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Dynamics of myelin content decrease in the rat stroke model

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Abstract. The majority of studies were usually focused on neuronal death after brain ischemia; however, stroke affects all cell types including oligodendrocytes that form myelin sheath in the CNS. Our study is focused on the changes of myelin content in the ischemic core and neighbor structures in early terms (1, 3 and 10 days) after stroke. Stroke was modeled with middle cerebral artery occlusion (MCAo) in 15 male rats that were divided into three groups by time points after operation. Brain sections were histologically stained with Luxol Fast Blue (LFB) for myelin quantification. The significant demyelination was found in the ischemic core, corpus callosum, anterior commissure, whereas myelin content was increased in caudoputamen, internal capsule and piriform cortex compared with the contralateral hemisphere. The motor cortex showed a significant increase of myelin content on the 1st day and a significant decrease on the 3rd and 10th days after MCAo. These results suggest that stroke influences myelination not only in the ischemic core but also in distant structures.

1. Introduction

Vascular diseases of the brain rank second among the leading causes of death after ischemic heart disease [1]. Studies of ischemic stroke focus primarily on neuronal death and recovery [2-5]. However, this disease affects absolutely all types of brain cells, including oligodendrocytes that form myelin sheath [6-8], a component which is crucial for normal functioning of the nervous system. The purpose of this research was to study the change in the degree of myelination of various structures of white and gray matter after modeling local ischemia of the brain.

2. Materials and methods of the research

The experiment was performed on 15 mature male Sprague-Dawley rats weighing 250-270 g. The ischemic stroke model was created by a reversible middle cerebral artery occlusion (MCAo) [2]. A nylon filament with a silicone tip (Doccol, USA) was introduced into the left internal carotid artery, which thus blocked the bloodstream in the MCA. The ischemic episode began with the introduction of the filament to the MCA and lasted 1 hour, after which the filament was removed. The operation was performed under isoflurane anesthesia (1.5-2% isoflurane in oxygen). During the operation, the body temperature of the animal was maintained at 37±0.5°C. After the operation, the animals were



randomly divided into three experimental groups: 1 day, 3 days and 10 days. On the expiration of this period, the animals underwent transcatheter perfusion with 4% paraformaldehyde under ether anesthesia. The brain was removed, and cryoprotection was performed in 10% and 20% solutions of sucrose, followed with freezing in liquid nitrogen vapor.

In order to assess the myelination after modeling the ischemic stroke, 10- μ m-thick brain sections were made on cryotome HM525 (Termoscientific, Germany), which were then stained with Luxol Fast Blue (LFB) to detect myelin. Photomicrographs of sections were taken with the AxioImager Z2 microscope (Carl Zeiss, Germany) and processed with ImageJ software. For the purposes of the study, several structures of white matter (corpus callosum, cc; commissura anterior, ca; and internal capsula, ic) and gray matter (caudoputamen, cp; cortex dorsalis, cxd; cortex ventralis, cxv; and thalamus, thal) were identified, as well as the area of ischemic lesion (L). In each of those, regions of interest (ROI) were selected, within which the optical density (OD) of staining with LFB (normalized by background) was estimated [9]. The contralateral hemisphere was used as the control, the OD of which was taken as 100%. The statistical processing of data was carried out in STATISTICA 10 software, the significance of differences between the hemispheres was assessed using the χ^2 criterion, and the differences at $p < 0.05$ were taken as statistically significant.

3. Results

The results are presented in Figures 1 and 2. As expected, ischemia resulted in a decrease in myelin content in the area of lesion itself ($p < 0.001$) at all follow-up times, but the LFB OD in the cp zone adjacent to the area of lesion significantly increased during the 1st day after the stroke modeling.

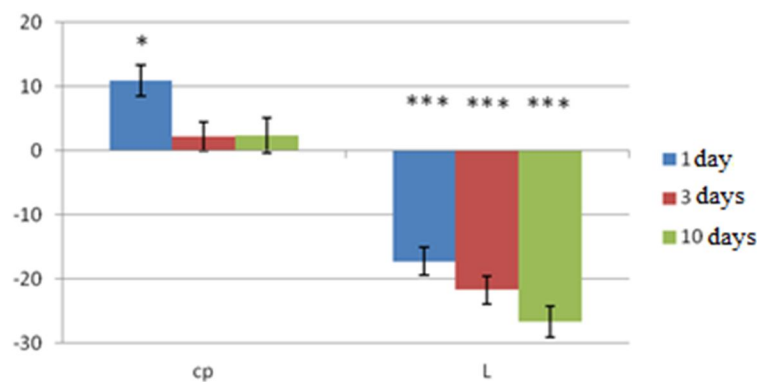


Figure 1. Changes in myelination in the area of ischemic lesion (L) and in caudoputamen of the affected hemisphere (cp) relative to the control hemisphere, expressed as a percentage. The mean \pm standard error of the mean. Significant differences between the hemispheres are * – $p < 0.05$, *** – $p < 0.001$.

Changes in myelination were also observed in the structures not directly affected by ischemia (Figure 2). Regarding the white matter structures, there was a significant decrease in myelin content in cc and ca observed during day 1, which persisted in cc on day 3. On day 10, there were no changes observed in these structures; however, in ic – the area most distant from the ischemic lesion – myelin content increased significantly (Figure 2).

Regarding the gray matter structures, with the exception of thal (the most remote from the zone of ischemic lesion of the structure), there were both an increase in myelination (cxd on day 1 $p < 0.05$; and cxv on days 1 and 3 after the surgery $p < 0.01$ and $p < 0.001$, respectively) and a decrease of this indicator relative to the control (cxd on days 3 and 10 $p < 0.01$ and $p < 0.05$, respectively) (Figure 2). Apparently, the changes in the degree of myelination of structures over time can be evidence of the

redistribution of myelin after ischemic exposure. Besides, in the structures of gray matter, the speed and/or extent of this redistribution can be more pronounced due to the greater water content.

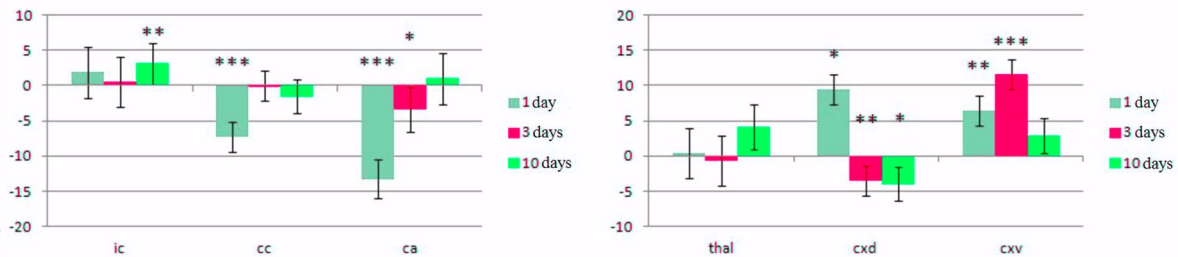


Figure 2. Changes in myelination of the structures of white (left) and gray (right) matter outside the area of ischemic lesion after modeling ischemic stroke relative to the control hemisphere, expressed as a percentage. Notation: internal capsule (ic), corpus callosum (cc), commissura anterior (ca), thalamus (thal), cortex dorsalis (cxd), cortex ventralis (cxv). The mean \pm standard error of the mean. Significant differences between the hemispheres are * - $p < 0.05$, ** - $p < 0.01$, *** - $p < 0.001$.

4. Conclusion

The results indicate that in various brain structures that do not even fall under the direct effect of ischemia, there are changes in the degree of myelination, the extent and direction of which depend on various factors, such as the remoteness from the source of ischemia and the initial degree of myelination (white or gray matter). Possible causes of this can be the density of matter in a particular structure, the percentage of water and other less obvious factors.

Acknowledgements

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