokina I, Glazacheva V, Akulov A, Nemirovich-Danchenko N, Romashchenko A, Tolstikova T, Mustafina L and Yarnykh V 2017 Histological validation of fast macromolecular proton fraction mapping as a quantitative myelin imaging method in the cuprizone demyelination model. *Sci Rep.* **7** 46686
- [8] Krutenkova E., Pan E., Khodanovich M. A 2015 Review of MRI Evaluation of Demyelination in Cuprizone Murine Model. *AIP Conference Proceedings* **1688** 030003.
- [9] Hillis J, Davies J, Mundim M, Al-Dalahmah O and Szele F. 2016 Cuprizone demyelination induces a unique inflammatory response in the subventricular zone. *Journal of Neuroinflammation* **13**(1) 190
- [10] Benetti F, Ventura M, Salmini B, Ceola S, Carbonera D, Mammi S, Zitolo A, D'Angelo P, Urso E, Maffia M, Salvato B, Spisni E 2010 Cuprizone neurotoxicity, copper deficiency and neurodegeneration *Neurotoxicology* **31** 509–517
- [11] Minagar A, Shapshak P, Fujimura R [et al.] 2002 The role of macrophage/microglia and astrocytes in the pathogenesis of three neurologic disorders: HIV-associated dementia, Alzheimer disease, and multiple sclerosis *Neurol. Sci* **202** 13–23.
- [12] Hatten M, Liem K, Shelanski M et al 1991 Astroglia in CNS injury *Glia* **4**(2) 233–243.
- [13] Hsieh J, Aimone J, Kaspar B [et al.] 2004 IGF-I instructs multipotent adult neural progenitor cells to become oligodendrocytes *J Cell Biol* **164** 111–122.