Favourable clinical course in an infant with severe deficiency of complex III of the respiratory chain combined with less severe deficiencies of complexes I, II and IV

Abstract An infant with severe deficiency of complex III combined with less severe deficiencies of complexes I, II and IV of the mitochondrial respiratory chain in skeletal muscle tissue presented with intra-uterine growth retardation, generalized hypotonia and delayed motor development. In the following 3.5 years muscle tone and motor development gradually normalized whereas the lactic acidosis and enzyme activities did not improve.

Conclusion This report documents a favourable clinical course in a child with combined respiratory chain deficiency despite persistent biochemical abnormalities.

Key words Mitochondria • Combined deficiency

Abbreviation RC respiratory chain

Introduction

The clinical presentation of patients with isolated or combined defects in the respiratory chain (RC) is variable. Neonatal or early infant manifestations usually predict a severe clinical course. A more benign course has rarely been reported. Five patients have been reported with a benign complex IV deficiency; in all of them, the enzyme activity normalized at the time of clinical improvement [2, 6, 7, 10, 11]. One report describes a benign complex I deficiency, but there are no data available regarding enzyme activity after clinical improvement [9]. Here we describe an infant with persistent severe deficiency of complex III combined with less severe deficiencies of complexes I, II and IV and a favourable clinical course.

Case report

This 1.7 kg (<3rd percentile) infant was born after 38 weeks gestation and had Apgar scores of 9 and 10 at 1 and 5 min, respectively. Length was 41 cm (<3rd percentile). The umbilical pH was 7.34. She was the third child of unrelated healthy parents. Two older siblings are healthy. The mother had two miscarriages.

Physical examination at birth revealed no abnormalities except for generalized hypotonia. During the first 5 weeks of life tube feeding became necessary because of diminished sucking. At 4 months of age she was readmitted to hospital because of failure to thrive. Physical examination showed a lively infant with a weight of 3840 g (percentile <10), length 54 cm (percentile <10), head circumference 39.8 cm (percentile 10). Axial hypotonia, mild hypertonia of the limbs, and atrophy of shoulder, arm and leg muscles were observed. The sucking reflex was moderately active and there were generalized stretch responses. No other abnormalities were noted.

Repeated blood analysis showed increased lactate concentrations (3–10 mmol/l; N < 2.1), increased lactate to pyruvate ratios (40–60; N 10–15), elevated concentrations of 3-OH-butyrate (0.18–1.60 mmol/l; N 0.02–0.09) and acetoacetate (0.06–0.34 mmol/l; N 0.016–0.040) and elevated 3-OH-butyrate to acetoacetate ratios (2.8–4.7; N < 2). Postprandial ketosis was present. Blood gas analyses showed a normal pH, CO₂, and base excess and slightly decreased bicarbonate (20 mmol/l). Lactate concentration in CSF was 5.3 mmol/l (N < 2.1). Urine organic acid profile showed an
increased concentration of lactate (3.0 mmol/mmol creatinine \( N < 0.1 \)) and increased concentration of 3-OH-butyric acid (0.22 mmol/mmol creatinine \( N < 0.06 \)). Creatinine, urea and transaminases were normal. Plasma alpha-tocopherol (9.5 \( \mu \text{mol/l; } N 15.6-43.8 \)) and beta-carotene (<0.01 \( \mu \text{mol/l; } N 0.10-0.80 \)) concentrations at the age of 3 months were reduced but normalized after supplementation. Electroencephalography and magnetic resonance imaging of the brain showed no abnormalities.

A defect in the RC was suspected and a biopsy of the vastus lateralis muscle was performed at the age of 4.5 months.

The clinical course was characterized by refusal to eat necessitating tube feeding. After her 3rd birthday her eating progressively improved and tube feeding was stopped. Her growth remained below but paralleled the 10th percentile curve despite adequate caloric intake and no signs of malabsorption. Despite persistent increased lactate concentrations in blood, the axial hypotonia and mild hypertonia of the limbs normalized in the 1st year. Her motor development was initially delayed (she sat unaided at the age of 1 year and walked at the age of 22 months) but normalized hereafter. Her motor development according to the Hoskins-Squires scale \([4]\) at 3.8 years was 3 years. The quality of the movements at this age was appropriate. Trunk tone was lower than normal. However, the tone elsewhere was normal. Assessment of motor development at 4.5 years was age adequate. There was never exercise intolerance. Psychological assessment according to the McCarthy Scale of Childrens Abilities \([5]\) at age of 3.9 years revealed a score of 68 (2 SD under average), at age 4.6 years the score had improved to 80 (more than 1 SD under average) with a homogeneous test profile on both occasions.

**Methods**

Surgical biopsies of the vastus lateralis muscle were performed, with informed parental consent, at age 4 months and age 3 years 8 months. Light microscopy, histochemical and enzyme-histochemical stainings were performed according to conventional procedures. Substrate oxidation rates, ATP production rates and mitochondrial enzyme activities in fresh muscle were measured in 600 \( g \) supernatants of muscle homogenates as described by Fischer et al. \([3]\) and Sperl et al. \([8]\). Complex III was measured using decyldiubiquinol and horse cytochrome c as electron donor and acceptor, respectively. Tween 20 (0.04%) was added to abolish the non-enzymatic reaction and to break the mitochondria (Bentlage, unpublished results).

In order to search for the molecular cause of the disorder, total cellular DNA was isolated from muscle tissue as previously described by De Vries et al. \([1]\). Using restriction digestion of PCR-generated fragments of mitochondrial DNA, the DNA of the patient was tested for pathogenic mutations. Southern blot analysis was used to search for mitochondrial DNA deletions. PvuII-digested DNA was separated on a 0.8% agarose gel and subsequently blotted to a nylon membrane. Hybridization with \( ^{32} \text{P} \)-labelled complete human mitochondrial DNA followed. Next, the gene for cytochrome b was sequenced using the dye terminator cycle-sequencing kit and the ABI A370 automated sequencer (Perkin Elmer).

**Results**

Examination of the first vastus lateralis muscle specimen showed normally distributed type I and type II fibres. Histochemical staining for oxidative enzymes and glycogen gave normal results, but fibres contained large fat deposits. Electron microscopy showed enlarged abnormal mitochondria with concentric cristae. Examination of the second vastus lateralis muscle specimen showed a high percentage of unequally distributed type I fibres (70 versus 50 normally). Muscle fibres still showed signs of delayed maturation at this age, but diameters were normal. There were no fibres with internal nuclei, and succinate dehydrogenase and cytochrome c oxidase stains were normal. Electron microscopy demonstrated abnormal mitochondria with too few cristae, degenerating mitochondria, and mitochondria with dense deposits suggestive for early crystalloid formation (Fig. 1).

**Fig. 1A–D** Abnormal mitochondria in vastus lateralis muscle. (A) many mitochondria with dense deposits (B) magnification of a mitochondrion in A (C) mitochondria with too few cristae (D) a degenerating mitochondrion. Arrows in A, B, and D point to dense deposits representing early crystalloid formation. Bars: 0.5 \( \mu \text{m (black)} \) and 0.1 \( \mu \text{m (white)} \).
The oxidation rates with pyruvate and malate as substrates and ATP + creatine phosphate production rates from these substrates were severely decreased (Table 1). The activity of rotenone-sensitive NADH:ubiquinone oxidoreductase (complex I) was diminished (52% and 18% of the lowest control value in the first and second biopsy, respectively). Succinate dehydrogenase (complex II) activity was 75% and 84% of the lowest control value, in the first and second biopsy respectively. Succinate:cytochrome c oxidoreductase (complex III) was diminished (37% of the lowest control value in both biopsies). The decylubiquinol:cyt c oxidoreductase (complex III) activity was undetectably low in the first biopsy and 11% of the lowest control value in the second biopsy. Cytochrome c oxidase (complex IV) was diminished (91% and 65% of the lowest control value in the first and second biopsy respectively). Pyruvate dehydrogenase complex (PDHC) was normal in both biopsies.

None of the tested pathogenic mutations: A3243G (MELAS), T3271C (MELAS), A4317G (MELAS), A8344G (MERRF), T8993G/C (NARP/Leigh syndrome), were detected. A deletion in the mitochondrial DNA of the patient was excluded. Hybridization with 32P-labelled complete human mitochondrial DNA revealed a single band of 16.5 kb representing the full-length linearized mitochondrial DNA. Cytochrome b sequencing showed one base substitution, G15043A, which does not change the aminoacid composition of the cytochrome b polypeptide.

### Table 1 Biochemical parameters of vastus lateralis muscle tissue

<table>
<thead>
<tr>
<th>Oxidation rates&lt;sup&gt;a&lt;/sup&gt; for [1-14C]Pyruvate + malate</th>
<th>1st biopsy age 4 months (%)&lt;sup&gt;e&lt;/sup&gt;</th>
<th>2nd biopsy age 38/15 years (%)&lt;sup&gt;e&lt;/sup&gt;</th>
<th>Control range</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>[U-14C]Malate + pyruvate + malonate</td>
<td>0.57 (16)</td>
<td>0.47 (13)</td>
<td>3.61-7.48</td>
<td>14</td>
</tr>
<tr>
<td>ATP + creatine phosphate production rates&lt;sup&gt;b&lt;/sup&gt; from Pyruvate + malate</td>
<td>4.25 (10)</td>
<td>4.76 (11)</td>
<td>42.1-81.2</td>
<td>14</td>
</tr>
<tr>
<td>Succinate + acetylcarnitine</td>
<td>n.d.</td>
<td>n.d.</td>
<td>7.2-18.4</td>
<td>14</td>
</tr>
<tr>
<td>Activity of NADH:Q&lt;sub&gt;1&lt;/sub&gt; oxidoreductase&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.050 (52)</td>
<td>0.017 (18)</td>
<td>0.096-0.197</td>
<td>13</td>
</tr>
<tr>
<td>Succinate dehydrogenase&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.050 (75)</td>
<td>0.056 (84)</td>
<td>0.067-0.177</td>
<td>11</td>
</tr>
<tr>
<td>Succinate:cyt c oxidoreductase&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.11 (37)</td>
<td>0.11 (37)</td>
<td>0.30-0.97</td>
<td>38</td>
</tr>
<tr>
<td>Decylubiquinol: cyt c oxidoreductase&lt;sup&gt;d&lt;/sup&gt;</td>
<td>n.d.</td>
<td>0.31 (11)</td>
<td>2.85-6.71</td>
<td>22</td>
</tr>
<tr>
<td>Cytochrome c oxidase&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.74 (91)</td>
<td>0.53 (65)</td>
<td>0.81-3.12</td>
<td>21</td>
</tr>
<tr>
<td>Pyruvate DH complex&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.045 (136)</td>
<td>0.041 (124)</td>
<td>0.033-0.122</td>
<td>25</td>
</tr>
<tr>
<td>Citrate synthase&lt;sup&gt;c&lt;/sup&gt;</td>
<td>124 (258)</td>
<td>78 (162)</td>
<td>48-162</td>
<td>32</td>
</tr>
</tbody>
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<sup>a</sup> nanomole substrate oxidized per hour per milliunits citrate synthase
<sup>b</sup> nanomole product formed per hour per milliunits citrate synthase
<sup>c</sup> milliunits per milligram protein
<sup>d</sup> milliunits per milligram protein
<sup>e</sup> Percentage of lowest control
n.d. not detectable

Discussion

This girl presented in the neonatal period with intrauterine growth retardation, generalized hypotonia and diminished sucking. There were no obvious signs of CNS involvement. As an infant she was flaccid but alert and responsive, in contrast to the lethargy associated with encephalopathy. Her motor development was delayed at first but normalized; other developmental aspects were adequate. There were no clinical signs of encephalopathy. MRI of the brain and electroencephalography at the age of 4.5 months, 2 and 4 years, showed no abnormalities. The lactate concentration in CSF was elevated but this may only reflect the high concentration in blood rather than CNS involvement. Biochemical studies in skeletal muscle showed a severe complex III deficiency combined with less severe deficiencies of complexes I, II and IV. A second biopsy specimen taken at a time when she was free of symptoms showed identical abnormalities. Molecular studies showed one base substitution in cytochrome b, which does not change the aminoacid composition of the cytochrome b polypeptide and therefore reflects a silent polymorphism.

This case illustrates that a severe deficiency of enzyme complexes of the respiratory chain in muscle, with significantly impaired overall substrate oxidation and ATP production rates, can have a favourable clinical course. Persistent biochemical abnormalities suggest that there are unknown adaptation mechanisms in skeletal muscle. Whether or not alpha-tocopherol and beta-carotene supplementation contributed to the favourable outcome remains unknown.

Acknowledgement We are gratefully indebted to A. J. M. Janssen for his skilled technical assistance.

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