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MRI study of the cuprizone-induced mouse model of multiple sclerosis: demyelination is not found after co-treatment with polyprenols (long-chain isoprenoid alcohols)

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Abstract. Multiple sclerosis is a neurological disorder with poorly understood pathogenic mechanisms and a lack of effective therapies. Therefore, the search for new MS treatments remains very important. This study was performed on a commonly used cuprizone animal model of multiple sclerosis. It evaluated the effect of a plant-derived substance called Ropren® (containing approximately 95% polyprenols or long-chain isoprenoid alcohols) on cuprizone-induced demyelination. The study was performed on 27 eight-week old male CD-1 mice. To induce demyelination mice were fed 0.5% cuprizone in the standard diet for 10 weeks. Ropren® was administered in one daily intraperitoneal injection (12mg/kg), beginning on the 6th week of the experiment. On the 11th week, the corpus callosum in the brain was evaluated in all animals using magnetic resonance imaging with an 11.7 T animal scanner using T2-weighted sequence. Cuprizone treatment successfully induced the model of demyelination with a significant decrease in the size of the corpus callosum compared with the control group ($p<0.01$). Mice treated with both cuprizone and Ropren® did not exhibit demyelination in the corpus callosum ($p<0.01$). This shows the positive effect of polyprenols on cuprizone-induced demyelination in mice.

1. Introduction

Multiple sclerosis (MS) is a neurological disorder of high social impact with poorly understood pathogenic mechanisms and a lack of effective therapies. For this reason, the search for new MS treatments is highly important. This study was carried out on a commonly used animal model of MS that is produced by administration of the neurotoxic agent cuprizone (CPZ). CPZ causes oligodendrocyte death followed by demyelination.

The CPZ model is a toxicological model that allows the study of acute and chronic demyelination caused by a primary loss of oligodendrocytes followed by spontaneous remyelination in the absence of inflammation [1]. This model of acute and chronic demyelination and spontaneous remyelination is simple to induce. The differentiation of inflammation, macrophage infiltration and demyelination in

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MS lesions is important [2], and CPZ treatment allows the study of demyelination independent of inflammation. This model does not allow the study of autoimmune mechanisms of MS, but it is highly suitable to elucidate cellular and molecular mechanisms of demyelination and remyelination independent of peripheral immune system activity [3].

Ropren® is isolated from conifer needles and is mainly composed of polyprenols (long-chain isoprenoid alcohols) that have hepatoprotective and immunomodulatory effects, have the ability to restore function after liver damage and have efficacy as a treatment for patients with chronic alcoholism [4]. The functional activity of this substance is extensively studied but it is clear that it is of great interest for pharmacology [5]. Polyprenols and their derivatives are essential components of cellular membranes, affect the membrane transport of proteins or peptides, contribute to nervous impulse transmission, and are also involved in the biosynthesis of glycoproteins and cholesterol [6]. This suggests that polyprenols (long-chain isoprenoid alcohols) could have a positive effect on myelin loss in pathological conditions, such as MS, and this study examines the effect of Ropren® on CPZ-induced demyelination.

2. Methods

2.4 Animals and treatment

The rationale, design, methods and animal ethics for this study were approved by Ethics Committee of Biological Institute of Tomsk State University.

Twenty-seven eight-week-old male CD-1 mice were obtained from the vivarium of the Institute of Pharmacology of the Siberian Branch of the Russian Academy of Science for use in this study. Mice were housed at 21 ± 2 °C, humidity 40% ± 2%, 12/12 h light/dark cycle, food and water was provided ad libitum.

After 10 days of quarantine, the animals were divided into three groups: “Control”, “Demyelination”, and “Demyelination+Ropren”. The CPZ animal model of MS was induced as previously described [1, 7]. The “Demyelination” and “Demyelination+Ropren” groups were fed the standard chow diet containing 0.5% of CPZ (Bis(cyclohexanone)oxaldihydrazone, Sigma-Aldrich) for 10 weeks. The “Control” group was fed the standard chow diet for 10 weeks. The animals in the “Demyelination+Ropren” group were injected by Ropren® (Solagran Limited) at a dose of 12 mg\kg intraperitoneally once a day from the 6th week of CPZ treatment. The animals of the other groups were injected with the same volume of the vehicle (vegetable oil).

2.2 *Ropren® substance*

Ropren® substance (95% polyprenols or long-chain isoprenoid alcohols) was isolated from the green verdure of *Picea abies* (L.) Karst as previously described [8]. Ropren® was supplied by (Solagran Limited).

2.5 2.3 MRI aqusition

On the 11th week, all animals underwent brain MRI examination with an 11.7 T small animal imager (Bruker BioSpec 117/16USR) with horizontal core. Imaging was performed under isoflurane anesthesia (1.5%–2% in oxygen) with respiratory monitoring (SA Instruments, Stony Brook, NY, USA) during the scan. The mice were placed on a heated bed (30°C) in order to maintain normal body temperature. The animals were placed in the prone position and were then slid into the magnet bore on an animal bed.

T2 weighted volume acquisition RARE sequence was used for in vivo MRI acquisition (spin echo-based RARE sequence, TR = 3500 ms, TE = 33 ms, RARE factor = 8, matrix: 224×224×80, slice thickness of 0.5 mm).

2.6 Image processing

Image analysis was performed using ImageJ software. Regions-of-interest (ROIs) corresponding to the corpus callosum were outlined manually (Figure 1). The size of the corpus callosum was evaluated as a sum of the area in three adjacent cross-sections (−0.82 ÷ −0.94 mm from bregma according to [8]). Delineation of corpus callosum was performed using a blind method, without the operator knowing the treatment group to which the animals belonged.

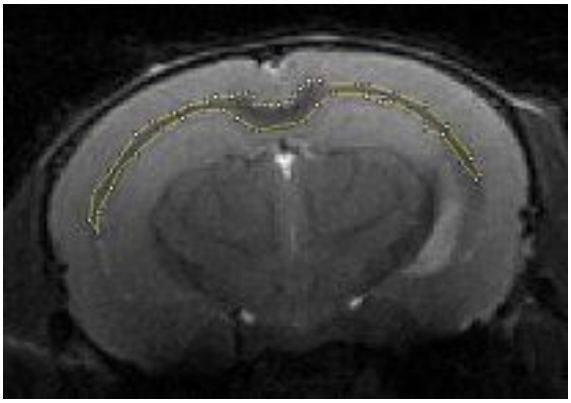

Figure 1. Delineation of the area of the corpus callosum using ImageJ software.

2.7 Statistical analysis

The significant differences between the groups were evaluated using a *t*-test for independent samples.

3 Results

Typical T2-weighted images of mice in the "Control", "Demyelination" and "Demyelination+Ropren" groups are shown on Figure 2. The images demonstrate a noticeable reduction of the corpus callosum in the mouse from the "Demyelination" group and the absence of visible signs of demyelination in the mouse from the control group and the mouse from the group treated with Ropren®.

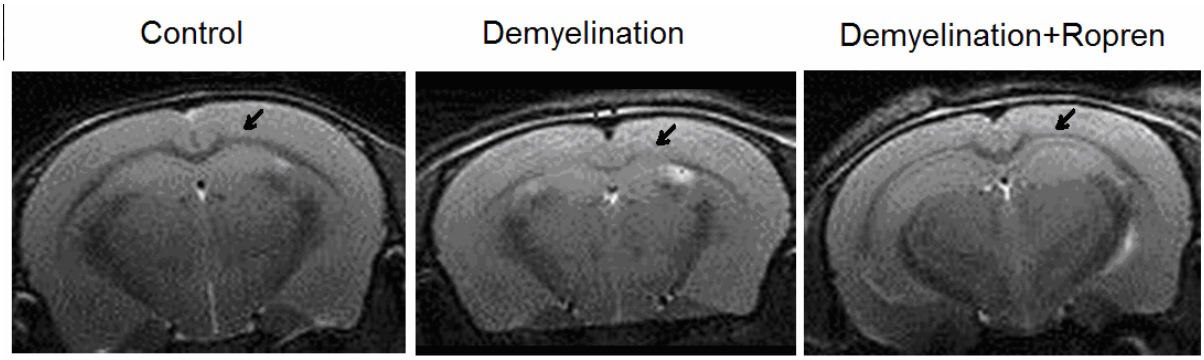

Figure 2. Typical examples of T2-weighted images of mice in the "Control", "Demyelination" and "Demyelination+Ropren" groups. The arrows indicate decreased size of the corpus callosum in the brain of the mouse from the "Demyelination" group and the normal size of the corpus callosum in mice from both the "Control" and "Demyelination+Ropren" groups.

Quantitative analysis showed that animals from the "Demyelination" group had a significantly smaller corpus callosum compared to the "Control" group ($p<0.01$) (Figure 3). CPZ administration reduced the corpus callosum by 19.5% on average. At the same time, mice treated with CPZ and

Ropren® have similar levels of demyelination of the corpus callosum to those seen in the control group, with no significant differences found between the "Control" and "Demyelination+Ropren" groups.

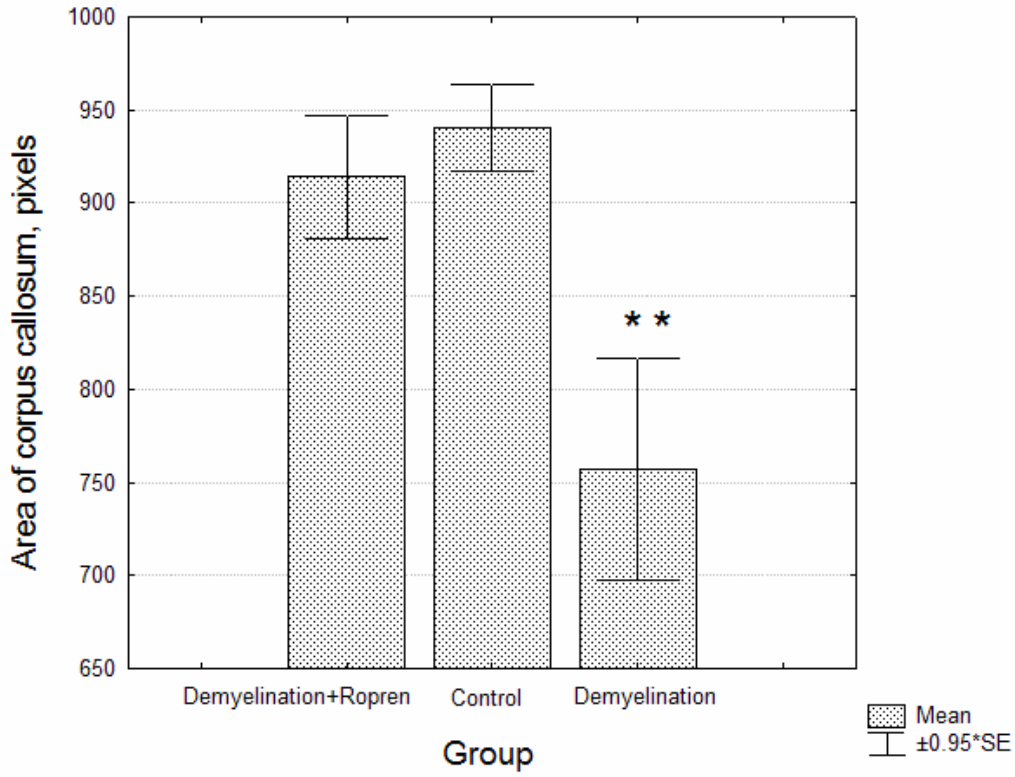

Figure 3. The significant decrease of the size of corpus callosum in cuprizone-treated mice compared to the control group. Mice treated with both Ropren® and cuprizone had similar levels of myelination of the corpus callosum as control animals.
** = $p<0.01$, *t*-test.

4 Discussion

The significant decrease of the size of the corpus callosum shows successful induction of the CPZ model of demyelination in CD-1 mice. The main finding is that after Ropren® injections, no damage of the corpus callosum was seen after 10 weeks of CPZ administration.

Long-chain polyisoprenoid alcohols, including polyprenols and dolichols, are a unique class of secondary metabolites within the isoprenoid natural product family [6]. Polyprenols and the phosphorylated derivatives comprise a small percentage of the total glycerophospholipid content in cellular membranes of bacteria (~1%) [9] and eukaryotes (~0.1%) [10].

Polyprenyl-phosphates act as oligosaccharide carriers during glycan biosynthesis, which is essential to many conserved cellular processes including N-linked protein glycosylation, C- and O-protein mannosylation, and bacterial cell wall biosynthesis. The production of dolichyl-phosphate is an important regulator of cell differentiation [11]. Inhibition of polyisoprenol biosynthesis resulted in abnormal gastrulation, which correlated with the inability of the cell to produce glycoproteins. Furthermore, addition of exogenous dolichol allowed for normal gastrulation, suggesting that dolichyl-phosphate is a limiting reagent for N-linked glycoprotein biosynthesis and subsequent cellular transformations [12]. The rate of dolichyl-phosphate and glycoprotein synthesis has also been linked to the growth rate of Chinese hamster ovary cells and cell division [13]. Other studies have confirmed

that dolichyl-phosphate is a rate limiting substrate in N-linked glycosylation and is thereby a key factor in cellular development [10, 14].

Polyprenols, as well as dolichols, contain polyisoprene units and also exhibit membrane-active properties affecting the viscosity and fluidity of membranes. Apparently, polyprenols could facilitate the membrane transport of proteins or peptides and contribute to nerve impulse transmission by binding glycoproteins with the polyisoprenoid fragment. Moreover, dolichol is involved in the biosynthesis of cholesterol that makes up one-third of the myelin sheath. Sterols and other lipids involved in their biosynthesis (dolichol) are the main agents for proteins passing through the membrane and are involved in the regulation of nerve impulse transduction [15]. Hartley et al. (2012) also suggests that dolichyl-phosphates may influence enzyme and pathway regulation, flippase-mediated glycan translocation across membranes, and macromolecular enzyme complex formation [6].

We suggest that Ropren®, with its composition of approximately 95% polyprenols, may either prevent myelin loss or restore myelin by several pathways. Polyprenols may serve as an exogenous source of isoprenoid chains that form endogenous metabolites, such as dolichol and cholesterol. Another pathway is the biosynthesis of glycoproteins, where phosphorylated derivatives of polyprenols are involved in the process of protein glycosylation and recognition of glycoprotein on the membrane, the recognition of antigens on the membrane, the fluidity and viscosity of membranes, the functioning of ion channels, and nerve impulse conduction, as well as an having an influence on regeneration through cell division, differentiation and development.

Polyprenols can accumulate at relatively high levels within many eukaryotic organisms; biosynthesis of these molecules is a complex, energy-dependent process and it seems unlikely that production of polyprenols would have been conserved in evolution if it did not serve an important role. Further work is necessary to establish the physical effects that polyprenols may exert on complex biological membranes and how polyprenol accumulation affects aging, disease, and intracellular transport processes [6].

5 Conclusions

CPZ treatment caused a significant reduction of corpus callosum associated with demyelination. Mice treated with Ropren® (the plant-derived substance containing 95% polyprenols) and CPZ showed a level of myelination of the corpus callosum similar to the level seen in control animals. The study suggests the positive impact of Ropren® on CPZ-induced demyelination and allow us to consider further testing of this substance as an effective treatment against MS. Future studies will also enable us to determine if Ropren® prevents demyelination or promotes re-myelination.

Acknowledgments

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