Metabolites predict lesion formation and severity in relapsing-remitting multiple sclerosis

Antoine M Klauser, Oliver T Wiebenga, Anand JC Eijlers, Menno M Schoonheim, Bernard MJ Uitdehaag, Frederik Barkhof, Petra JW Pouwels and Jeroen JG Geurts

Abstract

Background: Multiple sclerosis is characterised by white matter lesions, which are visualized with conventional T2-weighted MRI. Little is known about local metabolic processes preceding the appearance and during the pathological development of new lesions.

Objective: To identify metabolite changes preceding white matter (WM) lesions and pathological severity of lesions over time.

Methods: Fifty-nine relapsing-remitting MS patients were scanned four times, with six-month intervals. Imaging included short-TE MR spectroscopic imaging (MRSI) and DTI.

Results: Sixteen new lesions appeared within the MRSI slab in twelve patients. Glutamate increased (+1.0 mM (+19%), p=0.039) twelve to six months before new lesions appeared. In these areas, the increase of creatine and choline six months before until lesion appearance, were negatively correlated with radial diffusivity (ρ=-0.73, p=0.002 and ρ=-0.72, p=0.002). Increase of creatine also correlated with increase of axial diffusivity in the same period (ρ=-0.53, p=0.034). When splitting the lesions into 'mild' and 'severe' based on radial diffusivity, only mild lesions showed an increase in creatine and choline during lesion formation (p=0.039 and p=0.008 respectively).

Conclusion: Increased glutamate heralded the appearance of new T2-visible WM lesions. In pathologically ‘mild’ lesions, an increase of creatine and choline was found during lesion formation.

Abbreviations

0mp : zero month prior to appearance

12mp : 12 months prior to appearance

6mp : 6 months prior to appearance

AD : axial diffusivity

Cho : choline-containing compounds

DTI : diffusion tensor imaging

DTI-LM : DTI lesion mask

EDSS : Expanded Disability Status Scale

FWHM : full-width half-maximum

Glu : glutamate

GM: Grey matter

Ins : myo-inositol

mM: mmol/liter

MRSI : Magnetic resonance spectroscopic imaging

MRSI-LM : MRSI lesion mask

MS: Multiple sclerosis

NAWM: normal-appearing white matter

RD : radial diffusivity

SNR : signal to noise ratio

tCr : total creatine

tNAA : N-acetylaspartate and N-acetyl aspartylglutamate

VOI : volume of interest

WM: White matter

Introduction

MS is an inflammatory, neurodegenerative disease that is characterized by focal WM lesions, amongst other pathological changes.[1] Conventional T2-weighted MRI is an essential tool for diagnosis although the number of T2 WM lesions is a poor predictor of clinical disability.[2] More advanced MRI techniques are needed to be able to detect varying degrees of (subtle) tissue damage within lesions or in the NAWM, to better explain clinical disability. MRS imaging (MRSI) and DTI are two such techniques, giving information about metabolism and tissue microstructure respectively.[3-7]

One previous study investigated metabolite changes prior to lesion appearance in MS[8] and found an increased choline/creatine ratio in WM areas that later showed lesions. Metabolite changes during lesion formation have also been sparsely studied.[9-13] A few studies reported increases of total creatine and choline or a transient decrease of total N-acetylaspartate or its ratio to creatine when following acute, gadolinium-enhancing lesions over time. The relation between metabolite changes and microstructural tissue damage over time has not been investigated before.

In the present longitudinal study, we aimed to identify local metabolite changes both prior to and during WM lesion formation, using short echo-time MRSI. Changes in metabolite concentrations were related to changes in DTI-measured tissue damage in lesions, in order to study the predictive value of early metabolite changes in terms of lesion development and tissue damage.

Materials and methods

Study design

Fifty-nine relapsing remitting MS patients were included in this study and scanned four times, with a 6 month interval. The study is exploratory and no a priori sample size was estimated. Data, part of the same group, have been published previously.[14,15] Twenty-nine patients were starting natalizumab, nineteen patients were continuing interferon or glatiramer acetate and eleven patients were not using disease-modifying-drugs at the baseline measurement. Inclusion criteria were a diagnosis of clinically definite relapsing remitting MS,[16] and an age between eighteen and sixty-five years. Patients with a history of neurological or psychiatric conditions (besides relapsing-remitting MS), or a history of alcohol or drug abuse were excluded.

Standard Protocol Approvals, Registrations, and Patient Consents

The research protocol was approved by the institutional ethics review board, and written informed consent was given by all subjects before participation.

MRI Acquisition

MRI was performed on a 1.5T whole-body scanner (Siemens Sonata, Erlangen, Germany) with an eight-channel receiver head coil.

Metabolite spectra were measured with a 2D MRSI, point resolved spectroscopy (PRESS) sequence (TR/TE 3000/30 ms) on a 15mm thick slab, aligned to the pituitary-fastigium line, with the center touching the top of the corpus callosum (Fig.1A). Based on head size, the field-of-view was 160x160 or 140x160 mm, and the corresponding volume of interest (VOI) was 80x100 or 70x100 mm. The use of 16x16 phase-encodings resulted in a voxel size of 1.5 or 1.3 ml. Reference 2D-MRSI datasets were acquired without water suppression using both head and body coils as receiver. The phase encoded acquired data were filtered with a hamming function to reduce the outside voxel volume contamination.

DTI was performed by a diffusion-weighted echo-planar acquisition (TR 8.500 ms, TE 86 ms and isotropic resolution, 2x2x2 mm) including 60 volumes with noncollinear diffusion gradients (b value of 700 s/mm2) and 10 volumes without directional weighting.

Structural imaging included a 3D T1-weighted magnetization prepared rapid acquisition gradient-echo (MPRAGE) (TR 2700ms, TE 5ms, TI 950ms, 176 sagittal slices, 1.3 mm section thickness, 1.3x1.3 mm2 in-plane resolution), and a PD/T2-weighted turbo spin-echo (TR 3130 ms, TE 24 and 85ms, 46 contiguous 3 mm axial slices, 1x1 mm2 in-plane, aligned to the 2D MRSI slab. No T1-weighted contrast-enhanced imaging was performed.

Lesion masks

When a new lesion appeared in the MRSI slab during the study period, a lesion mask, i.e., a 3D volume delineating a region of interest, was manually created on the corresponding T2-hyperintensity. New T2-Hyperintense MS lesions were assessed by experienced neuroradiologists and only diagnosed new lesions were retained for manual segmentation. The lesion mask was then registered onto the exact same areas of the preceding timepoints, using FLIRT.[17] The WM surrounding the lesional area was also included, by dilating all lesion masks by 18mm. This reduced variability by including more MRSI voxels in the lesional masks. As a result, MRSI lesion masks (MRSI-LM) were createdat the appearance of the new T2 lesion (0mp), at 6 months prior to appearance (6mp) and at 12 months prior to appearance (12mp) (Fig.1C). WM lesions already visible at the first acquisition were dilated in plane by 2mm and included in a chronic lesion mask. Because this study focused on the metabolite changes preceding and during WM lesion formation metabolite concentrations in chronic lesion mask were dismissed and masked out. More details are given in the *on-line supplementary material.*

Metabolite quantification

The spectrum of each MRSI voxel was quantified using LCModel[20] resulting in absolute concentrations of N-acetylaspartate and N-acetyl aspartylglutamate (tNAA), total creatine (tCr), choline-containing compounds (Cho), myo-inositol (Ins) and glutamate (Glu) expressed in mmol/liter (mM). Glutamine levels were also quantified and checked in order to ensure a reliable Glu quantification (details given in *on-line supplementary material*). Quality assessment and reproducibility of the absolute metabolite quantification for these metabolites have been published previously.[14] See *on-line supplementary material* for full details.

Concentrations in lesion masks

The 3D T1 images were lesion-filled using LEAP[19] and segmented using SIENAX,[17] producing grey matter (GM) and WM masks. Overlaps with MRSI-LMs were removed resulting in GM and NAWM masks. These were then divided in frontal and parietal lobe sub-masks based onthe *ICBM 152* nonlinear 2009c atlas (Fig.1B).[20] More details about the steps are described in the *on-line supplementary material.*

Using the collective of these masks, brain tissue within the MRSI slab was divided into MRSI-LMs at 0mp, 6mp and 12mp, chronic lesion mask, frontal grey or NAWM and parietal grey or NAWM. Metabolite concentrations were then estimated within each area by a extrapolation analysis. This post-processing method extrapolates concentrations within all the masks depending on their partial volume contributions to each MRSI voxel. Changes in metabolite concentrations in MRSI-LM were calculated for every 6 months period between two acquisitions (Fig.1C): the prelesional period (differences between 12mp and 6mp in MRSI-LM) and the lesion formation period (differences between 6mp and 0mp in MRSI-LM). Additional details are given in the *on-line supplementary material.*

DTI within lesions

Diffusion tensor images were fitted to a tensor model resulting in 3D volumes of axial diffusivity (AD) and radial diffusivity (RD) in 10^{-5} mm^2/s, using DTIFIT.[17] DTI lesion masks (DTI-LM) were delineated identically to the MRSI-LMs (Fig.1C) but not dilated (due to the higher spatial resolution and SNR of the DTI measurements). The mean values for AD and RD were then calculated inside DTI-LM at 12mp, 6mp and 0mp. The DTI metric changes in DTI-LM between the different timepoints were then calculated in the same way as for the metabolite concentrations leading to ΔAD and ΔRD during the prelesional period (differences between 12mp and 6mp) and the lesion formation period (differences between 6mp and 0mp). AD and RD are known to increase markedly with the formation of a new WM lesion[21] and the level of RD found in new, enhancing lesions is thought to reflect the severity of the tissue damage.[22,23] Hence, the amount of ΔRD during the lesion formation period was considered as a degree of lesion severity.

DTI changes over time and the classification of lesions as 'mild' or 'severe'

To explore the change in DTI metrics occurring in the lesions, the significance of the changes of ΔAD and ΔRD was assessed during the prelesion and lesion formation periods. To identify possible metabolite markers specific to a lesion severity subtype, two subgroups of lesions were created. The lesions above the median ΔRD were defined as 'severe' and the lesions below the median as 'mild'.

Metabolite concentration changes during the prelesional period

To investigate whether and which metabolic changes occur before the appearance of new WM lesions, we measured the changes in concentrations during the prelesional period in the MRSI-LMs. If a metabolite showed a pre-lesional change, the same test was performed, as surrogate analysis, on the corresponding changes in concentration ratio with respect to tCr and additionally with MRSI-LM

at 12mp and 6mp mirrored in the contralateral hemisphere. Finally, the concentration changes in NAWM were also analyzed to check their stability.

As a posteriori analysis, the correlation was calculated between the significant changes in metabolite concentrations in the prelesional period and the changes in DTI metrics during the lesion formation period and the volume of the newly formed lesions.

Metabolite concentration changes during the lesion formation period and relations with lesion severity

The significance of the metabolite concentrations changes during the lesion formation period was determined either in all the lesions combined or in the 'mild' and 'severe' subgroups.

In an a posteriori analysis, the correlation between metabolite concentrations exhibiting significant changes and ΔAD and ΔRD during the same time period were assessed. Correlation coefficients were also computed between concentration changes during the lesion formation period and the volume of the newly formed lesions.

Statistical analysis

Statistical longitudinal assessments of metabolite concentrations were performed using a Wilcoxon signed-rank test between the applicable timepoints. The DTI metrics change were statistically analyzed with a Student's t-test on paired data of the applicable timepoints. Correlations coefficients were computed using the Spearman’s rank correlation.The tests were considered significant when the probability value, *p,* was less than 0.05 (values are given as mean ± standard deviation, unless indicated otherwise). Due to the exploratory nature of the study no adjustment for multiple comparisons was made.

Results

Subjects

Twelve patients showed one or more formations of new lesions within the MRSI slab. These patients had a mean age of 37 years (range 20-50) and mean disease duration of 4.6 years (range 0.16-13.3) at first acquisition. Only data of these subjects were retained for further analysis.

Five patients received interferon beta-1b or glatiramer acetate whereas seven patients were untreated. Mean (standard deviation) normalized brain volume, normalized WM volume and normalized GM volume[17] were respectively 1.39 L (±0.07 L), 0.68 L (±0.04 L) and 0.72 L (±0.04 L). The median T2 lesion load was 4.3 mL (interquartile range: 2.1-11.9 mL). Median Expanded Disability Status Scale (EDSS) was 2.0 (range 1-3.5). Spectral qualities of the VOI in the MRSI slab were comparable to a previous reproducibility study,[14] with a mean full-width at half-maximum (FWHM) of 5.18 Hz (±0.81 Hz) and a mean signal-to-noise ratio (SNR) of 10.8 (±1.35).

In total, sixteen newly forming WM lesions were monitored over time. Data in the prelesional period were only available for nine lesions (seven lesion appeared at the second acquisitions) and lesion formation period data were available for the entire sixteen lesions. AD and RD did not change during the pre-lesional period but increased during the lesion formation period (Table 1).

Pre-lesional period: glutamate increase

An increase of +19% on average (p=0.039) was measured for Glu during the pre-lesional period (ΔGlu = 1.0 ±1.3 mM) (Fig.2A, Table 1). Other metabolites remained stable within the pre-lesional period and Glu in the contralateral (control) region also showed no changes. Analysis of the change of concentration ratio Δ(Glu/tCr) confirmed the pre-lesional increase, on average of +23% (p=0.027, Δ(Glu/tCr)=0.26 ±0.31). The metabolite concentrations in NAWM did not show any significant changes in both the pre-lesion and the lesion formation period (Table e-1).

In the posthoc analysis, pre-lesional ΔGlu was associated with ΔAD of the lesion formation period (ρ = 0.86; p<0.003) (Fig.2B); pre-lesional ΔGlu was also associated with the volume of the new lesion (ρ=0.68; p=0.050) (Fig.2C) but not with ΔRD of the lesion formation period (Table 2).

Lesion formation period: Increase of tCr and Cho in developing 'mild' lesions

During the lesion formation period, only the subgroup of 'mild' lesions showed increases in metabolite concentrations: tCr was found to increase by 23% (p=0.039, ΔtCr = 0.89 ±0.86 mM) and Cho by 27% (p=0.008, ΔCho = 0.35 ±0.24 mM) (Table 1).

In the a posteriori analysis, the values of ΔtCr and ΔCho in all lesions during the lesion formation period were strongly associated with ΔAD and ΔRD in the same period. ΔtCr was anti-correlated to ΔRD (ρ -0.73, p = 0.002) and ΔAD (ρ -0.53, p = 0.034). ΔCho was anti-correlated with ΔRD (ρ -0.72, p = 0.002) (Fig.3C-D). ΔtCr and ΔCho were not correlated to the lesion volume (Table 2).

Discussion

This study investigated and related metabolite and diffusion changes prior to and during new WM lesion formation in MS. Glutamate increases were found to precede the development of new WM lesions on T2-weighted MRI scans. Furthermore, tCr and Cho increases were associated with lower increases of RD and AD in the developing lesions. And finally, pre-lesional Glu increase was indicative of higher subsequent lesion volumes and AD increases.

Pre-lesional period

Although a higher level of glutamate in NAWM and in acute WM lesions has been previously reported,[24,7] the observation of Glu rising prior to WM lesion appearance is new and important. The rise in Glu could be attributed to activated macrophages and/or microglia, which are known to cluster in the pre-lesional stages[25,26] and to produce Glu.[27] Other explanations might be subtle pathology of axons or abnormalities of Glu receptor/transporter biology leading to abnormal parenchymal Glu concentrations.[28]

The Glu increase during the pre-lesional period was strongly correlated with the AD increase, which is generally deemed to relate to axonal injury.[4,5,29-31] The early pathological changes discussed above may therefore be indicative of the extent of eventual axonal damage within newly developing WM lesions in MS. Moreover, a stronger increase of Glu in pre-lesional WM was also associated with higher subsequent lesion volumes. Although there is an obvious effect of the lesion volume on the metabolite estimated within MRSI-LMs (considering the dilation of the lesion mask, the amount of actual T2-hyperintense tissue in MRSI-LM would be greater for larger lesions than for smaller lesions), the correlation in Fig.2c is particularly important because the Glu changes were measured 6 months before the T2-hyperintensity appeared and before the actual lesion volume could be observed. This correlation suggests that changes of Glu in WM including and surrounding the future lesions could also predict their volume. Together, these results underline the relevance of Glu to predict both the extent and severity of the subsequently forming lesions.

The relevance of increased WM Glu as biomarker of MS progression and clinical disability has been already demonstrated in one recent clinical study[7] where the ratio Glu/tNAA was associated with decline of brain volume and higher clinical disability.

No changes in tCr or Cho were found in the pre-lesional period, in constrast with Tartaglia *et al.* .[8] This discrepancy could be explained by a difference in methodology. We investigated longitudinal absolute metabolite concentration changes, whereas Tartaglia et al. followed a cross-sectional approach. Additionally, the metabolite mask-matching analysis used in this study is beneficial for the precision of the results as the method corrects for intertwining of several tissue types (NAWM, GM, cerebrospinal fluid and lesional WM), and anatomical regions, known to differ natturally in terms of metabolite concentrations.[32]

Lesion formation period

In the lesion formation period, the lesions showing a high increase in tCre exhibited a small increase in AD and RD and vice versa. Likewise, when a high increase of Cho was observed, the increase in RD remained small. In fact, splitting the lesions into two groups ('mild' and 'severe') based on the RD increase, demonstrated that the effect is driven by the 'mild' lesions: increases of tCr and Cho were only present in the 'mild' lesion group. The increase of RD, which might reflect demyelination,[33] is a strong predictor for evolution of the new lesion into a black hole (a sign of permanent and severe tissue damage).[22,23] The metabolite variation in time and strong heterogeneity between lesions has already been described in magnetic resonance spectroscopy studies of acute WM lesions[9-12] but the origin of this variability remained unclear.

The observed increases of tCr and Cho could potentially be interpreted as a sign of remyelination, limiting damage in new lesions. This role for tCr was suggested before by a study that followed new contrast-enhancing lesions over time. These lesions showed increases of tCr, which was interpreted as a reflection of repopulation of the lesions with oligodendrocytes and, hence, remyelination.[13]

Our findings suggest that early metabolite changes predict the appearance and also the severity of newly forming lesions. Monitoring these changes could be useful for choice and timing of pharmacotherapeutical interventions, but also for understanding disease heterogeneity and clinical progression. Especially as the increasing Glu before lesion appearance could also be interpreted as an early leakage of the blood brain barrier causing inflammatory infiltrates stimulating clustering of activated glial cells.

Methodological considerations and future directions

Larger sample sizes, perhaps with multiple MRSI slabs, applied and measured, may be needed to support our results further. Furthermore, the actual time of lesion formation could not be determined more precisely in this study than with the six month window of the lesion formation period. However, this should not affect the validity of our results. Indeed, DTI metrics changes recorded in the WM lesions of MS patients were reported to appear during the first month[23] or two months[34] after enhancement, which falls entirely within the 6 months of the formation period taken into account here. Only the few lesions occurring in the last month of the formation period could have a possible incomplete increase of AD and RD that might have slightly reduced the overall effect.
It is important to mention that DTI metrics changes and metabolite changes are measured on different mask sizes and that the lesion volume could affect the results in certain cases. Indeed, due to the dilation, the actual relative content of T2-hyperintense tissue in MRSI-LMs of small lesions is considerably lower than for larger lesions. Therefore, it is important to consider this effect when interpreting correlation between metabolite changes in MRSI-LM and DTI metrics changes.
Imaging more frequently between time points and including contrast-enhancement could provide a more accurate characterization of metabolite changes as these are known to fluctuate in the month following the lesion formation.[12,13] Including a T1-weighted spin-echo sequence would enable the correlation of our findings to chronic T1-hypointensities, as a clinically used measure of (severe and/or lasting) tissue destruction.[35] Future studies are also required to observe whether a decrease of

tNAA follows eventually at longer follow-up, and how this would be related to our findings.

Conclusions

Our results show that a Glu increase precedes the appearance and predicts the volume of the new T2 lesions in the MS WM, and that tCr and Cho increases predict the severity of tissue damage within those lesions. These findings are of essence for understanding lesion development in MS, and for therapeutic strategies or disease prediction.

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