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BRIEF COMMUNICATION

^{1}H-^{31}P magnetic resonance spectroscopy: effect of biotin in multiple sclerosis

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Abstract

Biotin is thought to improve functional impairment in progressive multiple sclerosis (MS) by upregulating bioenergetic metabolism. We enrolled 19 patients suffering from progressive MS (5 primary and 14 secondary Progressive-MS). Using cerebral multinuclear magnetic resonance spectroscopy (MMRS) and clinical evaluation before and after 6 months of biotin cure, we showed significant modifications of: PME/PDE, ATP, and lactate resonances; an improvement of EDSS Neuroscore. Our results are consistent with metabolic pathways concerned with biotin action and could suggest the usefulness of MMRS for monitoring.

Introduction

Multiple sclerosis affects 2.3 million persons worldwide and remains the most common neurological cause of disability among young people. Recurrent relapsing form of MS accounts for 85% of the initial presentation with a clearly established inflammatory pattern.[1] A preliminary study suggested a potential clinical effect of biotin in nearly 13% of PP-MS and SP-MS[2]; whereas biotin effects in RR-MS were never studied. Moreover, quantitative and qualitative modifications of channels and pumps have been observed after inflammatory lesions during in vitro studies. In addition, mitochondrial dysfunction and energetic consumption of remyelination mechanism can lead to bioenergetic imbalance and render neuronal life-sustaining conditions impossible.[3,4]

$^{1}H/^{31}P$-MMRS has been used to explore cerebral metabolism modification in MS. These metabolites play a key role in energy production(PCr, ATP), and in the process of remyelination(Cho, PME, PDE), as well as TCA malfunction(lactate) and neuronal disability(NAA). Recent studies have shown variations of PCr and ATP in patient MS compared to healthy control.[5] One longitudinal MMRS study was realized to test efficiency of treatment on MS patient.[6] Due to the putative role of biotin in mitochondrial dysfunction,[7] we monitored ^{1}H-^{31}P metabolites by MMRS to assess biotin effect on mitochondrial impairment. The primary objective of our study was to assess differences in metabolic levels using MMRS before and after 6 months of biotin therapy. The secondary objectives were to evaluate clinical results and absence of adverse events over the same period of time.

Patients and Methods

Eligibility criteria and study design

We performed a monocentric cross-over study enrolling consecutive patients with MS treated at the Poitiers Hospital between November 2015 and November 2016. All subjects provided informed consent and met the following criteria: age more than 18 years; a diagnosis of

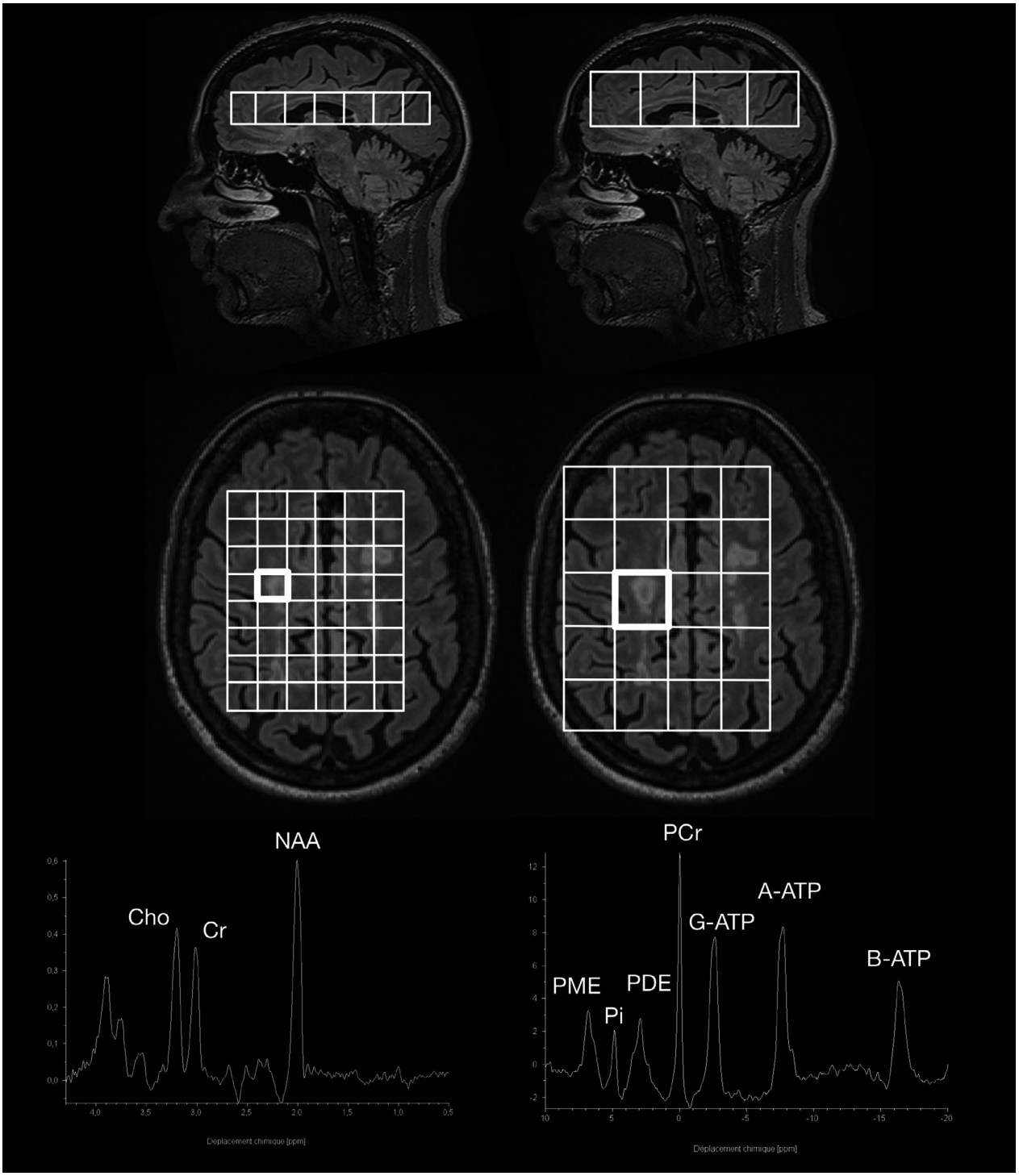

Figure 1. Example of MMRS VOI placement and resulting spectrum of white matter lesion.

clinically definite progressive MS (PP-MS, SP-MS)[8] for at least 12 months; and absence of inflammatory activity.[9] Exclusion criteria were as follows: acute clinical aggravation over the last 2 years; increased number of T2 lesions with reference to the most recent follow-up imaging occurring over at least 12 months; modification of MS treatment or corticosteroids 6 months before initiation of biotin therapy or during the study.

Clinical and radiological evaluations

In MS subjects, within a week of imaging procedures, neurological disability was assessed using the Expanded Disability Status Scale (EDSS) based on NEURO-SCORE,[10,11] and cognitive performance with the Computer Speed Cognitive Test(c).[12] Walking performance was evaluated with the perimeter during 6 min and Timed 25-Foot Walk(TW25F) test.[13]

Patients were given a 300 mg dose of Biotin(MD1003) daily by oral route in three doses.

MR data acquisition and processing

All patients underwent two brain MMRS examinations according to the same protocol just before Biotin treatment and 6 months later on 3T whole-body system Verio, (Siemens, Erlangen, Germany) using a double-tuned $^{31}P/^{1}H$ head coil. The MMRS protocol included 3D-FLAIR images, 2D ^{1}H-CSI semi-LASER, and ^{31}P-MRSI sequences. The locations of the MMRS-VOIs were determined from FLAIR images on the three orientation planes. The VOI was parallel to the AC-PC line going through the subcortical area. The voxels were chosen on WML and if there was no lesion in Normal Appearing White Matter (Fig. 1). To ensure the reproducibility of the protocol, the second examination was carried out by the same radiologist, and the VOI was localized at the same distance from the AC-PC line encompassing the subcortical area and the same voxel number in the CSI grid as in the first exam. The MMRS protocol was the following: ^{1}H-MRSI sequence parameters were as follows: outer volume saturation (OVS) with a voxel size of 4.5 mL ($15 \times 15 \times 20$ mm^3), TEs = 35-135 msec; for ^{31}P-MRSI, a 200mm 3D-MRSI slab was aligned to the ^{1}H-MRSI slice, thereby ensuring overlap of WML in the two scans for enhanced correlation. The ^{31}P protocol was standardized to CSI, TE = 2.3 msec, TR = 1000 msec with a matrix extrapolated to $16 \times 16 \times 16$ leading to a reconstructed voxel size of $25 \times 25 \times 25$ mm^3.

Table 1. Clinical results.

	Baseline	After treatment	*P*-value
EDSS mean (SD)	6.21 (0.61)	5.95 (0.91)	0.018
EDSS median (range)	6.5 (4.5–7.0)	6.0 (3.0–7.0)	0.018
EDSS, *n* (%)			
4.5–5.5	3 (15.7)	7 (36.8)	–
6–7	16 (84.3)	12 (63.2)	–
Improvement in EDSS, *n* (%)	7 (37)		–
Improvement and stability in EDSS, *n* (%)	12 (63)		–
TW25 (seconds) Mean (SD)	53.20 (92.55)	57.76 (102.66)	0.94
Test walk on 6 min (m) Mean (SD)	183.33 (96.21)	232.92 (128.69)	0.025
Improvement test walk on 6 min *n* (%)	5 (26)		–
CSCT correct answers *n* (SD)	39 (9.77)	40 (9.51)	0.3
CSCT mistakes *n* (SD)	0.72 (0.67)	0.47 (0.53)	0.08

Improvement was defined by an increase of at least 20% of Test Walk on 6 min. CSCT, computer speed cognitive test; EDSS, expanded disability status scale; SD, standard deviation; TW25, time to walk 25 feet.

Data processing

The MMRS raw data were transferred to an offline workstation and analyzed in the time domain with the JMRUI software tool[14] employing AMARES algorithm. In this interactive quantitation method the line-widths and concentrations are part of a nonlinear model and are

Table 2. ^{1}H-^{31}P results.

	Baseline mean ($\pm 3\sigma$)	After treatment mean ($\pm 3\sigma$)	*P*-value
Bioenergetic metabolites			
t-ATP	8.76 (1.68)	9.93 (2.13)	0.0003
PCr	1.98 (0.54)	2.19 (0.33)	0.001
PCr/t-ATP	0.23 (0.06)	0.22 (0.03)	0.63
PCr/Cr	0.030 (0.012)	0.031 (0.012)	0.65
PCr/Pi	3.46 (1.29)	3.31 (1.17)	0.32
PME/PCr	0.89 (0.27)	0.83 (0.27)	0.023
PDE/PCr	1.39 (0.63)	0.82 (0.27)	0.0002
Pi	0.58 (0.27)	0.67 (0.21)	0.005
pH	7.03 (0.021)	7.01 (0.018)	0.0004
Lac	3.17 (5.16)	2.15 (3.0)	0.02
Lac/Cr	0.06 (0.06)	0.03 (0.06)	0.005
Cr	67.18 (23.04)	72.75 (32.52)	0.001
Membrane metabolites			
PME/PDE	0.65 (0.3)	1.02 (0.48)	0.0002
PME	1.77 (0.24)	1.82 (0.45)	0.295
PE	0.96 (0.27)	1.19 (0.24)	0.0003
PC	0.80 (0.33)	0.61 (0.48)	0.002
PDE	2.68 (1.32)	1.83 (0.63)	0.0002
GPE	0.09 (0.21)	0.17 (0.63)	0.381
GPC	2.75 (1.41)	1.63 (0.63)	0.0002
Lip	4.79 (3.78)	4.89 (6.12)	0.91
Lip/Cr	0.59 (0.96)	0.50 (0.63)	0.34
Cho	96.7 (29.88)	88.54 (48.33)	0.011
Cho/Cr	1.45 (0.6)	1.22 (0.51)	0.001
Neuronal viability metabolites			
NAA	93.89 (34.38)	103.19 (31.17)	0.001
NAA/Cho	1.19 (0.54)	1.40 (0.54)	0.0003
NAA/Cr	1.40 (0.36)	1.43 (0.45)	0.836

t-ATP, total Adenosine Triphosphate; PCr, phosphocreatine; Pi, inorganic phosphate; Lac, lactate; Cr, creatine; ($\pm 3\sigma$), 99.73% confidence interval.

optimized by fitting the in vivo signal with a combination of metabolite signals by nonlinear least square techniques. For ^{1}H-MRSI, the absolute concentration of metabolites from signal intensity can be fitted to a simplified equation as published.[15] The concentration of the water of white matter is considered to be equal to 35.88 mmol/L.[15] The absolute quantification of metabolites measured using ^{31}P-MRSI is challenging due to the lack of water reference which can be used as a denominator. No corrections were carried out for the different ^{31}P T1 values for metabolites within the brain due to the long time lapses associated with accurate T1 measurements.

Statistical analysis

Quantitative variables collected before and after biotin administration were compared using the Wilcoxon matched pairs signed rank test. Correlations were performed with a nonparametric Spearman test. Unpaired quantitative data comparisons used a Mann–Whitney test. A P value less than 0.05 was considered significant. Statistical analyses were performed using Statview V5.0.

Results

Nineteen patients (women:11) were enrolled (5 PP-MS, 14 SP-MS), with an average age of 55.3 years(SD ± 8.5). Mean MS duration was 266.5 months(SD ± 105.6) and time to conversion was 113.1 months(SD ± 59.3) in patients with SP-MS. No significant difference was found between patients with PP-MS and SP-MS progressive forms regarding sociodemographics or baseline clinical characteristics. All patients presented WML.

We observed an improvement of EDSS in seven patients (37%) and absence of disability progression in 12 patients (63%). The test walk of 6 min was slightly improved for 37% of patient and was improved over 20% for five patients (Table 1).

We observed a significant increase in t-ATP level ($P = 0.0003$) without significant variation in PCr/t-ATP, PCr/Cr, and PCr/Pi. Moreover, we found a decrease in lactate($P = 0.02$) and pH normalization($P = 0.0004$). As regards membrane metabolites, we observed a significant increase in PME/PDE ratio ($P = 0.0002$) and PDE ($P = 0.0002$), particularly for GPC ($P = 0.0002$) (Table 2). In addition, we found a significant positive correlation between the perimeter of test walk on 6 min and the NAA/Cr ratio ($\rho = 0.727$; $P = 0.02$). No difference was observed for the other clinical parameters.

Discussion

Bioenergetic imbalance would be due to a mitochondrial dysfunction and energetic consumption to support neuron life-sustaining conditions and promote remyelination.[3,4] Biotin action by carboxylases activation (1) promote citric acid ring from amino acid catabolism then driving oxidative phosphorylation toward ATP production; (2) sustain neuronal viability and induce a lipid synthesis to the remyelination.[16] Hattingen et al have established the interest of investigating multiple sclerosis using MMRS,[17], demonstrating the importance of monitoring ATP, pHi, NAA, lactate, and free lipid resonance as well as PME and PDE and choline-containing compounds.[18,19]

In our study, we found an increase in the PME/PDE ratio ($P = 0.0002$) and a decrease in lactates ($P = 0.02$). Moreover, we observed pHi normalization ($P = 0.0004$) and increased ATP level ($P = 0.0003$). This is consistent with the regressive mitochondrial impairment due to biotin effects.[16]. However, these metabolite variations were not correlated with the EDSS improvement found in our study.[5]

Referring to the equation below, we believe that this result may be explained by oxidative phosphorylation normalization rather than chemical equilibrium displacement of the dephosphorylation reaction of PCr[20]. In that case, the PCr/Cr and PCr/Pi ratio stability observed in our study may not be surprising.

$$PCr + ADP^{-} + H^{+} \underset{F^{CK_r} = k_{-1}[ATP]}{\overset{F^{CK_f} = k_1[PCr]}{\rightleftharpoons}} ATP + Cr \underset{F^{ATPase_r} = k_{-2}[Pi]}{\overset{F^{ATPase_f} = k_2[ATP]}{\rightleftharpoons}} Pi + ADP$$

The decrease in choline/Cr and the increase in the PME/PDE ratio are related to the decline in membrane catabolism marked by decreased of GPC. As previously suggested,[18,19] these ratios may provide an early indicator of remyelination. Moreover, the significant increase in NAA/Cho ratio ($P = 0.0003$) is strongly correlated with the significant decrease in lactate ($\rho = -0.647$, $P = 0.009$), which seems to be consistent with the increase in the PME/PDE ratio, as evoked in [17].

Our results assessing clinical improvement, significant decrease in EDSS, significant increase in walking perimeter over 6 min, fit with previous studies(2,15). However, these features should be interpreted with caution, since in progressive MS, plateau phases are quite common and

spontaneous improvement in EDSS may occasionally occur. Furthermore, the impact of other factors (physiotherapy, concomitant diseases) upon disability and walking ability were not taken into account.

This was a preliminary MMRS study designed to test the impact of biotin in MS follow-up. The main limitation of our study is the small sample size In order to assess the link between treatment and MS evolution, each patient was his own control. The next step will be a placebo-controlled study including more patients to confirm our results. Expensive time acquisition caused us to limit the number of metabolites explored. Glutamate and glutamine were not evaluated.

In conclusion, we provide results suggesting a potential interest of MMRS to monitor biotin treatment response in progressive MS. Our results were significant and consistent with metabolic pathways concerned by biotin action. MMRS could be a useful biomarker of biotin therapeutic response.

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Author Contributions

PA, CG, MN, NM, RG, and JPN contributed to the study concept and design. PA, CG, MN, and SR contributed to data acquisition and analysis. PA, CG, RG, SR, GH, and JPN contributed to drafting the manuscript. PA and CG contributed equally.

Conflict of Interest

The authors declare that they have no conflicts of interest concerning this article.

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