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Research report

Quantitative analysis of lipid debris accumulation caused by cuprizone induced myelin degradation in different CNS areas

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ABSTRACT

Degradation of myelin sheath is thought to be the cause of neurodegenerative diseases, such as multiple sclerosis (MS), but definitive agreement on the mechanism of how myelin is lost is currently lacking. Autoimmune initiation of MS has been recently questioned by proposing that the immune response is a consequence of oligodendrocyte degeneration. To study the process of myelin breakdown, we induced demyelination with cuprizone and applied coherent anti-Stokes Raman scattering (CARS) microscopy, a non-destructive label-free method to image lipid structures in living tissue. We confirmed earlier results showing a brain region dependent myelin destructive effect of cuprizone. In addition, high resolution *in situ* CARS imaging revealed myelin debris forming lipid droplets alongwith myelinated axon fibers. Quantification of lipid debris with custom-made software for segmentation and three dimensional reconstruction revealed brain region dependent accumulation of lipid drops inversely correlated with the thickness of myelin sheaths. Finally, we confirmed that *in situ* CARS imaging is applicable to living human brain tissue in brain slices derived from a patient. Thus, CARS microscopy is potent tool for quantitative monitoring of myelin degradation in unprecedented spatiotemporal resolution during oligodendrocyte damage. We think that the accumulation of lipid drops around degrading myelin might be instrumental in triggering subsequent inflammatory processes.

1. Introduction

Multiple sclerosis (MS) is a disease of oligodendrocyte and myelin sheath affecting both the white and gray matter with diverse symptoms such as tremor, fatigue and paralysis (Hemmer et al., 2002; Trapp and Nave, 2008). In most cases the symptoms occur in a relapsing-remitting manner which usually devolves into a persistent progressive state after a decade following onset. MS is caused by demyelination and inflammation of axonal tracts. Since myelin acts as an insulator, injury of myelin sheaths wrapped around axons of neurons in the CNS it causes malfunction by impairing conduction of electric signals (Bando et al., 2008; Hamada and Kole, 2015). Moreover, the loss of myelin cause axonal (Huizinga et al., 2012; Kuhlmann et al., 2002; Lindner et al., 2009) and finally neuronal degeneration (Centonze et al., 2010).

The precise pathological mechanisms involved in MS are subject to considerable debate. Commonly MS is defined as an autoimmune disease initiated by an immune system dysregulation leading autoreactive T cells to enter the brain, induce microglia and macrophages to attack and erode myelin which finally causes the injury of oligondendrocytes (Frohman et al., 2006; Kornek and Lassmann, 2003; Stadelmann et al., 2011). This theory is corroborated by the fact that current effective treatments of MS are based on anti-inflammatory and immunomodulatory agents. Immune dysregulation is also supported by the results of genome-wide association (GWAS) studies showing an excessive number of genes influencing T-cell differentiation (Sawcer et al., 2011). However, mechanisms of MS initiation are still

Abbreviations: BODIPY, boron-dipyrromethene; CA, anterior commissure; CARS, coherent anti-Stokes Raman scattering; CB, cerebellum; CC, corpus callosum; CNS, central nervous system; FM, forceps minor of corpus callosum; GM, grey matter; MAG, myelin-associated glycoproteins; MOG, myelin–oligodendrocyte glycoproteins; EAE, experimental autoimmune encephalomyelitis; GWAS, genome-wide association studies

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