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Research Report

Oxidative injury in multiple sclerosis cerebellar grey matter

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ABSTRACT

Cerebellar dysfunction is a significant contributor to disability in multiple sclerosis (MS). Both white matter (WM) and grey matter (GM) injury occurs within MS cerebellum and, within GM, demyelination, inflammatory cell infiltration and neuronal injury contribute to on-going pathology. The precise nature of cerebellar GM injury is, however, unknown. Oxidative stress pathways with ultimate lipid peroxidation and cell membrane injury occur extensively in MS and the purpose of this study was to investigate these processes in MS cerebellar GM. Post-mortem human cerebellar GM from MS and control subjects was analysed immunohistochemically, followed by semi-quantitative analysis of markers of cellular injury, lipid peroxidation and anti-oxidant enzyme expression. We have shown evidence for reduction in myelin and neuronal markers in MS GM, coupled to an increase in expression of a microglial marker. We also show that the lipid peroxidation product 4-hydroxynonenal co-localises with myelin and its levels negatively correlate to myelin basic protein levels. Furthermore, superoxide dismutase (SOD1 and 2) enzymes, localised within cerebellar neurons, are up-regulated, yet the activation of subsequent enzymes responsible for the detoxification of hydrogen peroxide, catalase and glutathione peroxidase are relatively deficient. These studies provide evidence for oxidative injury in MS cerebellar GM and further help define disease mechanisms within the MS brain.

1. Introduction

The cerebellum is a major site for tissue injury in multiple sclerosis (MS), particularly in patients with progressive disease (Calabrese et al., 2010; Kutzelnigg et al., 2007; Redondo et al., 2014). Indeed, cerebellar dysfunction in MS is a significant contributor to disability, commonly progressing regardless of treatment with disease-modifying agents (Waxman, 2005). Cerebellar dysfunction in MS is thought to arise due to a combination of white matter (WM) and grey matter (GM) injury, similar to that which occurs elsewhere in the cerebral cortex and underlying white matter tracts. The role of GM injury in MS has received less attention than WM injury, yet pathological studies of MS GM have revealed evidence for extensive demyelination, inflammation and neuronal loss (Kutzelnigg et al., 2005; Lucchinetti et al., 2011; Mahad et al., 2008). Furthermore, cortical atrophy on MRI scans is a well-recognised feature of established progressive disease (Steenwijk et al., 2016). Understanding the causes of MS GM injury in the cerebellum may help design therapies to reduce injury in this part of the brain.

A major role for reactive oxygen species (ROS) in the pathophysiology of MS and central nervous system (CNS) inflammatory disorders has been demonstrated (Cross et al., 1998; Smith et al., 1999; van Horssen et al., 2011). An imbalance in cellular redox homeostasis, leading to oxidative stress, may be caused by a large number of biological mechanisms resulting in the overproduction of ROS (Murphy, 2009). Changes leading to high concentrations of ROS have the potential to cause tissue damage and cell death within the CNS (Haider et al., 2011). Increases in ROS also trigger the formation of toxic molecules, such as lipid peroxidation products (Keller and Mattson, 1998), which themselves are strong reactive electrophiles capable of perpetuating oxidative stress (Abarikwu et al., 2012; Matveychuk et al., 2011). Experimentally, ROS and their reactive products cause cellular injury to neurons (and their axons) and oligodendrocytes (Abarikwu et al., 2012; French et al., 2009; Li et al., 2005; Wilkins and Compston, 2005). In both pathological studies and animal models of CNS inflammation, ROS play a key role in promoting tissue damage (Cross et al., 1998; Haider et al., 2011; Smith et al., 1999; van Horssen et al., 2008).

There is a complex cellular interplay between oxidative injury and anti-oxidant defences, and cells possess a diverse array of

Abbreviations: MS, Multiple sclerosis; GM, grey matter; WM, White matter; SOD, superoxide dismutase; ROS, reactive oxygen species; CNS, central nervous system; GPX, glutathione peroxidase; CAT, catalase; CSF, cerebral spinal fluid; MAL, Malondialdehyde; 4-HNE, 4-hydroxynonenal; MBP, myelin basic protein; HLA, human leucocyte antigen

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mechanisms to reduce ROS that build up during normal physiological processes. These include anti-oxidant enzymes such as superoxide dismutase (SOD), catalase, glutathione peroxidase (GPX) and glutathione reductase. Failure of endogenous cell protection mechanisms that operate early in the disease course is postulated to be a major reason for on-going tissue damage in MS (van Horssen et al., 2011). Specifically, imbalances in the levels of oxidative stress molecules and anti-oxidant defences may be important in this respect, and precise identification of any imbalances may allow for the development of targeted therapeutic interventions which may help crucially tip the balance between cell death and survival. Indeed, a number of neurological conditions including amyotrophic lateral sclerosis, X-linked adrenoleucodystrophy and adrenomyeloneuropathy all highlight the important balance between oxidative stress and anti-oxidant defences (Moser et al., 2007; Rosen, 1993).

To date, studies have shown alterations in the levels of specific endogenous anti-oxidant enzymes and markers of oxidative stress in serum, cerebrospinal fluid (CSF) and brain tissue derived from patients with MS (Calabrese et al., 2002; Lund-Olesen, 2000; Tajouri et al., 2003; van Horssen et al., 2008). However, a clear understanding of precise mechanisms of tissue injury in MS is lacking, particularly in key pathological sites such as the cerebellum. In this study we have studied oxidative injury and the expression of anti-oxidant molecules in MS cerebellar grey matter.

2. Results

2.1. Antibody specificity

All primary antibodies used for immuno dot-blotting were tested for their specificity against their chosen antigens using western blotting techniques. Under the experimental conditions used, all antibodies displayed specific bands as described on manufacturer data sheets and/or relative to their reported molecular weights and were therefore considered suitable for use in immuno dot-blotting techniques (Figs. 2(f), 3(e) and 5(c)).

2.2. Characterisation of cerebellar grey matter

Regions of demyelination (determined by myelin basic protein (MBP) staining) were seen in cerebellar grey matter (GM) tissue samples. Demyelination was typically seen exclusively within GM cerebellar above and below the layer of the Purkinje cells (supra- and infra-ganglionic layer) extending into the granular layer (cortical; Fig. 1(a)); or extending from white matter into the granular layer (leucocortical). As previously reported using the same patient cohort, all MS cases (and no control cases) showed areas of cortical and leucocortical demyelination within the grey matter. 29.4% (+/− 11.5 SEM) of the cerebellar cortex was demyelinated, representing 13.7% (+/− 8.5 SEM) with leucocortical demyelination and 15.7% (+/−7.2 SEM) with purely cortical demyelination (Redondo et al., 2014). Control sections immunolabelled for the macrophage/microglial marker human leucocyte antigen (HLA)-DP, DQ, DR, showed few positive cells in the

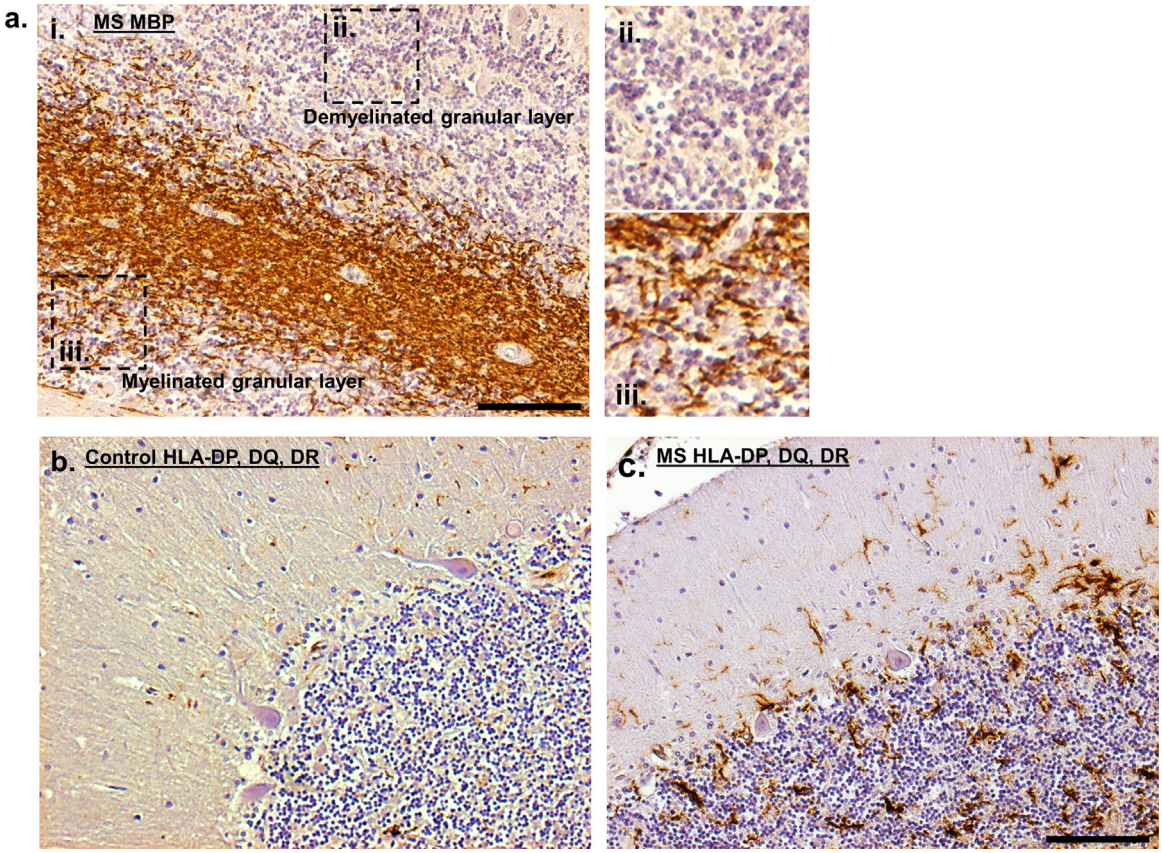

Fig. 1. Demyelination and microglial infiltration in MS and control cerebellar grey matter. (a) MS section showing DAB (brown) labelling of myelin basic protein (MBP) demonstrating demyelination within the granular layer of MS cerebellum. The hatched areas in (i) represent the higher magnified images (ii/iii); (b) Control and (c) MS sections DAB (brown) immunolabelled with HLA-DP, DQ, DR showing an influx of microglial cells spread throughout the cerebellar grey matter in MS brain. (Scale bar = 100 μm).