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Abstract

The magnetization transfer ratio reflects the integrity of tissue structure, including myelination and axonal density. Mean magnetization transfer ratio fell in 18 untreated patients with multiple sclerosis both in normal appearing grey (-0.25 pu/year, $p < 0.001$) and white matter (-0.12 pu/year, $p = 0.004$). Conversely, mean magnetization transfer ratio was stable in 20 alemtuzumab-treated patients (grey matter: -0.01 pu/year, $p = 0.87$; white matter: -0.02 pu/year, $p = 0.51$). The gradient difference in grey matter was 0.25 pu/year ($p < 0.001$) after age-adjustment. These data suggest that in multiple sclerosis alemtuzumab protects against tissue damage in normal-appearing grey matter, perhaps by preventing new lesion formation.

Keywords

magnetization transfer, multiple sclerosis, alemtuzumab

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Introduction

Alemtuzumab is a humanized monoclonal antibody; phase III trials of its use in multiple sclerosis are complete, awaiting publication. In a phase II trial (CAMMS223), alemtuzumab significantly reduced the risk of relapse and sustained accumulation of disability compared with interferon- β 1a.¹

Alemtuzumab depletes lymphocytes, which reconstitute, a process prone to autoimmunity but forming an altered immune system that effectively combats infection. The new lymphocyte pool has a predominance of T cells with a regulatory phenotype ($CD4^+CD25^{hi}FoxP3^+$)² and may, in vitro, secrete neuro-protective factors.³

The magnetization transfer ratio (MTR) is an MRI-derived measure that probes the proton-pool bound to macromolecules, thus providing information on tissue structural integrity. In an 8-year follow-up study, baseline grey matter MTR and change in lesion MTR over the first year were associated with the subsequent development of disability.⁴ Post mortem, reduced MTR correlates with reduction in myelin content and axonal density.⁵

We investigated potential mechanisms of disability prevention after alemtuzumab by comparing the MTR changes seen with those in a natural history cohort studied using the same scanner and MT sequence.

Patients and methods

Patients

All patients had early relapsing–remitting MS and were imaged at the Institute of Neurology, University College London. Alemtuzumab-treated patients were participants in the CAMMS223 trial (ClinicalTrials.gov identifier: NCT00050778). A blinded neurologist determined their Expanded Disability Status Score (EDSS) every 3 months and they were imaged annually for 3 years, between 2003 and 2006. The natural history controls were identified from a longitudinal cohort observational imaging study (subjects who had not received disease-modifying treatment); EDSS was assessed every 6 months and imaging performed

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annually for 2 years, between 1998 and 2004. For both groups all patients completing the imaging protocol at the Institute of Neurology were included.⁶

Imaging

Imaging used a 1.5 Tesla Signa (General Electric, Milwaukee, USA); the 2D interleaved dual spin echo MT imaging sequence performed has been described previously.⁷ MTR was calculated on a voxel-by-voxel basis according to $\{(M_0 - M_S)/M_0\} \times 100$ where M_S and M_0 represent the signal intensities with and without the MT pulse. The scanner was upgraded in April 2004 resulting in a small increase in subsequent MTR measurements; statistical analysis was adjusted to correct for this.

Image post-processing

T₂-weighted lesions were defined on proton density images, with reference to the T₂-weighted images, using a semi-automated contouring method implemented with JIM 5.0_21 (Xinapse Systems, UK). After masking of lesions, grey and white matter were segmented using the T₂-weighted images in SPM8 (Wellcome Department of Cognitive Neurology, Institute of Neurology, London). Partial volume voxels were minimized through the use of a stringent threshold of probability for tissue classification

(70%). Mean MTR values were generated for normal-appearing grey and white matter and lesions. The same investigator identified lesions for all participants.

Statistical methods

Rates of change of MTR were estimated using linear mixed models which regress the MTR values over time with random subject intercepts and slopes; by including group and group \times time interaction terms, differences in gradient between the patient groups were estimated and tested, as well as the within-group gradient estimates. By adding an indicator for data points observed after the scanner upgrade, all estimates were adjusted for a small change attributable to it. Potential confounding of differences between groups was examined by adding age, gender and disease duration covariate terms and interactions with time. Analyses were carried out in Stata 11.2 (Stata Corporation, USA). The difference in change in lesion volume was compared using the Mann-Whitney *U*-test in PASW Statistics 18.0 (IBM, USA).

Results

The demographic, disability and MRI data are shown in Table 1. The improvement in disability after alemtuzumab

Table 1. Demographic, disability, MRI and MTR data.

	Alemtuzumab treated patients (n = 20)			Untreated patients (n = 18)		
Age (years: median: interquartile range)	32 (30.3–34.9)			41 (36.7–43.2)		
Disease duration (years: median: interquartile range)	1.4 (1.1–2.5)			1.5 (1.3–2.1)		
Sex (% male)	30%			15%		
Baseline EDSS (median: interquartile range)	1.75 (1–2.5)			1.0 (0–2)		
Final EDSS (median: interquartile range)	1.0 (0.25–1.9)			2.0 (1–2.5)		
T ₂ lesion volume mm ³ (median: interquartile range)	Baseline	Final		Baseline	Final	
	3572 (2620.5–6513.5)	2656 (1426–5165.25)		4326 (3398–9528)	6566 (2729–8918)	
Baseline MTR (pu): mean (SD)	Grey Matter	White Matter	T ₂ Lesion	Grey Matter	White Matter	T ₂ Lesion
	30.6 (0.61)	36.1 (0.64)	33.4 (2.10)	30.6 (0.92)	36.2 (0.53)	30.3 (1.91)
MTR gradient pu/year and p-value*	–0.007 p = 0.872	+0.02 p = 0.511	–0.23 p = 0.251	–0.25 p < 0.001	–0.12 p = 0.004	–0.08 p = 0.679
(95% confidence interval)	(–0.09 to +0.08)	(–0.06 to +0.11)	(–0.61 to +0.61)	(–0.33 to –0.18)	(–0.20 to –0.04)	(–0.47 to +0.30)

*From regression analysis; null hypothesis gradient = 0
EDSS, Expanded Disability Status Scale; MTR, magnetization transfer ratio.

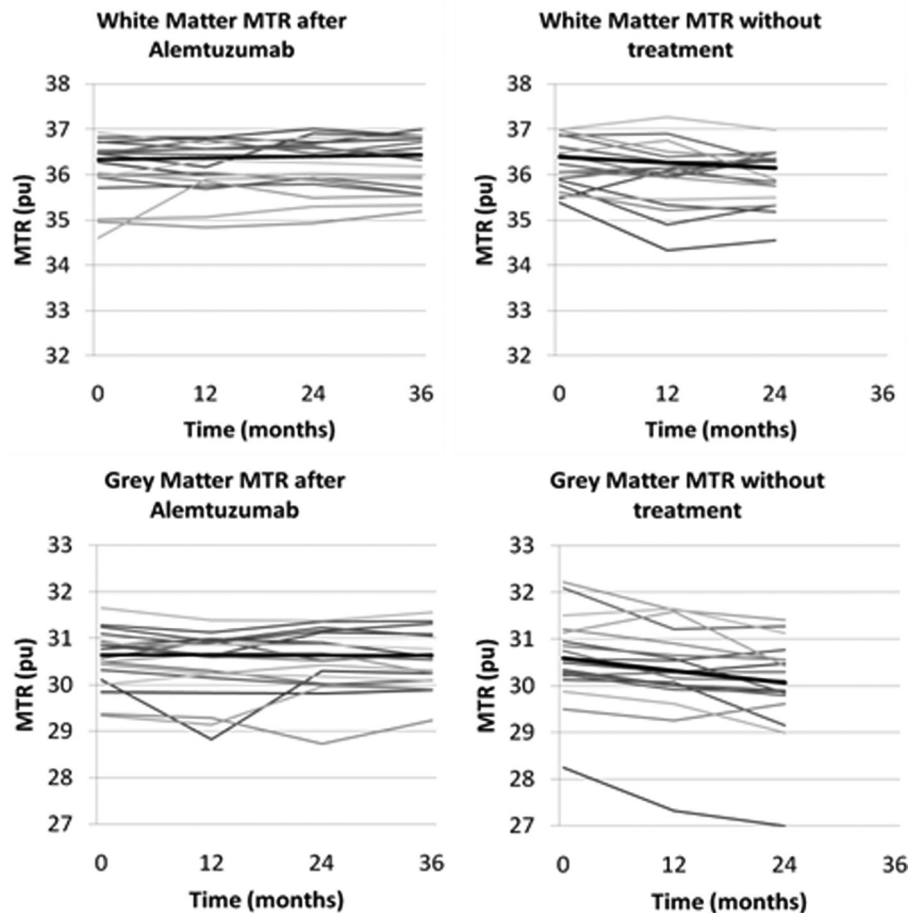


Figure 1. MTR mean for each subject represented by coloured lines. Fitted model for MTR trajectory shown in black

and worsening in untreated patients is representative of previous trials. The T2-weighted lesion volumes fell after alemtuzumab (median -26% interquartile range -43% to -19%) and continued to increase in the control group (median $+5\%$ interquartile range -12% to $+37\%$), $p > 0.001$.

MTR mean fell over 2 years in the control group both in normal-appearing grey (-0.25 pu/year, 95% confidence interval [CI] -0.33 to -0.18 , $p < 0.001$) and white matter (-0.12 pu/year, 95% CI -0.20 to -0.04 pu/year, $p = 0.004$). In the alemtuzumab group, MTR mean was unchanged over 3 years for both normal-appearing grey (-0.007 pu/year, 95% CI -0.09 to $+0.08$, $p = 0.872$) and white matter ($+0.02$ pu/year, 95% CI -0.06 to $+0.11$, $p = 0.511$). The difference between groups reached significance for the grey matter (difference in gradients 0.25 pu/year, 95% CI 0.14 – 0.36 , $p < 0.001$). Age adjustment eliminated the significant difference between gradients for white matter. Lesional MTR mean was stable in both groups (alemtuzumab treated -0.23 pu/year, 95% CI -0.61 to $+0.16$, $p = 0.251$; untreated -0.08 pu/year, 95% CI -0.47 to $+0.30$, $p = 0.679$).

Discussion

These data suggest that alemtuzumab prevents the accumulation of tissue damage in normal-appearing grey matter seen in subjects not receiving disease-modifying treatment. MTR reduction in MS is correlated with demyelination and axonal loss. Neuropathological studies have highlighted the high frequency of demyelinating lesions in MS grey matter.⁸ Cortical grey matter lesions are generally not seen on conventional sequences; therefore, it is likely that focal lesions are included in the grey matter reported here (in contrast to the better separation achieved for white matter). A possible explanation for the stable grey matter MTR after alemtuzumab is that it restricts the formation of grey matter demyelinating lesions, in addition to its effect in white matter. This supports alemtuzumab's significant effect on cerebral atrophy, compared with interferon beta-1a, in the CAMMS223 trial.¹ Future use of newer sequences, better able to identify cortical grey matter lesions, such as double inversion recovery and phase sensitive inversion recovery could investigate this potential therapeutic effect more specifically.⁹ It is also

possible that alemtuzumab has additional neuroprotective effects that contribute to stabilising the grey matter MTR.³

This study suggests the potential of MTR measurement to detect a significant treatment effect, even in a small cohort. We recognise the limitations of this study's size and use of historical controls; however, the same sequence and scanner were used for both groups and statistical adjustment performed for factors that might have influenced the between-group differences (age, sex, disease duration and the scanner upgrade). In these cohorts the white matter MTR gradient appears to steepen with age and this may explain the between-group difference for this tissue.

MTR has recently been recommended as an outcome measure in clinical trials with particular interest in treatments that prevent demyelination or enhance remyelination.¹⁰ Using MTR in phase III trials has two attractions: first, the potential to explore new therapeutic mechanisms with relevance to tissue integrity including myelination; and, second, the opportunity to determine clinical correlations in large studies.

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