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BRAIN RESEARCH

Research Report

Regional differences between grey and white matter in cuprizone induced demyelination

Viktoria Gudi[a,b,1], Darius Moharregh-Khiabani[a,1], Thomas Skripuletz[a], Paraskevi N. Koutsoudaki[a,b], Alexandra Kotsiari[a], Jelena Skuljec[a,b], Corinna Trebst[a], Martin Stangel[a,b,*]

[a]Department of Neurology, Hannover Medical School, Hannover, Germany
[b]Center for Systems Neuroscience, Hannover, Germany

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ABSTRACT

Cuprizone feeding is a commonly used model to study experimental de- and remyelination, with the corpus callosum being the most frequently investigated white matter tract. We have previously shown that demyelination is also extensive in the cerebral cortex in the cuprizone model. In the current study, we have performed a detailed analysis of the dynamics of demyelination in the cortex in comparison to the corpus callosum. Prominent and almost complete demyelination in the corpus callosum was observed after 4.5–5 weeks of 0.2% cuprizone feeding, whereas complete cortical demyelination was only observed after 6 weeks of cuprizone feeding. Interestingly, remyelination in the corpus callosum occurred even before the termination of cuprizone administration. Accumulation of microglia in the corpus callosum started as early as week 3 reaching its maximum at week 4.5 and was still significantly elevated at week 6 of cuprizone treatment. Within the cortex only a few scattered activated microglial cells were found. Furthermore, the intensity of astrogliosis, accumulation of oligodendrocyte progenitor cells and nestin positive cells differed between the two areas investigated. The time course and dynamics of demyelination differ in the corpus callosum and in the cortex, suggesting different underlying pathomechanisms.

1. Introduction

Multiple sclerosis (MS) is a chronic immune-mediated disease of the central nervous system (CNS), characterised by demyelinating white matter lesions, glial scar formation, and axonal loss. In recent years studies have shown that demyelination and neuronal damage also affect the cerebral cortex. The underlying pathophysiological mechanisms seem to differ between white and grey matter since cortical demyelinating lesions are associated with an intact blood brain barrier, alleviated infiltration of lymphocytes and mild astrogliosis (Bo et al., 2003; van Horssen et al., 2007). Furthermore, the remyelinating capacity of grey matter lesions seemed to be higher compared to white matter lesions (Albert et al., 2007).

However, the detailed pathomechanisms of grey and white matter demyelination and subsequent remyelination are still only poorly understood. To address these questions animal models like the commonly used murine cuprizone model are valuable. In this model, young adult mice are fed with the copper chelator cuprizone (bis-cyclohexanone oxaldihydra-

* *Corresponding author*. Department of Neurology, Hannover Medical School, 30625 Hannover, Germany. Fax: +49 511 532 3115.
E-mail address: stangel.martin@mh-hannover.de (M. Stangel).
[1] Both authors contributed equally.

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zone) leading to reproducible demyelination of the corpus callosum within weeks (Matsushima and Morell, 2001; Torkildsen et al., 2008). Previously, we have shown that cuprizone administration also causes demyelination of the cerebral cortex with subsequent remyelination after withdrawal of the toxin (Skripuletz et al., 2008).

In order to understand the mechanisms of white and grey matter demyelination we compared the dynamics of cuprizone induced demyelination and glial reactions in the cortex and the corpus callosum in detail.

2. Results

2.1. *De- and remyelination follow a different pattern in the cortex and corpus callosum*

To determine the sequential loss and re-expression of myelin proteins (PLP, MBP, CNPase and MOG) during cuprizone treatment histochemical and immunohistochemical stainings were performed. As depicted in Figs. 1B and C, PLP immunoreactivity in the cortex continuously decreased and reached the minimum at week 6 of the cuprizone treatment. The time course for MBP was nearly the same, except for week 6, where in some animals small amount of MBP was visible in the cortex (data not shown). As previously described (Skripuletz et al., 2008), LFB staining was not sensitive enough to reveal cortical demyelination. The low content of the myelin proteins MOG (2% of all myelin proteins) and CNPase (4% of all myelin proteins) made an exact analysis of the cortical myelination pattern difficult and was therefore not performed. The dynamics of myelin protein expression were quite different in the corpus callosum. As previously described there was remyelination despite ongoing cuprizone administration (Lindner et al., 2008). After 3 weeks of cuprizone feeding the expression of all four myelin proteins was slightly reduced in the corpus callosum (Fig. 1D). Maximal degradation of MBP and CNPase was detectable after 4.5 weeks of cuprizone exposure. At week 5 of cuprizone feeding re-expression of MBP and CNPase was evident and further increased after 5.5 and 6 weeks of treatment. Maximal loss of the myelin proteins MOG and PLP was observed after 5 weeks of cuprizone feeding followed by re-expression at weeks 5.5 and 6. The different temporal patterns for degradation and re-expression of MBP and PLP myelin proteins during cuprizone treatment are shown in Fig. 1E on the MBP/PLP double stained sections of the corpus callosum. For LFB the peak of demyelination was observed after 4.5 weeks of cuprizone treatment.

2.2. *Grey and white matter show a different density of oligodendrocytes and oligodendrocyte precursor cells (OPCs)*

To follow the oligodendroglial cellular response we used Nogo-A as a marker for mature oligodendrocytes and NG2 for OPCs. In control mice, Nogo-A positive cells were found in high numbers in the white matter structures, e.g. corpus callosum (Fig. 2A1). In the cortex of control animals the density of oligodendrocytes was remarkably lower. Nogo-A positive cells were preferentially located in the cellular layers 5 and 6. Upon cuprizone treatment the amount of oligodendrocytes was strongly reduced in both grey and white matter structures (Figs. 2A2–A7) with a maximum reduction at 3 and 4 weeks of cuprizone feeding (Figs. 2C, D, $p<0.0001$ for both). Thereafter, oligodendrocytes began to reappear in both areas.

OPCs were found widely distributed in all brain structures including corpus callosum and cortex in control animals (Figs. 2B1, E, F). These cells had a stellate shape with long, branched processes (Figs. 2I, J). In the cortex of control animals the density of OPCs was lower as compared to the corpus callosum. Upon cuprizone treatment the number of NG2 positive cells increased and reached the peak after 4.5 weeks ($p<0.0001$) (Figs. 2B1–B7; C) followed by a decrease. At week 6 of cuprizone feeding the amount of OPCs was still elevated in the cortex in comparison to controls. In the corpus callosum the number of NG2 positive cells increased significantly after 4 weeks of cuprizone treatment ($p<0.0001$) and reached their maximum at 4.5 weeks, followed by a marked decrease (Figs. 2B1–B7; D). However, at week 6 of cuprizone feeding the amount of OPCs in the corpus callosum was still elevated as compared to controls (Fig. 2G).

Thus, the dynamics of oligodendroglial cells are comparable between corpus callosum and cortex despite the obvious difference in myelination.

2.3. *Accumulation of microglia in the cortex is diminished in comparison to the corpus callosum*

Accumulation of activated microglia was studied by mac-3 staining. In untreated control animals activated microglia were absent (Fig. 3A1). In the corpus callosum the amount of mac-3 positive cells increased significantly after 3 weeks of cuprizone treatment, and reached a peak at week 4.5

Fig. 1 – Comparison of demyelination in the corpus callosum and cortex. (A left) Schematic diagram of the mouse brain in coronal section. The blue line shows the investigated middle part of corpus callosum. The red line marks the area of the cortex analysed with the different layers shown in A right. (B) Comparison of demyelination in the corpus callosum and cortex as judged by scoring of PLP. Score of 0 represents complete myelin protein loss, score of 4 represents normal myelin protein amount in the cortex. For corpus callosum: score of 0 represents complete loss of myelin protein, score of 3 normal amount of investigated myelin protein. (C) PLP-stained sections representing peak demyelination in the corpus callosum at week 5 and in the cortex with the peak at week 6 of cuprizone administration. (D) Time course for the expression of myelin proteins PLP, MBP, MOG, and CNPase as well as for myelin stained by LFB-PAS in the corpus callosum. Score of 0 represents complete myelin loss, score of 3 represents normal myelin amount. (E) MBP/PLP double (MBP in red, PLP in green) stained sections of the corpus callosum. Degradation of MBP occurred after 4 and 4.5 weeks of cuprizone treatment, followed by a recovery phase at week 5. Note, PLP degradation occurred late at week 5 with re-expression at week 5.5 of cuprizone diet.