A mitochondrial encephalomyopathy with a partial cytochrome c oxidase deficiency of muscle


From the Institutes of Neurology, Pediatrics and Submicroscopic Morphology, University Hospital Nijmegen, The Netherlands

SUMMARY A 16 year old girl showed delayed psychomotor development. In infancy, exercise intolerance, cerebellar signs, deteriorated with increasing intercurrent infections, and disturbances of breathing and cardiac rhythm became manifest. From the age of 7 years there was chronic progressive psychomotor deterioration, with hypotonia, a bilateral pyramidal and cerebellar syndrome, and mild epilepsy. CSF pyruvate and lactate levels were elevated, and lactate content was elevated in the urine. There was an abnormally high rise of lactate levels on moderate exercise and an abnormal response to pyruvate loading. Quadriceps muscle biopsies obtained at age 10 and 16 years showed ragged-red fibres, and a decreased cytochrome c oxidase activity and cytochrome aa₃ content. Cytochrome c oxidase activity in fibroblasts was normal. Clinical signs and symptoms in association with a disturbance of mitochondrial energy metabolism led us to a diagnosis of probable Leigh syndrome.

In the clinical spectrum of mitochondrial myopathies Petty et al. identified three subgroups: (1) chronic progressive external ophthalmoplegia (CPEO) and limb weakness, (2) proximal weakness with fatigue, (3) predominantly or exclusively central nervous system (CNS) disease. The recognition that CNS disease is a prominent feature in a subgroup of the mitochondrial myopathies prompted Shapira et al. to expand the concept of mitochondrial myopathy to mitochondrial encephalomyopathy. This latter group of disorders encompasses the syndromes of Alpers, Leigh, dysmyelination, myoclonus epilepsy associated with ragged-red fibres (MERRF) and a syndrome indicated by the acronym MELAS (mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes).

We present a girl with a mitochondrial encephalomyopathy and a clinical diagnosis of probable Leigh syndrome and a partial cytochrome c oxidase deficiency of muscle.

Case reports

The patient is the youngest child of healthy non-consanguineous parents. There is one healthy sib. The oldest sib died at age 12 after an accident. Family history reveals no neurodegenerative disorders. Two sibs of the father suffer from epilepsy. Pre-, peri- and postnatal periods of the patient were uneventful. Psychomotor development was delayed. She was able to sit without help at age 18 months and walk at age 3 years. She spoke her first words at age 2½ years. Motor performance was poor and deteriorations often with slow recovery was noted with intercurrent infections. During these periods of deterioration, frequent sighing, tachy- and bradypnoea, and tachy- and bradycardia were evident. Occasionally she had partial oculomotor nerve paresis on the left, that subsided within one week. Progressive exercise intolerance became evident. At age 5 years, neurological examination revealed myopathic and cerebellar signs, brisk tendon reflexes and flexor plantar responses. Total IQ (WISC-R) was 95. At age 7 years, a slowly progressive psychomotor deterioration set in, and...
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absence-like epileptic manifestations appeared, that were treated with ethosuximide.

At age 10 years, she was referred to our department. Examination revealed a mentally retarded girl, with normal height, weight and skull circumference. There were no degenerative stigmata. General physical examination was normal. There was generalised hypotonia, muscle wasting, and proximal muscular weakness, with increased lumbar lordosis, genu recurvatum and a positive Gowers' sign. Facial expression was poor with mild bilateral ptosis, and monotonous dysarthric speech. Cerebellar involvement was shown by ataxia of rump and extremities, and marked dysdiadochokinesis. Tendon reflexes were brisk with spontaneous ankle clonus and bilateral Babinski signs. Steady slowly progressive deterioration of the neurological status occurred in the following years. She is now 16 years old with total IQ 53. She has been free of seizures and without antiepileptic medication for the last 4 years.

Electrophysiological and radiological investigations

Electroencephalogram showed focal (right temporal) and generalised epileptic discharges. Electromyography and nerve conduction velocities were normal. Somatosensory, brainstem auditory and visual evoked potentials were within the normal ranges. Radiographs of skull, chest, hand and vertebral column, cerebral computed tomography and cerebral nuclear magnetic resonance imaging were normal. The electrocardiogram was normal and the echocardiogram showed no signs of cardiomyopathy.

Laboratory investigations

Standard blood, urinary and CSF studies revealed no abnormality. Serum levels of glucose, pyruvate, lactate, β-hydroxybutyrate and acetoacetate, their ratios, and responses of glucose, pyruvate and lactate to oral glucose loading (1.75 g/kg) were in the normal ranges. Lactate excretion was elevated in one 24-hour urine sample (131 pmol/mmol creatine, normal <100). The McArdle/ Fischbein ischaemic exercise test yielded a normal rise of serum lactate and ammonia levels. Moderate exercise (walking for a few minutes) gave a rise of serum pyruvate to 335 μmol/l (normal 60–155 μmol/l under resting conditions) and of serum lactate to 6480 μmol/l (normal 460–1720 μmol/l under resting conditions); the lactate-pyruvate ratio was 18.2 (normal up to 15) and serum pH remained normal with a base excess of −3.9. An intravenous pyruvate loading test, (500 mg/kg) showed an abnormal response in that there was a rise of the lactate level after 5 minutes post-infusion to a maximum at 15 minutes.

In the CSF, the levels of glucose, β-hydroxybutyrate and acetoacetate were normal. The levels of pyruvate (222 μmol/l, normal 85–132 μmol/l) and lactate (1900 μmol/l, normal 1200–1600 μmol/l) were elevated with a normal lactate-pyruvate ratio.

Histopathological studies

Quadriceps muscle biopsies were performed at the age of 10 and 16 years. Histopathological studies showed no abnormalities except for the occurrence of ragged red fibres in both specimens. The cytochrome c oxidase stain showed a normal appearance in most fibres, but the ragged-red fibres showed strongly increased activity (fig. a). Electronmicroscopy revealed no structural abnormalities of the mitochondria, and crystal-like inclusions did not occur. Cytochrome c oxidase activity was normal in all fibres investigated (fig. b).

Biochemical studies

In fresh homogenate of quadriceps muscle obtained by biopsy at age 10 years, pyruvate oxidation rate and activities

Fig Cytochrome c oxidase stain. The fibres show a normal activity except for the ragged-red fibre in the middle, that shows a strong increase of activity (bar = 50 μm). (b) Electronmicrograph of section stained for cytochrome c oxidase activity. Normal activity is seen in all mitochondria (x 42,000).
Table. Results of biochemical studies in the 600 g supernatant of the two muscle tissue specimens

<table>
<thead>
<tr>
<th>Patient</th>
<th>Controls</th>
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<tbody>
<tr>
<td></td>
<td>1980</td>
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**Oxidation**

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<tr>
<th>Reaction</th>
<th>1980</th>
<th>1986</th>
<th>Range</th>
<th>Mean, SD</th>
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<tbody>
<tr>
<td>[1→14C]pyruvate + malate</td>
<td>208*</td>
<td>273-705</td>
<td>473, 117</td>
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<tr>
<td>[1→14C]pyruvate + carnitine</td>
<td>237*</td>
<td>266-641</td>
<td>504, 169</td>
<td>20</td>
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<tr>
<td>[U→14C]malate + pyruvate + malonate</td>
<td>270*</td>
<td>320-996</td>
<td>621, 211</td>
<td>20</td>
<td></td>
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<tr>
<td>[U→14C]malate + acetyl carnitine + malonate</td>
<td>343*</td>
<td>317-1155</td>
<td>575, 220</td>
<td>20</td>
<td></td>
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<tr>
<td>[U→14C]malate + acetyl carnitine + arsenate</td>
<td>196*</td>
<td>198-517</td>
<td>294, 80</td>
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**ATP metabolism**

<table>
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<th>1986</th>
<th>Range</th>
<th>Mean, SD</th>
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<tr>
<td>ATP + CrP production from pyruvate</td>
<td>2075†</td>
<td>3354-9993</td>
<td>5910, 2168</td>
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<td>ATP + CrP production/pyruvate oxidation</td>
<td>10-0</td>
<td>8-8-150</td>
<td>11-8, 1-8</td>
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**Enzyme activities**

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<th>1986</th>
<th>Range</th>
<th>Mean, SD</th>
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<td>Citrate synthase</td>
<td>41†</td>
<td>48-146</td>
<td>77, 33</td>
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<tr>
<td>Cytochrome c oxidase</td>
<td>41</td>
<td>73-284</td>
<td>194, 92</td>
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<td>Succinate-cytochrome oxidoreductase</td>
<td>8-7§</td>
<td>10-33</td>
<td>18, 7</td>
<td>9</td>
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<tr>
<td>NADH:O2 oxidoreductase</td>
<td>15-3</td>
<td>4-5-23</td>
<td>13, 7</td>
<td>7</td>
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*nmol [14CO2/hr/mg protein; †nmol/hr/mg protein; ‡μmol/min/mg protein; §nmol cytochrome c reduced/min/mg protein; ¶nmol NADH oxidized/min/mg protein.

of citric acid cycle and respiratory chain were evaluated by measuring [14CO2] production from [1→14C]pyruvate and [U→14C]malate.12 ATP production by muscle homogenate was measured according to Ruitenbeek et al.13 The activity of cytochrome c oxidase was measured in muscle homogenate and in isolated mitochondria. Cytochrome content was measured in isolated mitochondria.15 Protein was assayed according to Lowry et al.16

At age 16 years a quadriceps muscle specimen was obtained by needle biopsy. Carnitine content,17 and activities of cytochrome c oxidase,14 citrate synthase,18 succinate-cytochrome c oxidoreductase19 and NADH:O2 oxidoreductase20 were measured, after storage of the muscle at −70 °C. Oxidative metabolism was also evaluated in cultured fibroblasts by measuring [14CO2] production rate from [1→14C]pyruvate and [2→14C]pyruvate,21 and the activities of cytochrome c oxidase12 and citrate synthase.17

**Results**

The table shows the biochemical findings in both muscle specimens. [14CO2] production rates from [1→14C]pyruvate and [U→14C]malate by muscle homogenate were slightly diminished. ATP + CrP production with pyruvate as substrate was also decreased. The ratio of the ATP + CrP production to the arsenite sensitive pyruvate oxidation was normal. Total carnitine content (carnitine + acylcarnitines) was normal in muscle homogenate. The specific activity of cytochrome c oxidase in muscle homogenate and in isolated mitochondria was about 20% of the mean control value. This enzymatic defect is associated with a lack of cytochrome aa3. The ratio of the content of this protein of the cytochrome c oxidase complex to the content of cytochrome b and c + c1 is diminished (0-59:1.1:20; control ratio 1-08:1.1:52). [14CO2] production rates from (1→14C)pyruvate and (2→14C)pyruvate by fibroblasts, and the activities of cytochrome c oxidase and citrate synthase were normal.

**Discussion**

We describe a 16-year-old girl with a slowly progressive degenerative neurological disorder characterised by myopathy, mental retardation, pyramidal, cerebellar and ocular signs, frequent sighing, marked fluctuations of respiratory and cardiac rate, and long-lasting deteriorations with intercurrent infections are other clinical characteristics.

Laboratory investigations showed an elevation of lactate excretion in 24-hour urine. Lactate concentrations showed an abnormal increase on moderate exercise, and after pyruvate loading. In the CSF, levels of pyruvate and lactate were elevated. These findings are suggestive of a defect in pyruvate metabolism in this patient.

At the age of 10 and 16 years quadriceps muscle biopsy specimens showed ragged-red fibres. Biochemical studies showed a slight decrease of [14CO2] production from pyruvate and malate, and a decrease of ATP production from pyruvate to one-third of the mean control value. Cytochrome c oxidase activity in muscle homogenate as well as in isolated muscle mitochondria was decreased to 20% of the mean control value. These data and the lack of cytochrome aa3 protein point to a disturbance of mitochondrial oxidative metabolism at the level of cytochrome c oxidase. Involvement of the central nervous system and elevation of pyruvate and lactate in CSF make it likely that the same defect was present in CNS. Although the disorder had a multisystem character, no defect could be detected in fibroblasts.
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In various patients with partial or complete cytochrome c oxidase deficiency, an overall decrease or complete lack of histochemical reactivity of cytochrome c oxidase have been reported. Apparently the residual activity of cytochrome c oxidase in our patient was sufficient to give normal light- and electron-microscopic appearance.

Cytochrome c oxidase deficiency clinically presents in a heterogeneous fashion: it can manifest as a fatal infantile myopathy with or without De Toni-Fanconi-Debré syndrome or as a benign spontaneously remitting myopathy of infancy. Cytochrome c oxidase deficiency has also been reported in two types of mitochondrial encephalomyopathies: the syndromes of Alpers and Leigh. Complete lack of histochemically demonstrable cytochrome c oxidase activity has been reported in individual fibres in patients with chronic progressive ophthalmoplegia, but biochemical studies most often revealed normal cytochrome c oxidase activity. The deficiency of cytochrome c oxidase described in Menkes' kinky hair disease has a secondary nature, due to secondary deficiencies of copper dependent enzymes, one of which is cytochrome c oxidase.

Our patient fits in with the clinical picture of Leigh syndrome and we have made a clinical diagnosis of Leigh syndrome in our patient.

Recently, a patient with a childhood encephalomyopathy with cytochrome c oxidase deficiency in muscle and platelets, resembling our patient, was reported by Angelini et al. This patient, an 8 year old boy, had muscle wasting with proximal weakness, ataxia and mental impairment, but no pyramidal signs. There was parental consanguinity in this case, pointing to an autosomal recessive mode of inheritance.

In other patients with Leigh syndrome, the deficiency of cytochrome c oxidase was present in skeletal muscle, heart muscle, brain, kidney, liver and cultured fibroblasts. Normal enzyme activities have been reported in liver tissue, and in liver tissue and cultured fibroblasts of Leigh patients with a cytochrome c oxidase deficiency of muscle.

There are strong indications of the existence of tissue-specific isoforms of the complex enzyme cytochrome c oxidase, that might explain the differential involvement of tissues. The apoprotein consists of multiple subunits, three of which are encoded by mitochondrial DNA, whereas the rest is encoded by nuclear DNA. As mitochondrial DNA is maternally transmitted, a non-mendelian type of inheritance is possible in cases of cytochrome c oxidase deficiency. However, this has until now not been documented in respiratory chain defects, and autosomal recessive inheritance seems most probable.

To elucidate aetiological mechanisms, enzyme abnormalities will have to be identified at the molecular level with modern techniques of immuno-cytochemistry and molecular genetics. Immunodetection allows identification of the enzyme subunit composition. The amount of immunologically reacting protein was normal in two Leigh patients with cytochrome c oxidase deficiency, and diminished or absent in patients with a fatal myopathy with or without renal dysfunction. As human mitochondrial DNA has been fully sequenced, it is likely that mutations can be demonstrated at DNA level within a few years. Definition of biochemical errors at the molecular level will disclose different molecular defects of cytochrome c oxidase that constitute the basis of the different phenotypic expressions of the enzyme deficiency.

This investigation is part of the research program "Disorders of the Neuromuscular System" of the University of Nijmegen.

References

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