1HMRS Study in Siblings with Disorder of Respiratory Chain Complex I Activity without Established Molecular Background

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Abstract

We report the results of follow-up study of the siblings with disorder of respiratory chain complex I activity without established molecular background. The dynamics of the metabolic changes was studied during two years using the ¹H NMR \textit{in vivo} method. The spectra were acquired from the cerebellum. The NAA relative levels as well as lipids and lactate were found to be the most sensitive markers of disturbances of mitochondrial processes. The MRS findings are clearly related to the patients' symptoms.

Keywords: Mitochondrial disorder, Complex I deficiency, ¹H MRS in vivo

Introduction

Defects of mitochondrial oxidative phosphorylation are known to be associated with an increasing number of diseases, and may give rise to any symptom, in any organ or tissue dependent upon mitochondrial energy supply [1]. Complex I deficiency (#MIM 252010) is the most frequently observed mitochondrial disorder and no curative therapy as yet exists.

The aim of this study was to characterize the dynamics of the metabolic changes in the siblings with the disorder of respiratory chain complex I activity without established molecular background. ¹H NMR method was applied and the follow-up period was over two years.

Methods

NMR measurements were done using the whole-body 2T MRI/MRS system operating at a proton resonance frequency of 81.3 MHz. ¹H NMR \textit{in vivo} spectra were acquired from the left cerebellum (PRESS sequence, TR 1500 ms, TE 35/136 ms and 100 acquisitions; voxel volume 1.5×1.5×1.5 cm³). The metabolite ratios were calculated using the user independent method of spectra analysis [2]. The activity of mitochondrial respiratory chain complexes was determined spectrophotometrically in muscle homogenates according to the method described in ref. [3].

Results

Two siblings (KKO, male, 11 years, and KKA, female, 9 years) have been under the neurological supervision since the second month of life. The measurements of the enzymatic activities of the respiratory chain complexes in a muscle postnuclear supernatant showed evidence of clear complex I deficiency (Table 1). MRI performed twice during their childhood revealed the cerebellar atrophy.

The ¹H NMR \textit{in vivo} acquired twice (at the age of 9 and 7, and at 11 and 9, in KKO and KKA, respectively) gave similar results for both the children (Figure 1a, b): NAA/tCr
is reduced (>2SD from the normal mean) and Cho/NAA is significantly increased, however the ratios are stable in time; Cho/tCr is normal, which confirms the NAA level drop; AlaLip/tCr is high, but relatively stable in time and LacLip/tCr is high but stable within the observation period only for KKA; for KKO it increases markedly. The other metabolite ratios are normal.

Table 1. Lactate level in plasma and cerebrospinal fluid (CSF) and enzymatic activities of respiratory chain complexes measured in muscle postnuclear supernatant

<table>
<thead>
<tr>
<th>Parameter</th>
<th>KKO, 14 months</th>
<th>KKA, 5 months</th>
<th>Normal values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma lactate [mM]</td>
<td>1.4</td>
<td>1.3</td>
<td>0.6-2.3</td>
</tr>
<tr>
<td>CSF lactate [mM]</td>
<td>2.1</td>
<td>1.0</td>
<td>0.8-2.2</td>
</tr>
<tr>
<td>CSF protein [mg/dl]</td>
<td>12</td>
<td>26</td>
<td>&lt;40</td>
</tr>
<tr>
<td>KKO, 11 years</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma lactate [mM]</td>
<td>7.0</td>
<td>5.6</td>
<td>0.6-2.3</td>
</tr>
<tr>
<td>CSF protein [mg/dl]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Citrate synthase (CS) [nmol/min/mg protein]</td>
<td>120</td>
<td></td>
<td>96-150</td>
</tr>
<tr>
<td>Complex I (%CS)</td>
<td>&lt;3</td>
<td></td>
<td>8-18</td>
</tr>
<tr>
<td>Complex II (%CS)</td>
<td>6.5</td>
<td></td>
<td>7-12</td>
</tr>
<tr>
<td>Complex III (%CS)</td>
<td>75</td>
<td></td>
<td>45-94</td>
</tr>
<tr>
<td>Complex II+III (%CS)</td>
<td>4.2</td>
<td></td>
<td>6-10</td>
</tr>
<tr>
<td>Complex IV (%CS)</td>
<td>17</td>
<td></td>
<td>19-34</td>
</tr>
</tbody>
</table>

The parents did not agree for the lumbar puncture

Fig. 1. Dynamics of the metabolic changes in (a) patient 1 (KKO) and (b) patient 2 (KKA)
Discussion

In the present study, the proton NMR spectroscopy shows a decreased cerebellum NAA, although not progressing, whereas the values of Cho/tCr are not different from that for the control group (Figure 1a, b). The choline level stability indicates chronic and slowly progressive demyelinating processes [4-6]. In case of acute alterations in cell membrane integrity increase in choline is usually seen, whereas impairment of the membrane maintenance due to reduced energy production, possible in mitochondrial diseases, may lead to choline reduction. In all the spectra the intense Lac/Lip band is observed. If it reflected exclusively dysmyelination and demyelination, the higher Cho/tCr ratios should be expected. Thus, the high lipid signals seen in the siblings’ spectra indicate presumably also the disturbance of lipid metabolism. Recent findings indicate that complex I may be considered an important factor in the regulation of oxidative phosphorylation. It is also claimed to be the main site of radical oxygen species (ROS) production in mitochondria. The impairment of mitochondrial complex I activity attributable to ROS-induced phospholipid damage may increase the electron leak from the electron transport chain, generating more ROS and perpetuating a cycle of oxygen-radical–induced damage, which ultimately leads to a decrease in oxidative phosphorylation and to brain failure [7, 8].

Conclusions

The dynamics of the metabolic changes observed by means of $^1$H NMR in vivo reflects the patients’ neurological status. The NAA relative levels as well as lipids and lactate are found to be the most sensitive brain markers of the disturbances of mitochondrial processes in complex I deficiency. Though in complex I deficiency lactate levels in blood and in cerebrospinal fluid are highly informative, $^1$H NMR in vivo, as a noninvasive brain biochemical biopsy, can improve the diagnostic accuracy and allows treatment monitoring.

Acknowledgements

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References