Eur J Pediatr (1995) 154:915–918

ORIGINAL PAPER

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Neonatal De Toni-Debré-Fanconi syndrome due to a defect in complex III of the respiratory chain

Received: 9 September 1994
Accepted: 10 February 1995

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Abstract A patient with neonatal expression of severe De Toni-Debré-Fanconi syndrome is presented. Because of early signs of renal tubulopathy together with a large urinary excretion of lactate, 3-hydroxybutyrate and citric acid cycle intermediates, a mitochondrial disorder was suspected and muscle and liver biopsies were performed. Biochemical investigations in both tissues revealed a defect in the respiratory chain at the level of complex III. In this patient renal dysfunction was the primary symptom, and hyperlactataemia, an important clue for a mitochondrial disorder, was lacking.

Conclusion Complex III deficiency should be included in the differential diagnosis of neonatal De Toni-Debré-Fanconi syndrome.

Key words De Toni-Debré-Fanconi syndrome · Respiratory chain defects · Complex III

Abbreviation *ATP* adenosine triphosphate

Introduction

The renal tubulopathy De Toni-Debré-Fanconi syndrome is seen in different disorders of the mitochondrial respiratory chain with impaired adenosine triphosphate (ATP) production required for proper renal tubular transport activity (Fig. 1). It is relatively common in fatal infantile myopathy due to cytochrome c oxidase (complex IV) deficiency [2, 9, 15] and has also been reported as a feature of complex I and combined complex III/IV deficiencies [6, 7, 11]. Moreover, De Toni-Debré-Fanconi syndrome is invariably involved at a late stage of Pearson syndrome, a mitochondrial disorder with a mitochondrial DNA deletion affecting different organs including the kidneys [4, 8, 10, 13]. Here we report the neonatal expression of severe renal tubulopathy associated with complex III deficiency of the respiratory chain in muscle and liver.

Case report

A 2-month-old baby was admitted to the hospital because of failure to thrive. He was born by normal delivery after an uncomplicated 42-week pregnancy. Birth weight was 2.5 kg, length was 48 cm. He was the fourth child of a consanguineous Turkish couple. Family history was unremarkable.

On admission the child presented with a mild upper respiratory tract infection, laryngeal stridor due to tracheomalacia, hyperpnoea, wide open fontanelles and sutures, rickets, irritability, muscle weakness and insufficient response to stimuli. He was mildly dehydrated; weight was 3.0 kg. Laboratory tests showed metabolic acidosis (pH 7.28, bicarbonate 15 mEq/l, base excess – 11) with otherwise normal serum electrolytes, glucose and creatinine. Transaminases (about 150 U/l) and alkaline phosphatase (1250 U/l) were elevated. The latter indicated active rickets. Inorganic phosphate (0.46 mmol/l and 1.4 mg/dl) and uric acid (53 µmol/l and 0.89 mg/dl) were decreased. Serum triglycerides (3.3 mmol/l and 2.92 mg/dl) were moderately elevated. Blood lactate (1.7–2.9; normal < 2.0 mmol/l) and pyruvate (0.077; normal < 0.065 mmol/l) concentrations were normal or mildly elevated. Serum amino acids, including alanine, were normal. The urinary hyperexcretion of amino acids of all classes, glucose, bicarbonate and phosphate indicated proximal tubulopathy.

At the age of 4 months biochemical investigation also included the analysis of urinary organic acids. There were markedly in-

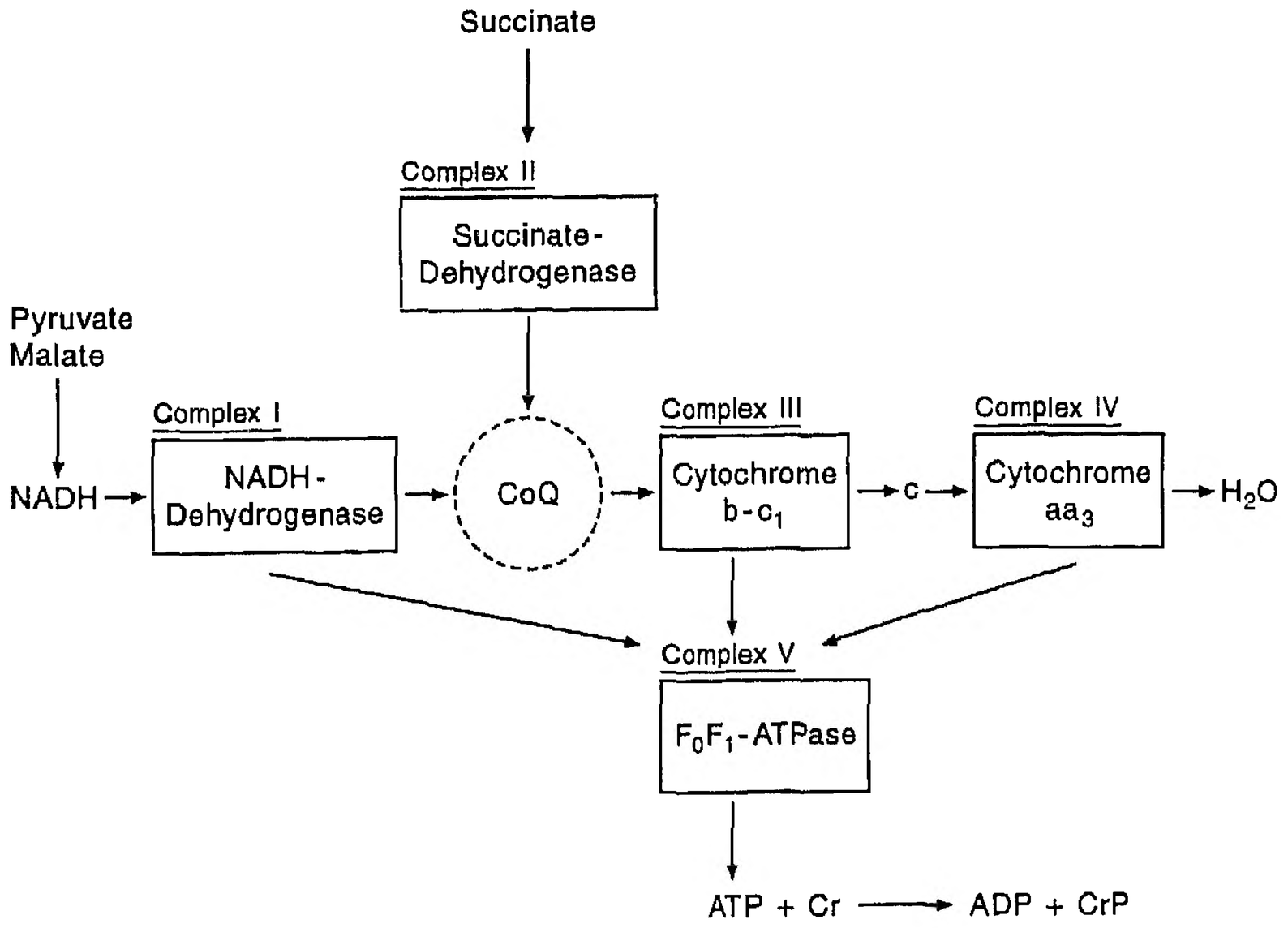

Fig. 1 Schematic presentation of the mitochondrial respiratory chain (*CoQ* coenzyme Q)

Table 1 Urinary organic acid and alanine excretion (mmol/mol creatinine) in our patient, in a 15-month-old child with cystinosis, in a 16-year-old boy with severe ifosfamide nephropathy and in controls

	Patient	Cystinosis	Ifosfamide nephropathy	Controls $n = 320$
Lactate	28,500	800	150	< 200
3-Hydroxy-butyrate	25,000	5,400	35	< 80
Fumarate	260	45	1	< 20
Malate	1020	120	3	< 50
Succinate	850	0	25	< 150
Alanine	12,020	850	500	< 160

creased amounts of lactate and 3-hydroxybutyrate (Table 1) together with a complex pattern of organic acids (fumarate, succinate, and malate), suggestive of a respiratory chain defect. In CSF, lactate and amino acid concentrations were normal. Serum total and free carnitine levels were also normal.

From the age of 4 months the patient was treated symptomatically with bicarbonate, phosphate supplements, 1,25-dihydroxycholecalciferol, and carnitine. Later he also received vitamin K (40 mg/day) and riboflavin (100 mg/day). However, his clinical condition deteriorated and the child died of respiratory distress at the age of 7 months. No autopsy was performed and no post mortem kidney tissue was available.

Methods

Because of the early occurring De Toni-Debré-Fanconi syndrome and massive urinary excretion of lactate, a mitochondrial disorder was suspected. In order to obtain mitochondrial material for biochemical and morphological investigations a surgical muscle biopsy of the quadriceps and a needle biopsy of the liver were performed.

Substrate oxidation rates, ATP production rates, mitochondrial enzyme activities and carnitine content were measured in 600 g supernatants of muscle homogenates as described previously [3, 12]. Complex III activity was measured using decylubiquinol as a substrate according to Zheng et al. [16] with modifications. Light microscopy, histochemical and histo-enzymological stainings were done according to standard procedures. Mitochondrial DNA was analysed as reported earlier [1]. Other laboratory investigations were performed according to routine procedures.

Results

Biochemical investigation in fresh skeletal muscle supernatant revealed that oxidation rates with pyruvate, malate and succinate as substrates and ATP+creatine phosphate production rates with pyruvate and malate were both severely diminished (< 20% of lowest control values). These data, as well as the activities of the respiratory chain subunits in muscle and liver and carnitine concentration in muscle, are listed in Table 2. Due to the small amount of liver tissue, only few activities could be measured. Table 2 shows that complex III activity was most severely decreased (in muscle 13% of the lowest control value). The activities of succinate: cytochrome c oxidoreductase and NADH: O_2 oxidoreductase, which both measure also complex III activity [14], were less reduced (50% of the lowest control values). These values point to a severe deficiency of complex III of the respiratory chain. A complex III deficiency (59% of the lowest control value) was also found in the liver. Carnitine concentration (total and free) was reduced in muscle.

Histopathological findings in muscle revealed a normal fibre structure with an increased lipid content in type I fibres. Ragged red fibres were not seen. On electron microscopy there were no structural abnormalities of the mi-

Table 2 Biochemical parameters of muscle and liver tissue

	Patient	Control range	
Muscle			
Oxidation rate[a] for			
[1 - ^{14}C] pyruvate + malate	56	273–705	(n = 19)
[U - ^{14}C] malate + pyruvate + malonate	38	320–996	(n = 19)
ATP + creatine phosphate production rate[a] from			
pyruvate + malate	535	1833–8075	(n = 25)
succinate + acetylcarnitine	142	814–1527	(n = 7)
Activity[b] of			
citrate synthase	45	48–162	(n = 32)
cytochrome c oxidase	54	68–437	(n = 54)
succinate: cytochrome c oxidoreductase	11	22–89	(n = 35)
decylubiquinol: cytochrome c oxidoreductase	32	253–619	(n = 10)
NADH: Q_1 oxidoreductase	5.3	4.4–25.9	(n = 28)
NADH: O_2 oxidoreductase	12	24–87	(n = 9)
pyruvate dehydrogenase complex	4.4	2.7–8.2	(n = 15)
Concentration[c] of			
carnitine, total	1.6	2.7–4.6	(n = 21)
carnitine, non esterified	1.2	2.2–4.2	(n = 21)
creatine, total	20	15–36	(n = 9)
Liver			
Cytochrome c oxidase[a]	71	37–53	(n = 4)
Succinate cytochrome oxidoreductase	3.1	6.0–51	(n = 4)
Decylubiquinol: cytochrome c oxidoreductase	41	69–107	(n = 4)

[a] nmoles · hr^{-1} · mg^{-1} protein
[b] mU · mg^{-1} protein
[c] µmoles · g^{-1} wet weight

tochondria. Some muscle fibres contained fat vacuoles close to the mitochondria. Liver morphology was without specific abnormalities. No mitochondrial DNA deletions or frequently occurring mutations were found.

Discussion

We describe a patient in whom onset of severe De Toni-Debré-Fanconi syndrome in early infancy lead us above all to look for a biochemical defect at the level of mitochondrial oxidative phosphorylation. This was done under the assumption that the energy requiring process of renal tubular transport might be severely disturbed due to a failure of the respiratory chain to produce adequate amounts of ATP. Since kidney tissue could not be obtained for biochemical investigations, we strived to demonstrate the assumed respiratory chain defect in other organs such as muscle and liver. In both tissues a severely decreased activity of complex III of the respiratory chain was found giving rise to a significantly impaired oxidative phosphorylation in whole mitochondria.

Usually, patients suspected of having a mitochondrial disorder are selected after demonstration of elevated lactate concentrations in blood and/or CSF. Due to the absence of evidently increased lactate concentrations in both body fluids, a defect in the respiratory chain could have been overlooked in our patient. It might be possible that the high renal clearance of lactate (daily loss: 14–20 mmoles) caused by the disrupted tubular reabsorption was responsible for the almost normal lactate levels. Interestingly, another case of severe respiratory chain defect in muscle (deficient cytochrome-c-oxidase activity) and De Toni-Debré-Fanconi syndrome also with absence of severely elevated blood lactate levels has been reported [9]. However, with respect to the extreme amounts of urinary lactate, 3-hydroxybutyrate, and other organic acids, as compared with other patients with a serious form of tubulopathy due to cystinosis or ifosfamide toxicity (Table 1), it seems that part of these metabolites originates also from high production in the kidney itself. According to earlier reports [4, 13] an increased production and excretion of lactate and particularly of intermediates of the citric acid cycle was explicitly attributed to tubulopathy due to mitochondrial disorders.

The block at the complex III level impairs oxidation of both NAD-linked and FAD-linked substrates (Fig. 1). In most previous publications the diagnosis of complex III (ubiquinol:cytochrome c oxidoreductase) deficiency was concluded indirectly from a reduced activity for both succinate:cytochrome c oxidoreductase and rotenone-sensitive NADH: O_2 oxidoreductase and a normal activity of succinate dehydrogenase [5, 14]. In our patient complex III activity was measured directly by a spectrophotometric assay, using decylubiquinol as a substrate. This activity was severely reduced in skeletal muscle and liver. The re-

duced rate of pyruvate, malate, and succinate oxidation, as well as of ATP production in fresh muscle suggest that the overall respiratory chain activity in vivo was significantly impaired by the complex III deficiency. The low muscle carnitine concentration is a secondary phenomenon which is frequently found in respiratory chain disorders.

Complex III deficiency is a relatively rare type of mitochondrial disorder. Moreover, among the small number of patients (ca. 20) described with a defect of complex III there is great variability in the clinical presentation, including tissue specific defects such as pure skeletal muscle disease and pure fatal cardiomyopathy, as well as progressive encephalopathy [5]. A severe complex III deficiency in muscle and liver could recently also be demonstrated in tissues (stored at –80°C) from the neonate with severe lactic acidosis and renal tubulopathy, described by Sperl et al. [11] by using decylubiquinol as a substrate in the measurement of complex III activity (residual activity 16% of the lowest control value). Thus, the present and the previously reported case expand the clinical spectrum of complex III defect to include neonatal expression of severe renal tubulopathy.

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