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Magnetic resonance imaging characterization of different experimental autoimmune encephalomyelitis models and the therapeutic effect of glatiramer acetate

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ABSTRACT

The roles of inflammation and degeneration as well as of gray matter abnormalities in multiple sclerosis (MS) and its animal model experimental autoimmune encephalomyelitis (EAE) are controversial. We analyzed the pathological manifestations in two EAE models, the chronic oligodendrocyte glycoprotein (MOG)-induced versus the relapsing–remitting proteolipid protein (PLP)-induced, along the disease progression, using advanced magnetic resonance imaging (MRI) parameters. The emphasis of this study was the overall assessment of the whole brain by histogram analysis, as well as the detection of specific affected regions by voxel based analysis (VBA) using quantitative T2, magnetization transfer ratio (MTR) and diffusion tensor imaging (DTI). Brains of EAE-inflicted mice from both models revealed multiple white and gray matter areas with significant changes from naïve mice for all MRI parameters. Ventricle swelling was more characteristic to the PLP-induced model. Decreased MTR values and increased apparent diffusion coefficient (ADC) were observed mainly in MOG-induced EAE, indicative of macromolecular loss and structural CNS damage involvement in the chronic disease. The MS drug glatiramer acetate (GA), applied either as prevention or therapeutic treatment, affected all the MRI pathological manifestations, resulting in reduced T2 values and ventricle volume, elevated MTR and decreased ADC, in comparison to untreated EAE-inflicted mice. In accord, immunohistochemical analysis indicated less histological damage and higher amount of proliferating oligodendrocyte progenitor cells after GA treatment. The higher brain tissue integrity reflected by the MRI parameters on the level of the whole brain and in specific regions supports the in situ anti-inflammatory and neuroprotective consequences of GA treatment.

Introduction

Multiple sclerosis (MS) is a chronic disease of the central nervous system (CNS) in which pro-inflammatory processes that target self myelin constituents lead to multifocal demyelination (Lassmann, 2008; Lassmann et al., 2007). In addition, increasing evidence indicates that the axonal and neuronal pathology, which is crucial to the impairment associated with the progressive disease course, begins at early disease stage (Bruck, 2008; Trapp and Nave, 2008). Diffuse abnormalities in the gray matter and in normal-appearing brain tissue are currently recognized as central components of MS (Geurts and Barkhof, 2008). Yet, the relative roles of the diverse pathological manifestations in this multifaceted disease are highly controversial. In this respect, deeper investigation of the various experimental autoimmune encephalomyelitis (EAE) models, induced by exposing susceptible mice strains to different myelin antigens and which mirror different aspects of MS, may lead to better understanding. Indeed, we have recently demonstrated that the proteolipid protein (PLP)-induced relapsing–remitting model is characterized mainly by widespread myelin damage, whereas in the chronic model induced by oligodendrocyte glycoprotein (MOG), axonal degeneration and neuronal loss are more prevalent (Aharoni et al., 2011).

MS diagnosis and assessment has progressed dramatically by the application of magnetic resonance imaging (MRI). At present, MRI is an essential tool for treatment management and evaluation of therapeutic impact (Polman et al., 2011). In addition, MRI methodologies are employed to investigate various EAE models in order to study the detrimental processes occurring within the CNS and their relevance to the human disease, as they allow direct correlation of radiological

Abbreviations: MS, Multiple sclerosis; EAE, Experimental autoimmune encephalomyelitis; GA, Glatiramer acetate; MOG, Oligodendrocyte glycoprotein; PLP, Proteolipid protein; CNS, Central nervous system; MTR, Magnetization transfer ratio; DTI, Diffusion tensor imaging; VBA, Voxel based analysis.

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and histopathological findings. Advance MRI parameters that are sensitive to the microstructural changes occurring in the tissue can serve as biomarkers for different aspects of the disease pathology (Vigeveno et al., 2012).

Quantitative T2, which is sensitive to water content and tissue composition, has the potential to reflect pathological changes such as edema and myelin damage (MacKay et al., 2006). Increased T2-weighted signals were demonstrated in the corpus callosum of rats inflicted by MOG-induced EAE, which is manifested by focal lesions (Serres et al., 2009), and in a mouse model combining cuprizone-induced demyelination and MOG induced EAE (Boretius et al., 2011).

Magnetization transfer ratio (MTR) — the ratio with and without magnetization transfer, is considered a measure of the macromolecular structure and myelin water fraction, both potential measures of the myelin sheath integrity (Dousset et al., 1992; Whittall et al., 1997; Wolff and Balaban, 1989). Studies using the focal MOG-induced EAE in rats revealed correspondence between reduced MTR values and demyelination within the lesions (Rausch et al., 2009; Serres et al., 2009). Reduction in MTR was found also in the corpus callosum in the cuprizone–MOG combined mouse model (Boretius et al., 2011), and in normal appearing white matter in brains of chronic-progressive EAE-induced guinea pig (Gareau et al., 2000).

Diffusion tensor imaging (DTI), which measures the magnitude and directionality of water diffusion in the tissue, serves as an indicator for axonal integrity. Apparent diffusion coefficient (ADC) indicates the average diffusion in the tissue (Basser and Pierpaoli, 1998). Positive correlation between clinical scores and ADC values in the external capsule (Verhoye et al., 1996), as well as in the corpus callosum (Serres et al., 2009), were demonstrated in the rat brain using chronic-relapsing and focal EAE models, respectively.

Both MTR and DTI have been investigated as measures of demyelination, axonal damage and myelin integrity (Budde et al., 2008, 2009; DeBoy et al., 2007; Filippi and Agosta, 2009; Serres et al., 2009; Vigeveno et al., 2012). As such they are linked to the neurodegenerative component of MS and complement the established MRI readouts of inflammation. The current study was prompted by the notion that further usage of these MRI measurements to study the whole brain, as well as various specific regions in different EAE models and the effect induced by treatment, may contribute to the elucidation of in situ pathological and therapeutic mechanisms.

Glatiramer acetate (GA, Copaxone®), a synthetic polypeptide of L-alanine, L-lysine, L-glutamic acid and L-tyrosine (Teitelbaum et al., 1971), is an approved MS drug which is widely used as first-line disease-modifying therapy (Aharoni, 2010; Carter and Keating, 2010). In MS patients, GA treatment has been shown to modify various MRI parameters that indicate disease activity and severity. These include the reduction in mean number of gadolinium-enhancing lesions, the number of new enhancing lesions, the volume of enhancing lesions and the change in the volume and number of lesions on T2-weighted images (Carter and Keating, 2010; Comi et al., 2001; Sormani et al., 2005). The mechanism of action of GA was extensively studied in several EAE models. These studies attributed the therapeutic activity of GA to immunomodulation, mainly by the induction of anti-inflammatory Th2/3 and T-regulatory cells that penetrate the CNS and induce in situ bystander suppression (Aharoni et al., 2000, 2003, 2010; Farina et al., 2005). During recent years, cumulative results indicated that, in addition to its immunomodulatory activity, GA induces neuroprotective and repair processes within the CNS, as manifested by the decrease in neurological damage and demyelination as well as by increased expression of neurotrophic factors, neurogenesis and remyelination (Aharoni et al., 2005a, 2005b, 2008, 2011; Azoulay et al., 2005).

In the present study we used these advanced MRI methodologies, namely quantitative T2, MTR and DTI, to investigate the two widely used MS models: the PLP-induced relapsing–remitting EAE and the chronic EAE form induced by MOG. The various manifestations revealed at different time points during disease course, as well as following treatment by GA, were analyzed both for the whole brain by histogram analysis and for specific areas indicated by voxel based analysis (VBA). We report herewith on differences in the MRI parameters characteristic to each EAE model and a beneficial effect of GA treatment in their restoration.

Materials and methods

Mice

C57BL/6 and (SJL/JxBALB/c)F1 mice were purchased from Harlan (Jerusalem, Israel). Female mice, 8–12 weeks of age, were used and kept under specific pathogen free (SPF) environment. All experiments were approved by the Institutional Animal Care and Use Committee of the Weizmann Institute.

Induction and evaluation of EAE

Chronic EAE was induced in C57BL/6 mice by injecting a peptide consisting of amino acids 35–55 of myelin oligodendrocyte glycoprotein (MOG), synthesis by Genscript (Piscataway, NJ, USA). Relapsing–remitting EAE was induced in (SJL/JxBALB/c)F1 mice by the peptide encompassing amino acids 139–151 of proteolipid protein (PLP) synthesized by Genscript. Mice were injected subcutaneously at the flank, with 200 μl emulsion containing 200–300 μg of the encephalitogenic peptide in incomplete Freund's adjuvant enriched with 3 mg/ml heat-inactivated *Mycobacterium tuberculosis* (Sigma, St. Louis, MO, USA). Pertussis toxin (Sigma), 250 μg/mouse, was injected intravenously immediately after the encephalitogenic injection and 48 h later. Mice were examined daily. EAE was scored as follows: 0—no disease, 1—limp tail, 2—hind limb paralysis, 3—paralysis of all limbs, 4—moribund condition, and 5—death.

Glatiramer acetate (GA, Copaxone, Copolymer 1)

GA consists of acetate salts of synthetic polypeptides containing four amino acids L-alanine, L-glutamate, L-lysine, and L-tyrosine (Teitelbaum et al., 1971). GA from batch 242905809, with an average molecular weight of 7.7 kDa, obtained from Teva Pharmaceutical Industries (Petah Tiqva, Israel) was used throughout the study. GA treatment was applied by consecutive 7–8 daily subcutaneous injections, in 0.1 ml phosphate buffered saline, either as a prevention treatment starting one day following disease induction, or as a suppression treatment beginning after the appearance of clinical manifestations. In the first experiment GA doses of 0.1, 0.5 and 2.0 mg per mouse were tested. In subsequent experiments the dose of 2 mg/mouse was used. Layouts of the GA treatment schedules are demonstrated in panel A of Figs. 3–5. Mice that were not treated by GA (untreated control) were similarly injected by PBS.

Magnetic resonance imaging (MRI)

Brain MRI measurements were performed using 9.4 Tesla BioSpec Magnet, at three time points along disease progression (TP1, TP2, TP3), namely at days 13, 20, 27 and days 11, 17, 27 for the MOG and the PLP models, respectively, 5–9 mice per group. During imaging, mice were anesthetized with 2–2.5% isofluorane and were placed in a 35 mm diameter birdcage coil. An MR compatible small animal monitoring system was used to monitor respiratory rate and body temperature of the anesthetized mouse was maintained during imaging.

The MRI protocol included the following sequences:

Quantitative T2: T2 multi-slice multi-echo (MSME) sequence was performed with sixteen echoes collected at intervals of 10 ms from TE = 10 ms to TE = 160 ms, with TR = 3000 ms and with NA = 2, FOV 22 mm^2, 14 slices, slice thickness of 1 mm and a

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