Decreased Creatine in NAWM Suggest a Reduced Gliosis in Natalizumab Treated MS Patients

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Introduction

Clinically Definite Multiple sclerosis (CDMS) is a demyelinating inflammatory disease, which leads to the formation of focal lesions. However, it also leads to diffuse pathologies mainly due to axonal damage, gliosis and atrophy in normal appearing white matter (NAWM) [1]. These processes can be assessed using ^{1}H-magnetic resonance spectroscopy (MRS), by which a number of metabolites can be measured. N-acetylaspartate (NAA) is a neuronal marker [2] and reflects the axonal density in NAWM. The MRS signal of NAA is difficult to separate from that of N-acetylaspartate glutamate (NAAG), thus often the sum of NAA+NAAG (tNA) is reported. Decreased NAA and tNA concentrations have been reported in NAWM of MS patients, compared with healthy controls (HC) [3]. Creatine + Phosphocreatine (tCr), *myo*-Inositol (mIns) and Choline containing compounds (tCho) are more abundant in glia cells and increased levels of these metabolites has been reported in NAWM in CDMS patients compared to HC [3, 4]. Glutamate (Glu) is a neurotransmitter, but increased levels of Glu have also been shown to induce apoptosis of oligodendrocytes [5]. The MRS signal of Glu is difficult of differentiate from glutamine (Gln), thus the sum of total glutamine, glutamate (tGlx) is often reported. A number of MS therapies aim for a reduction of the inflammation of CNS, based on the hypothesis that it is the inflammatory response that fuels the axonal damaging processes. Treatment of patients with Natalizumab prevents the migration of potential inflammatory disease-promoting cells into the CNS, therefore resulting in reduced intrathecal inflammation [6]. However, it is not known whether natalizumab treatment also promotes axonal integrity. The aim was to assess the effect of natalizumab on NAWM in CDMS patients using quantitative MRS.

Materials and Methods

25 CDMS patients (median age 40 years, range 24-48 years at inclusion), selected for treatment with Natalizumab, were included in the study. All patients were examined three times; before the treatment (baseline), after one year of treatment (1 year follow-up) and after three years of treatment (3 year follow-up). Single voxel MRS was performed using PRESS, TE 30 ms, TR 3 s. A quantitative transverse relaxation rate R_2 volume was acquired using a *Multi Echo GRAdient Spin Echo* sequence. LCModel ver 6.2 (Provencher, Canada) was used for the quantification of MRS-spectra. The internal water was used for referencing and the influence of R_2 relaxation on the internal water was corrected for using the quantitative R_2 volume using the method described in [7] A group of 18 healthy controls (HC) were included (median age 48 years, range 27-72 years). All MR examinations were performed using an Achieva 1.5 T MR scanner (Philips Healthcare, The Netherlands). Statistical tests were performed using JMP 8 (SAS Institute Inc. USA). For the group comparisons, a mixed linear model was created for each separate metabolite. 'Group', 'Age' and 'Lateral' were used as fixed factors, each individual was treated as a random effect. Differences between the groups were tested using the Tukey post-hoc test. Changes in absolute metabolite concentrations were tested by creating linear regression models with metabolite concentration as dependent variable, and time as independent.

Result

19 patients remained in the study after 3 years (4 patients interrupted the treatment, 2 patients dropped out by choice) Table 1 shows the mean and standard error of metabolite concentrations, and also the significance level of the difference between MS patients and HC. tCho, mIns, tGlx increased significantly in the patient group at all time points. In contrast, the concentrations of tNA and NAAG decreased. A tendency of increased tCr concentration at baseline compared to HC was also observed ($p = 0.06$). In addition, regression analysis showed a decreasing tCr concentration ($p = 0.03$) with respect to time (Fig. 1), no other metabolites changed significantly with time.

Table 1	Healthy controls		MS baseline		MS 1 year follow-up		MS 3 years follow-up	
	Mean	*SE*	Mean	*SE*	Mean	*SE*	Mean	*SE*
NAA	10.75	*0.20*	10.57	*0.26*	10.48	*0.22*	10.40	*0.20*
NAAG	2.56	*0.17*	1.42	*0.23***	1.12	*0.19****	1.57	*0.17***
tNA	13.32	*0.25*	12.02	*0.28**	11.61	*0.25****	11.97	*0.24***
tCr	6.64	*0.09*	7.06	*0.13*	6.97	*0.10*	6.74	*0.09*
tCho	2.50	*0.07*	2.83	*0.08**	2.84	*0.07***	2.81	*0.06**
mIns	5.62	*0.21*	7.15	*0.26****	7.22	*0.22****	7.11	*0.21****
tGlx	10.39	*0.36*	12.41	*0.54**	12.27	*0.42***	12.22	*0.38***

* $p < 0.05$, ** $p < 0.01$, ***$p < 0.001$ Tuky post-hoc test of difference between MS patients and Healthy controls.

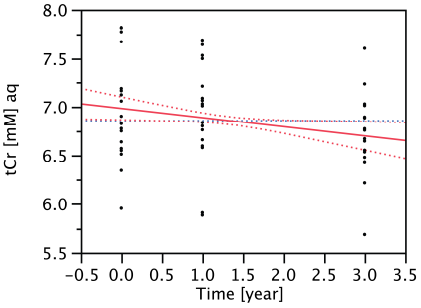

Fig. 1. Linear regression of total creatine with repect to time showed a significant change ($p = 0.03$).

Discussion and Conclusion

The higher concentrations of tCho, mIns, and tGlx and lower concentrations of tNA in CDMS, compared with healthy subjects, agrees with observations of similar cohorts in previous reports [3]. The elevated levels of tCho, mIns and the trend of elevated tCr suggest a process of gliosis in NAWM in these patients [4]. The correlation between decreased tCr concentration and length of the therapy can be interpreted as a positive treatment effect of natalizumab in reducing the extent of pathological gliosis in NAWM. In contrast, the increased levels of mIns, tCho, and tGlx did not correlate with time. This latter observation might be explained by a stabilization effect of the therapy on the metabolism in glia. Either the concentrations of these metabolites are actually unchanged as suggested, which would be a positive effect of the treatment, or alternatively the time span of the measurements was too short (3 years). Yet another possibility is that that the number of patients presently included (25 patients) was too small for observing the anticipated small concentrations changes (mIns, tCho, and tGlx).

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