Magnetisation transfer ratio in optic neuritis is associated with axonal loss, but not with demyelination

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A B S T R A C T

Background: Pathophysiological basis of Magnetisation Transfer Ratio (MTR) reduction in multiple sclerosis still remains a matter of controversy. Optic nerve represents an ideal model to study the consequences of axonal loss and demyelination on MTR since effects of disease on the optic nerve are clinically apparent and potentially quantifiable by objective means. By measuring the latency of multifocal visual evoked potentials (mfVEP) (measure of optic nerve conduction) and Retinal Nerve Fiber Layer (RNFL) thickness (measure of axonal damage) we investigated the effect of neurodegeneration and demyelination on MTR after an episode of optic neuritis (ON).

Methods: 23 patients with a single unilateral episode of ON and 10 healthy volunteers were enrolled. Orbital MRI including MTR protocol, Optical Coherence Tomography and Multifocal VEP were performed at post-acute stage of ON.

Results: Average MTR of affected eye was significantly reduced as compared to the fellow eye and normal controls. There was a highly significant correlation between MTR and measures of axonal loss (RNFL thickness and mfVEP amplitude), which was independent on the level of demyelination. While latency delay also correlated significantly with MTR, correlation became non-significant when adjusted for the degree of axonal loss. There was a significant reduction of MTR in a group of patients with extensive axonal damage, while MTR remained normal in a group of patients with extensive demyelination, but little or no axonal loss.

Conclusion: Results of this study indicate that reduction of optic nerve MTR after an episode of ON has a strong association with the degree of axonal damage, but not with demyelination.

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Introduction

Multiple sclerosis (MS) is a chronic inflammatory neurodegenerative disease of the central nervous system (CNS). It is a leading cause of non traumatic neurological disability in young adults in developed countries. Approximately 1 million people worldwide suffer from the disease (Weinshenker, 1991). While MRI is proven to be a very sensitive tool in confirming the diagnosis of multiple sclerosis (MS) and monitoring of treatment trials, it is pathologically non specific. Although MRI provides some insight into temporal characteristic of MS lesions (i.e. gadolinium-enhanced T1 imaging (Katz et al., 1993)), it cannot distinguish between oedema, inflammation, demyelination and axonal loss (Blumhardt et al., 1977; Zivadinov and Cox, 2007).

It has been suggested that non-conventional MRI techniques such as Magnetisation Transfer Ratio (MTR) may provide a more specific characterisation of underlying pathological processes. MTR imaging is a measure of the exchange of protons between free water and macromolecules in membranes. It is believed to be affected by either dilution of protons caused by oedema or loss of tissue structure, in particular structure of myelin. While animal research has demonstrated a strong relationship between the level of myelination and MTR (Deloire-Grassin et al., 2000; Zaaraoui et al., 2008), the situation is more complicated in human studies which are primarily based on post-mortem examination. Some studies have found a strong correlation between MTR reduction and the level of demyelination (Barkhof et al., 2003; Deloire-Grassin et al., 2000; Douset et al., 1992; Schmierer et al., 2004), while others demonstrated correlation between MTR with axonal density (Gass et al., 1994; van Waesbergh et al., 1999) or both (Mottershead et al., 2003). As new methods for precise tracking of MTR changes in MS brains are now becoming available (Chen et al., 2008; Dwyer et al., 2009) it is important to verify the true nature of its underlying pathophysiology.

The optic nerve represents an ideal model to study MS due to the fact that it subserves a single class of functions which are easily identifiable and measurable in vivo. Thus axonal loss in post-acute period can be assessed by measuring the thinning of RNFL, which is
caused by retrograde degeneration subsequent to inflammatory-mediated axonal transection (Costello et al., 2006; Frohman et al., 2008; Trip et al., 2005) and amplitude of the VEP, while latency of the VEP provides a measure of demyelination. Conduction block in acute optic neuritis normally recovers within a few weeks, during which inflammation subsides, ion channels are reconstructed and conduction resumes, although often in a slower, continuous mode (Smith and Waxman, 2005). Therefore, the degree of conductivity delay through the lesion, as detected by the VEP latency and found in a high proportion of patients with optic neuritis, is most likely a manifestation of the extent of inflammatory demyelination (Davies et al., 1998; Halliday et al., 1972; McDonald, 1977). This direct association between latency delay and degree of optic nerve myelination was recently confirmed using an animal model (Martin et al., 2006).

While a number of studies have use optic nerve inflammation in an attempt to clarify the relationship of MTR with demyelination and axonal loss, this still remains uncertain. In some studies MTR was shown to correlate with a presumed measure of demyelination (VEP latency) and not correlate with a measure of axonal loss (visual acuity or VEP amplitude)(Hickman et al., 2004; Thorpe et al., 1995); in others the opposite results were obtained(Inglese et al., 2002; Melzi et al., 2007). This may relate to the heterogeneous nature of the pathological changes after optic neuritis, which at different times may result in varying degrees of axonal loss and demyelination or a combination of both.

In an attempt to resolve the problem of heterogeneity seen in previous studies, the aim of current investigation was to examine the relationship between MTR and each of the pathological conditions (axonal loss and demyelination) by minimising the effect of each factor on another one. This was achieved by separating optic neuritis patients into two groups: one with significant axonal loss and another with no axonal loss, but extensive chronic demyelination using Optical Coherence Tomography and visual evoked potentials.

Methods

Subjects

Twenty three consecutive patients who had suffered a single clinical episode of ON and no previous clinical demyelinating events were recruited specifically for this study. ON was diagnosed by a neuro-ophthalmologist based on clinical findings. Exclusion criteria were atypical presentation, bilateral and/or recurrent ON and a history of other ocular or neurological diseases. No other data from these patients presented in other publications.

Ten age-matched healthy volunteers were also examined (as controls) using MTR protocol. The eligibility criteria for control subjects included 6/6 vision in both eyes (visual acuity was recorded using Snellen charts) and normal ophthalmic and neurological examination. One eye of each normal subject was selected randomly for analysis. To assess MTR test–retest variability 5 normal subjects were imaged twice.

Procedures followed the tenets of the Declaration of Helsinki. Ethics approval was obtained from the University of Sydney Ethics Committee. Written informed consent was obtained from all participants.

It has been previously reported that acute inflammation and oedema may affect the level of MTR (Gareau et al., 2000; Melzi et al., 2007; Odrobina et al., 2005). Therefore, only patients with a minimum of 6 months after acute ON were enrolled.

In the setting of multiple sclerosis demyelination can occur without axonal loss whereas axonal loss is typically accompanied by demyelination. Therefore, to investigate the separate effects of demyelination and axonal loss on MTR, patients were sub-divided into two groups based on degree of axonal loss. Patients with RNFL thickness asymmetry between affected and fellow eyes exceeding 95% confidence interval of RNFL thickness asymmetry in normal popula-

MfVEP recording and analysis

Multifocal mVEP testing was performed using the Accupmap™ (ObjectVision Pty. Ltd., Sydney, Australia) employing standard stimulus conditions described in detail elsewhere (Klistorner and Graham, 2001). Briefly, the stimulus consisted of a cortically scaled dartboard pattern of 58 segments (eccentricity up to 24°). Each segment contained a 4 × 4 grid of black (1.1 candela per square metre) and white (146 candela per square metre) checks (Michelson contrast 99%), which reversed patterns according to a binary pseudorandom sequence.

The visual stimulus was generated on a 21 in. display. All recordings were performed monocularly. Four gold cup electrodes (Grass, RI, USA) were used for bipolar recording with 2 electrodes placed 4 cm on either side of the inion, one electrode 2.5 cm above and one 4.5 cm below the inion in the midline. Electrical signals were recorded along 4 channels: as the difference between superior and inferior; left and right, and obliquely between horizontal and inferior electrodes. Visual evoked responses were amplified 1 × 10⁴ times and band-pass filtered 1 to 20 Hz.

Opera™ software correlated the pattern reversal binary sequence with the electrical signals recorded and a response for each segment was obtained. The largest peak-trough amplitude within the interval of 70–210 ms was determined for each channel. For amplitude analysis, the wave of maximal amplitude among the four channels was automatically selected by the software to create a combined topographic map (Fig. 1B) (Graham and Klistorner, 1999). Latency analysis was performed as follows: there were four traces (four channels) for each eye recorded for every individual segment. Amplitude of the traces from all four channels of both eyes from a single segment of the visual field was analysed as described above and the amplitude of the largest wave was recorded. The second peak of the largest wave was automatically determined for latency measurement by a specially designed algorithm. The same channel and the same peak (minimum or maximum) were then used for latency analysis for that particular segment in the other eye. Values of both amplitude and latency, which were used for the final analysis were calculated by averaging the amplitude and latency of individual sectors.

Optical Coherence Tomography

OCT was performed using OCT-3 (Stratus™, version 3.0, Carl Zeiss, Dublin, CA) The Fast RNFL protocol, consisting of three circular scans with diameters of 3.4 mm centred on the optic disc, was used to acquire the data. Pupils were not dilated. The OCT scan was considered acceptable if signal strength score was 7 or more. Mean total RNFL thickness was assessed. Average RNFL thickness was analysed.

MTR technique

MRI was performed on a Philips 3-T imager (TR 4.97/TE 2.45 echo train 35 nsa 4, recon matrix 268/189 and TFE factor 35). Volume obtained had sub-millimetre (0.9 mm) resolution with coverage from the mid part of the globe to the chiasm 20 slices were taken and MTR was calculated on every alternate optic nerve segment (2 mm thick)
using on-resonance frequency (Hickman et al., 2004; Melzi et al., 2007; Papanikolaou et al., 2002).

Region of interest (ROI) was plotted with a software driven oval and signal intensity was measured at the same point in the coronal plane through the length of optic nerve at a gap of 2 mm. ROI dimensions varied between 3 and 1.5 mm² depending on the size of optic nerve.

The segment of the optic nerve immediately behind the globe and the one through the course of optic foramen was completely excluded. MTR was calculated as follows (Hickman et al., 2004):

$$100 \times \frac{M_0 - M_s}{M_0};$$

where $M_s$ and $M_0$ represent signal intensities with and without the saturation pulse.

Statistical analysis

Statistical analysis was performed using SPSS 11.0 for Windows (SPSS, Chicago, IL, USA). Mean amplitude and latency were compared between groups using one-way ANOVA and Tukey post-hoc test. Pearson’s correlation and linear regression analysis were used to examine the relationships between various parameters. Partial correlations were used to control the effect of possible confounding factors. Significance was assessed at the $p < 0.05$ level.

To reduce between-subject variability, inter-eye asymmetry (difference between affected and fellow eyes), rather than absolute values of amplitude and latency mfVEP, RNFL thickness and MTR were used for correlation.

For comparison of averaged MTR the eye in the normal control group was selected randomly.

Result

Demographic data is presented in Table 1.

In normal controls MTR inter-subject coefficient of variability was 14.3% and intra-subject coefficient of variability was 6.7%.

Average MTR of affected eye of ON patients was significantly reduced as compared to the fellow eye and normal controls (19.9 (5.6), 23.6 (4.9)μ and 25.9 (3.1)μ respectively ($p = 0.005$, one-way ANOVA). Post-hoc analysis revealed a significant difference between MTR of affected and fellow eyes ($p = 0.033$) and between MTR of affected and normal control eyes ($p = 0.009$). Fellow eye MTR was not significantly different from normal control MTR ($p = 0.4$).

There was a highly significant correlation between inter-eye asymmetry values of MTR and RNFL thickness ($r = 0.71$, $p = 0.0001$) when the entire patient cohort was analysed (Fig. 1A). Correlation remained significant when controlled for mfVEP latency (partial $r = 0.54$, $p = 0.019$).

Similar correlation was also found between MTR and mfVEP amplitude ($r = 0.73$, $p = 0.0001$) (Fig. 1B). Again, correlation remained significant when controlled for mfVEP latency (partial $r = 0.49$, $p = 0.04$).

Correlation of MTR with latency of mfVEP, while not as strong as the former two, also reached a significant level ($r = 0.46$, $p = 0.042$) (Fig. 1C). When, however, regression was controlled by RNFL thickness or mfVEP amplitude, correlation lost significance (partial $r = 0.34$, $p = 0.15$ and partial $r = 0.25$, $p = 0.32$ respectively).

“Axonal loss” and “non-axonal loss” sub-group analysis

Average RNFL thickness asymmetry in “axonal loss” and “non-axonal loss” groups were 29.4 (5.9)μ and 9.3 (5.8)μ respectively. Average latency asymmetry in the “non-axonal loss” group was 20.2 (8.7)ms indicating considerable degree of demyelination. Age and gender distribution were similar between groups.

There was a significant difference in MTR values between “axonal loss” and “non-axonal loss” groups ($p < 0.001$, One-way ANOVA) (Table 2). Post hoc analysis (Tukey t-test), however, demonstrated that only the affected eye in the “axonal loss” group was significantly different from the rest ($p < 0.01$ for all). When compared to normal

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<tr>
<th>Table 1</th>
<th>Demographic data. Mean (SD).</th>
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<tbody>
<tr>
<td></td>
<td>ON patients</td>
</tr>
<tr>
<td>Number of subjects</td>
<td>23</td>
</tr>
<tr>
<td>Male/female ratio</td>
<td>7/16</td>
</tr>
<tr>
<td>Age</td>
<td>36.9 (9.3)</td>
</tr>
<tr>
<td>Visual acuity:</td>
<td></td>
</tr>
<tr>
<td>6/6 or better</td>
<td>10</td>
</tr>
<tr>
<td>6/12–6/6</td>
<td>9</td>
</tr>
<tr>
<td>&lt;6/12</td>
<td>4</td>
</tr>
<tr>
<td>Time since onset of ON</td>
<td>16.2 (12.7) months</td>
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</tbody>
</table>
controls, again only the affected eye in the “axonal loss” group was significantly different ($p<0.001$).

The range of latency asymmetry delay in “non-axonal loss” sub-group was considerable (10–36 ms) indicating a wide spectrum of demyelination. To investigate the effects of the degree of demyelination on MTR (while excluding the confounding effect of the axonal loss), MTR asymmetry in this sub-group was correlated with mfVEP latency asymmetry. There was no correlation between the two parameters ($r = 0.09$, $p = 0.5$) (Fig. 2).

The observation that MTR is not directly related to the degree of demyelination, but rather reflects the level of axonal damage is exemplified by the following individual cases.

Fig. 3A shows an example of the mfVEP of a patient with extensive latency delay (average asymmetry 36 ms), but little axonal loss (preserved VEP amplitude and minimal RNFL asymmetry) in the affected (right) eye. This patient demonstrated no MTR asymmetry. On the other hand, the patient shown in Fig. 3B demonstrates dramatic axonal loss (practically absent VEP amplitude and large RNFL asymmetry (41 μ) which was associated with significant reduction of MTR in the ON eye.

**Discussion**

Combined MRI and histopathological studies in animals and human postmortem MS studies have demonstrated correlations in MTR with inflammation, axonal loss, gliosis and demyelination but it is difficult to separate these components in the brain. Although assessment of optic neuritis is a good model to clarify this, previous studies have led to conflicting results.

Thorpe et al. (1995) in a study of 20 patients with optic neuritis observed a reduction of MTR in affected eyes, which correlated significantly with full-field VEP latency (demyelination), but not with visual acuity (axonal loss). Whereas in the study of Inglese et al. (2002) there was a good correlation of MTR with visual acuity ($r = 0.49$), but not with VEP latency ($r = -0.10$).

A longitudinal study of MTR in 21 patients with optic neuritis by Hickman et al. (2004) used amplitude and latency of full-field VEP to access a possible role of MTR as a marker of remyelination or axonal loss. It demonstrated MTR decline over time with a nadir at about 240 days and subsequent (although not significant) increase. There was no correlation between any of the MTR variables and logMAR visual acuity or visual field mean deviation. There was also no significant direct linear relationship between MTR and electrophysiological measurements; although there was evidence that patients with higher time-averaged MTR values had shorter time-averaged VEP latencies.

A reduction of MTR in ON eyes with good visual recovery was reported by Melzi et al. (2007). While the authors did not find correlation of MTR reduction with any of the VEP parameters they showed delayed latency and reduction of optic nerve volume at the end of a 12 months follow up period which may indicate combined effect of both demyelination and axonal loss.

The recent study by Trip et al. (2007) demonstrated a significant correlation between the level of optic nerve MTR with both axonal loss (RNFL thinning) and demyelination (VEP latency delay). The authors admitted, however, that since axonal loss following optic neuritis inevitably results in loss of myelin, they were unable to estimate relative contribution of the two pathological processes. Therefore, none of the studies were able to delineate with a high degree of certainty the relationship between axonal loss, demyelination and MTR in optic neuritis. The disparity may be a result of the heterogeneous nature of the group with varying levels of demyelination and axonal loss in an individual subject confounding the group analysis. Furthermore only full field visual evoked potentials were used which may result in the misinterpretation of P100 latency in the presence of a scotoma (Klistorner et al., 2008b).

In the current study we demonstrated that both axonal loss, as measured by RNFL thickness and mfVEP amplitude, and demyelination, as measured by mfVEP latency, correlate significantly with the degree of MTR reduction, which is in agreement with the most recent study on the subject (Trip et al., 2007). However, since new techniques such as OCT and mfVEP permit assessment of the level of axonal loss and demyelination in optic nerve with a high degree of precision, it is possible now to study the effects of demyelination and axonal loss on MTR in isolation. We recently demonstrated that the degree of remyelination in optic nerve has limited time-window and is independent of the level of initial myelin loss and, therefore, severe inflammation may result in residual (chronic) demyelination, which is not necessary accompanied by extensive axonal loss (Klistorner et al., 2010). Therefore, we were able to minimise the confounding effect of axonal loss in addition to demyelination. This is achieved in two ways: first by adjusting for degree of demyelination (latency delay) when analysing the relationship between axonal loss and MTR or adjusting for the degree of axonal loss (mfVEP amplitude reduction and RNFL thinning) when analysing the relationship between demyelination and MTR and secondly, by the separate analysis of cases with predominant axonal loss or predominant demyelination. To the best of our knowledge, this type of analysis has not been performed before.

Our results indicate that the degree of demyelination, as assessed by latency delay, does not relate to the level of MTR reduction in cases without significant axonal damage. The dominant role of axonal loss in optic nerve MTR reduction is evidenced by the finding of a significant partial correlation of MTR asymmetry with RNFL thickness and amplitude of mfVEP in the patient’s entire cohort after adjustment for latency delay. However, when the confounding factor of severe structural damage was removed, correlation between the level of demyelination and reduction of MTR was no longer apparent.

This becomes even more apparent by separating patients into groups based on the degree of axonal loss. Thus, analysis of the “non-axonal loss” group (the group with significant degree of latency delay, which is indicative of demyelination) demonstrates no average MTR asymmetry and no correlation between the degree of latency delay and MTR. Contrary to that, the group with severe axonal loss shows significant MTR reduction.

Overall our result, while limited by a small sample size and averaged rather than lesional MTR analysis, demonstrates a strong

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<th>ON eye</th>
<th>Fellow eye</th>
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<tr>
<td>Whole patient group</td>
<td>19.9 (5.6)</td>
<td>23.6 (4.9)</td>
</tr>
<tr>
<td>“Axonal loss” group</td>
<td>16.7 (3.3)</td>
<td>23.2 (3.6)</td>
</tr>
<tr>
<td>“Non-axonal loss” group</td>
<td>24.0 (5.3)</td>
<td>24.1 (6.3)</td>
</tr>
<tr>
<td>Normal controls</td>
<td>25.8 (3.7)</td>
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</table>

**Fig. 2.** Correlation between MTR asymmetry and mfVEP latency asymmetry in “demyelination” group.
relationship between MTR and axonal degeneration, but not demyelination in optic nerves of patients with previous episode of ON. As such it is questionable whether MTR is capable of clarifying structural changes occurring in individual MS lesions and confidently demonstrating remyelination. This is important when considering its use in clinical trials of remyelinating therapies.

**Conclusion**

Results of this study indicate that the reduction of optic nerve MTR after an episode of ON has a strong association with the degree of axonal damage, but not with demyelination.

**Acknowledgment**

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**References**
