IN VIVO LOCALIZED NMR PROTON SPECTROSCOPY OF NORMAL APPEARING WHITE MATTER IN PATIENTS WITH MULTIPLE SCLEROSIS

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SUMMARY

We used a rapid long TE proton magnetic resonance spectroscopic (MRS) sequence in the normal appearing white matter of 11 patients with definite multiple sclerosis (MS) localizing the volume of interest in the centrum semi-ovale. The metabolic changes were compared to the same area in 11 normal brains. We found a significant decrease in NAA/Cr ratios and a borderline significance of increase in Cho/Cr ratios in patients with MS. A discriminant analysis was performed on these data. This allowed to obtain a simple ratio, NAA/(Cho + Myo), which discriminated MS patients from controls. Our results indicate that normal appearing white matter on MRI is biochemically abnormal in patients with MS. In addition MRS could be routinely used after a standard MRI examination in patients with MS for clinical correlations, total load of the disease assessment and monitoring clinical trials.


RÉSUMÉ

RMN de proton spectroscopique localisé in vivo chez des patients atteints de sclérose en plaques dont la substance blanche paraît normale.

Nous avons utilisé une séquence de RMN spectroscopique rapide et à long TE chez 11 patients atteints de sclérose en plaques définie (MS) et dont la substance blanche paraissait normale en IRM, le volume d’intérêt étant le centre semi-ovale. Les modifications métaboliques ont été comparées, dans la même zone, avec 11 cerveaux normaux. Nous avons observé une diminution significative des rapports NAA/Cr (N-acetyl-aspartate/créatine) et une augmentation flairant la signification des rapports Cho/Cr (Choline/créatine) chez les patients atteints de MS. Une analyse discriminante a été effectuée sur ces données. Cela a permis d’obtenir un rapport simple, NAA/(Cho + Myo) qui séparait les patients des contrôles. Nos résultats indiquent qu’une substance blanche paraissant normale en IRM est anormale en biochimie chez les patients atteints de sclérose en plaques. Par ailleurs, le RMN spectroscopique pourrait être utilisé régulièrement après un examen RMN standard chez les patients atteints de sclérose en plaques pour évaluer les corrélations cliniques et l’état total de la maladie, et pour surveiller les essais cliniques.

Mots-clés : Spectroscopie protonique. Substance blanche d’apparence normale.

INTRODUCTION

Since the first description of multiple sclerosis (MS) lesions on magnetic resonance imaging (MRI) [1], considerable progress has been achieved to improve the sensitivity of the technique in this indication. In addition, MRI has brought great insights in understanding the « in vivo » natural history of MS [2, 3]. Besides, it is now almost systematically combined with clinical assessment to monitor therapeutic trials performed in this disease. Nevertheless, up to 5 % of MRI examinations in cases of clinically or laboratory supported definite MS remain normal, and « MS suggestive lesions » in patients presenting with isolated symptoms involving the central nervous system are not necessarily predictive of the onset of MS [4-7]. In addition, its specificity needs to be improved in the early stages of the disease.
In the past years, magnetic resonance spectroscopy (MRS) has emerged as a new method for an old technique, allowing to evaluate biochemical characteristics of a localized volume in the brain [8, 9]. Applied to MS, it seems to give information on the biochemical parameters of the lesions [10-14] and their temporal evolution [15, 16]. It was then attempting to correlate spectroscopic changes with the histological state of the lesions: inflammation, myelin breakdown, neuronal loss, gliosis. The aim of our study was to investigate using MRS, the normal appearing white matter (NAWM) in patients with definite MS. If MRS allows to detect white matter anomalies ignored with conventional MRI, then it should be used systematically in the exploration of white matter diseases such as MS. The routine use of this method supposes an easy, rapid and reproducible sequence.

MATERIAL AND METHODS

Subjects

Eleven patients with definite multiple sclerosis (MS) [17], 5 with a relapsing remitting and 6 with a secondary progressive course, aged 25-48 years (mean 37.2y), and eleven controls aged 21-64 years (mean 32y) were included in this study. MRS examination was performed immediately after a conventional MRI prescribed as a part of diagnosis and/or evaluation of the activity of the disease. Control patients were undergoing a MRI examination for headaches (9 patients), facial trauma without brain involvement (1 patient) and localized sellar tumor (1 patient). All MRI of MS patients showed on T2 wi hypersignals areas very suggestive of the disease [2]. All of control patients had a normal MRI of the brain.

MRI and MRS

MRIs were acquired on a whole body MR-scanner (Signa, GE Medical Systems, Milwaukee) operating at 1.5T using a standard quadrature head coil. For all patients, at least the following 3 sequences covering the whole brain were undertaken: 1-T1 wi spin-echo sequence (500-12) in the sagittal plane (matrix 512 x 256, 5 mm/1.5 mm). 2-Short T2 wi fast spin-echo sequence (4500-17) in an axial plane parallel to the neuro-optical plane (matrix 512 x 256 ; 4 mm/0.4 mm). 3-Long T2 wi fast spin-echo sequence in the coronal plane (4500-85) orthogonal to the previous one (matrix 512 x 256 ; 5 mm/0.5 mm).

MRS spectra were acquired with the PROBE-SV module (GE). A stimulated echo acquisition mode (STEAM) sequence was used (TE 136 ms., TR 1,500 ms., TM 13.7 ms.) [8]. The spectral width of the acquisition was set to 2,500 Hz and 2,048 samples were collected. The parallelepipedic volume where the spectrum was acquired was graphically prescribed on the T2 wi axial view, in a way that neither MRI abnormal appearing white matter, nor grey matter was embedded. The volume of interest varied from 5 to 8 ml and was localized in the centrum semi-ovale (fig. 1). Four series of 8 excitations with alternate phases were acquired without water suppression, then 16 series were done with water suppression. These raw data were transferred on a Sun Sparc 2 workstation (Sun Microsystems, Mountains View, California) with SAGE data analysis software (GE Medical Systems). The processing was made with a macro, the main steps of which are listed below.

Water suppressed data were corrected with the non suppressed ones in order to remove phases variations due to eddy currents [18]. The creatine peak was identified by the operator. The corrected data was windowed (variable exponential multiplied by a constant 8 Hz gaussian window) such that the width of the creatine peak was made up for the specific T2 relaxation time. These data were zero filled up to 8,192 points before the Fourier transformation. Zero and first order phase corrections were then performed automatically. The 4 main peaks appeared singlet like : myoinositol (Myo ; ppm : 3.56), choline (Cho ; ppm : 3.22), creatine (Cr ; ppm : 3.03), N-acetyl-aspartate (NAA ; ppm : 2.02) and were separately fitted with Marquardt’s algorithms using lorentzian models. When the signal to noise ratio corresponding to a metabolite was less than the quarter of the average signal to noise ratio estimated for the four peaks, a warning message was displayed. This only occurred for the myoinositol.

Data analysis

Every metabolite peak amplitude was normalized to the one of the creatine. The data were thus considered as three value profiles (Myo/Cr, Cho/Cr, NAA/Cr) measured on the population. A repeated measures analysis of variance (RM-ANOVA) was performed on them [19]. Comparisons between the two groups for each metabolite were done with t-tests (two-tailed, Bonferroni’s correction for three comparisons). Linear discriminant function was derived in order to classify the populations all standard statistical package (Stagraphics STSC Inc. Rockville, Maryland). An uniform discriminant ratio was built, achieving the same result. Non parametric Mann Whitney test was used to assess the significance of the discriminant ratio.

RESULTS

Figure 2 shows the spectra of a representative patient of each group. Relative quantification results are displayed on table 1. The signal to noise ratios were not different in both groups (p = 0.48).

The RM-ANOVA rejected the hypothesis of metabolite profiles parallelism (p , 0.0008). The mean normalized values for the three metabolites in both groups are shown on figure 3 with their 95% confidence intervals. Multiple comparisons were done showing that NAA/Cr was significantly decreased in patients with MS (p , 0.0008). Whereas Cho/Cr was increased with a borderline
significance ($p < 0.025$; for an overall error rate of 0.05, each one of the comparison was to be done at the 0.016 level).

The linear discriminant function was found to be:

$$W = -3.51 \frac{\text{NAA}}{\text{Cr}} + 4.37 \frac{\text{Cho}}{\text{Cr}} + 6.70 \frac{\text{Myo}}{\text{Cr}} - 1.27.$$  

$W$ was positive for MS patients and negative for all controls but one. The mean value of the scores was $-1.56$ for MS patients and $-1.56$ for controls. The standardized discriminant function coefficients showed that Ch/Cr and Myo/Cr contribution had almost the same magnitude (respectively 0.69 and 0.79), obviously in the opposite way to NAA/Cr ($-1.06$). This led us to assess $R = \frac{\text{NAA}}{\text{Cho + Myo}}$ as a discriminant ratio.
Fig. 2. – Spectra after their acquisition and analysis: representative control patient in a; representative patient with multiple sclerosis in b.

Fig. 2. – Spectres après leur acquisition et leur analyse : patient représentatif de la population témoin en a ; patient représentatif de sclérose en plaques en b.

Table 1. – Ages and relative quantification results of NAA/Cr, Cho/Cr and Myo/Cr ratios. The signal to noise ratios are also represented; they were not different in both groups (p = 0.48) (MS = multiple sclerosis patients; C = control patients)

<table>
<thead>
<tr>
<th># pat</th>
<th>age</th>
<th>cat</th>
<th>NAA/Cr</th>
<th>Cho/Cr</th>
<th>Myo/Cr</th>
<th>S/N</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>34</td>
<td>MS</td>
<td>2.05</td>
<td>1.11</td>
<td>0.58</td>
<td>8.45</td>
</tr>
<tr>
<td>2</td>
<td>41</td>
<td>MS</td>
<td>2.00</td>
<td>1.65</td>
<td>0.44*</td>
<td>7.68</td>
</tr>
<tr>
<td>3</td>
<td>45</td>
<td>MS</td>
<td>1.48</td>
<td>1.32</td>
<td>0.50</td>
<td>12.17</td>
</tr>
<tr>
<td>4</td>
<td>29</td>
<td>MS</td>
<td>2.00</td>
<td>1.30</td>
<td>0.68</td>
<td>8.65</td>
</tr>
<tr>
<td>5</td>
<td>39</td>
<td>MS</td>
<td>1.64</td>
<td>1.45</td>
<td>0.43</td>
<td>7.14</td>
</tr>
<tr>
<td>6</td>
<td>40</td>
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<td>1.04</td>
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<td>11.24</td>
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<tr>
<td>7</td>
<td>26</td>
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<td>1.29</td>
<td>0.47</td>
<td>20.40</td>
</tr>
<tr>
<td>8</td>
<td>48</td>
<td>MS</td>
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<td>1.06</td>
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<tr>
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<td>7.93</td>
</tr>
<tr>
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<tr>
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<tr>
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<tr>
<td>13</td>
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<td>C</td>
<td>2.52</td>
<td>1.10</td>
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</tr>
<tr>
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<tr>
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<tr>
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<td>C</td>
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<td>1.23</td>
<td>0.59</td>
<td>7.70</td>
</tr>
</tbody>
</table>

Table 2 illustrates that cut-off value 1.25 for R permits to assign patients to their respective group, except for one control. This corresponds to a significant difference at the confidence level of p , 0.0001).

DISCUSSION

We investigated normal appearing white matter in patients with definite MS using a long TE STEAM sequence with localized proton MRS and compared it to patients free of a disease involving the brain. STEAM sequence was preferred to PRESS (point resolved spectroscopy), which is also accessible, because it yields a better spatial accuracy in the location of the sample where the spectrum is done. The relatively long TE makes the water suppression easier than shorter ones [20]. It also lowers the contributions of metabolites from the glutamate/glutamine group, proteins, glucose, and from the free lipids.

* Indicates the occurrence of the warning message.
We observed biochemical anomalies in NAWM on MRI in patients with MS. Analyzed separately, NAA/Cr ratio was significantly reduced in patients with MS, whereas Cho/Cr increase was on the limit of significance at the global confidence level of 95%. There was no significant change in the ratio of Myo/Cr. When these peaks were studied together, a discriminant linear function allowed to distinguish MS patients from controls. The given function and ratio are only valid for the acquisition sequence and the data processing described. Peak amplitudes and ratio are only valid for the acquisition sequence and/or density. Previous studies have reported a reduction in NAA/Cr ratios both in acute [12, 24, 25] and chronic lesions in MS [25]. NAA, a precursor of neurotransmitters (N-acetyl-aspartate-glutamate) [24-28] is formed by mitochondria [29]. Experimental and clinical studies support its presence exclusively in neurons and their process [28, 30-35], although it was described in oligodendrocyte progenitor cells (O2A cells) [36]. Serial studies in MS showed regional discrepancies in abnormal metabolite signals within and around hypersignal anomalies. NAA peak anomalies remained abnormal in follow-up indicating a possible irreversible damage in the central nervous system [12]. The decrease of NAA/Cr in NAWM in patients with MS could reflect an early neuronal dysfunction and/or loss, and be a witness of mitochondrial impairment in these areas. Our study confirms the results of others in NAWM showing a reduction of NAA/Cr ratio surrounding hypersignal anomalies. NAA peak anomalies in abnormal metabolic signals within and around normal appearing white matter of patients with MS using both phosphorus and proton MRS [23]. NAA peak may be a reliable marker for neuronal function and/or density.

Choline and its derivatives are present in all cell membranes [38] and are thought to be an index of phospholipid metabolism. Its increase was described in association with demyelination [14, 39], but experimental studies in animals with exclusive inflammatory EAE argue for its involvement in inflammation [39]. Thus choline change could be associated with both mechanisms [12].

Inositol which could serve as a marker for astrocytes [40] and may therefore be associated with active gliosis [41]. Together with choline it could also reflect the breakdown of myelin membrane phospholipids. Although inositol peak is thought to be detected with short Te sequences, we
could evidence changes in most of studied patients. With our acquisition settings, this peak could only detect some of the compounds present and having a relatively long relaxation time. Systematic studies of this peak in the same region with comparative short and long TE could answer this question.

Our results in NAWM of MS patients are consistent with those of other studies based on MRI T1 and T2 relaxation times measurement [42-45] magnetization transfer MRI [46, 47] or diffusion MRI [48, 49]. They could reflect histological changes described in normal white matter of postmortem samples of patients with MS [50-54].

The biochemical changes we describe in NAWM in MS patients are not specific for the disease and could not be used as diagnosis criteria. Nevertheless they may constitute valuable pragmatic classification criteria for patients at risk of MS in whom no white matter signal alteration is visible on classic MR imaging. The choice among the different discriminant functions to determine the best one, strongly depends upon the studied population. An important issue is the possible effect of age on metabolites peaks relative amplitudes. In our control population the NAA/Cr ratio was negatively correlated with age (NAA/Cr = 3.64 – 0.041 × age; \( R^2 = 0.759; \ p = 0.0005 \)). This suggests that the ages range [21-48] may have been too large or that such studies have to be done with a strict age matched design.

CONCLUSION

Using an easy rapid and reproducible localized proton MRS sequence, we describe biochemical abnormalities in NAWM of MS patients. One ratio and a linear combination of relative peak amplitudes allow both to discriminate MS patients from controls. The approach with discriminant analysis could easily be extended to more than two classes of patients and could evidence more specific metabolites profiles. We emphasize the fact that this study doesn’t assess whether these functions are specific for MS or not. Nevertheless, they could help to determine the total activity of the disease, to better understand its pathophysiology, and to more precisely monitor clinical trials and target the efficacy of new therapeutics.

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RÉFÉREnces


