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Research Report

Increased microglial catalase activity in multiple sclerosis grey matter

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ABSTRACT

Chronic demyelination, on-going inflammation, axonal loss and grey matter neuronal injury are likely pathological processes that contribute to disease progression in multiple sclerosis (MS). Although the precise contribution of each process and their aetiological substrates is not fully known, recent evidence has implicated oxidative damage as a major cause of tissue injury in MS. The degree of tissue injury caused by oxidative molecules, such as reactive oxygen species (ROS), is balanced by endogenous anti-oxidant enzymes which detoxify ROS. Understanding endogenous mechanisms which protect the brain against oxidative injury in MS is important, since enhancing anti-oxidant responses is a major therapeutic strategy for preventing irreversible tissue injury in the disease. Our aims were to determine expression and activity levels of the hydrogen peroxide-reducing enzyme catalase in MS grey matter (GM). In MS GM, a catalase enzyme activity was elevated compared to control GM. We measured catalase protein expression by immune dot-blotting and catalase mRNA by a real-time polymerase chain reaction (RT-PCR). Protein analysis studies showed a strong positive correlation between catalase and microglial marker IBA-1 in MS GM. In addition, calibration of catalase mRNA level with reference to the microglial-specific transcript AIF-1 revealed an increase in this transcript in MS. This was reflected by the extent of HLA-DR immunolabeling in MS GM which was significantly elevated compared to control GM. Collectively, these observations provide evidence that microglial catalase activity is elevated in MS grey matter and may be an important endogenous anti-oxidant defence mechanism in MS.

1. Introduction

Multiple sclerosis (MS) is an inflammatory disease of the central nervous system characterised by significant levels of oxidative stress. Although classically described as a white matter (WM) disease, in recent years the importance of grey matter (GM) injury in MS has been emphasised as a likely substrate for some of the more 'cortical' features of MS, such as cognitive dysfunction (Calabrese et al., 2009). Cortical atrophy on MRI scans is a well-recognised feature of

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established progressive disease. Pathological studies of MS GM have revealed evidence for demyelination, inflammation and neuronal loss (Kutzelnigg et al., 2005, Lucchinetti et al., 2011). The precise relationship between inflammation and GM neuronal loss is not clear, although studies have revealed higher levels of neuronal injury in areas of GM with greater lesion activity characterised by inflammatory cell infiltrates (Mahad et al., 2008).

A major mechanism by which inflammatory cells produce tissue injury is through oxidative stress pathways. Oxidative molecules such as nitric oxide, superoxide, hydrogen peroxide and peroxynitrite are released from inflammatory cells as part of their physiological function to protect against invading pathogens. However, reactive oxygen species (ROS) may also cause damage to endogenous DNA, RNA and cellular proteins and, if present in high enough concentrations, can cause cell death (Haider et al., 2011). Experimentally, ROS cause cellular injury to neurons (and their axons) and oligodendrocytes (Li et al., 2005; Wilkins and Compston, 2005). In MS, there is now almost overwhelming evidence implicating activation and generation of ROS as a major cause of tissue injury (Cross et al., 1998; Smith et al., 1999; van Horssen et al., 2011). Oxidative stress is kept in check by a number of endogenous anti-oxidant enzymes and it is likely that the balance of oxidative stress and anti-oxidant response mechanisms may be crucial in determining the degree of tissue injury. Of particular interest is catalase, which catalyses the detoxification of hydrogen peroxide (itself formed as a by-product of superoxide dismutase activity on superoxide ions).

Endogenous anti-oxidant molecules are expressed widely and there is a complex interplay between oxidative injury and endogenous anti-oxidant defence mechanisms which determines the degree of cellular injury induced by ROS. In MS, oxidative damage coincides with enhanced anti-oxidant enzyme expression, but the precise pattern and cellular origin of anti-oxidant enzyme expression are not completely clear (van Horssen et al., 2008). In this study we focused on determining activity and expression pattern of the major hydrogen peroxide reducing enzyme catalase within MS grey matter. Understanding endogenous anti-oxidant molecule expression may lead to enhanced therapeutic strategies for the disease.

2. Results

2.1. *Catalase activity is significantly increased in MS grey matter*

As an assessment of anti-oxidant capacity, we measured activity of the peroxisomal enzyme catalase. Catalase activity was measured in homogenates prepared from MS ($n=25$) and control ($n=14$) grey matter. Post-mortem delay was significantly greater in the control (mean=21.05 h, SE=1.957, median=22) than the MS group (mean=16.59 h, SE=0.5816, median=16; $P<0.05$). However, within two groups (MS and control GM) there was no significant relationship between catalase activity and post-mortem delay ($P=0.25$). Regression analysis of the contributions to catalase activity of the presence/absence of MS indicated a strong positive association between MS and levels of catalase ($P<0.0001$; Fig. 1). Analysis of samples based on the presence or absence of demyelination within tissue blocks (determined by MBP labelling) revealed no difference in catalase activity dependent on the presence of demyelination (demyelinated block: mean catalase activity 12.92 nmol/min/mg total protein ±0.75 SEM; non-demyelinated block: mean catalase activity 12.63 nmol/min/mg total protein ±0.91 SEM; $P=0.34$)

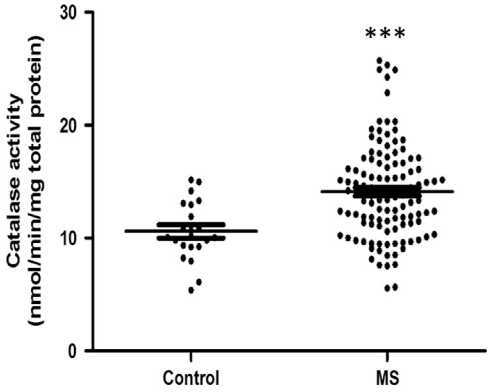

Fig. 1 – Catalase activity in multiple sclerosis (MS) grey matter and control grey matter. The mean values ± SE are shown for samples from 25 cases of multiple sclerosis grey matter and 14 controls. Catalase activity is significantly increased in multiple sclerosis grey matter ($P<0.0001$).

2.2. *Catalase protein levels positively correlate with IBA-1 protein levels in MS grey matter*

Levels of catalase protein were evaluated in grey matter via immune-dot blotting. Protein levels of glyceraldehyde 3-phosphate dehydrogenase (GAPDH, as a pan-cellular protein), neuron specific enolase (NSE, as a neuronally expressed protein) and an ionised calcium-binding adapter molecule-1 (IBA1, which is expressed in activated microglia) were also measured. Levels of catalase, NSE and IBA-1 were expressed relative to GAPDH expression levels from the tissue sample to account for any cell loss. We analysed the data to determine if there was a correlation between the neuron-specific marker (NSE) and the catalase expression or between the microglia-specific marker (IBA-1) and the catalase expression. No correlation existed between NSE (controlled to GAPDH expression) protein levels and catalase (controlled to GAPDH expression) protein levels (Fig. 2A). However, there was a strong positive correlation between IBA-1 (controlled to GAPDH expression) protein levels and catalase (controlled to GAPDH expression) protein levels in homogenates examined (Fig. 2B).

2.3. *Catalase gene expression is increased in MS grey matter relative to AIF-1 expression*

We analysed catalase gene expression relative to the expression of a number of genes. In order to determine whether there were cell-type specific gene transcription changes we expressed catalase RT-PCR values relative to cell-specific