Eur J Pediatr (1994) 153:745–750

ORIGINAL PAPER

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Long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency: a severe fatty acid oxidation disorder

Received: 11 November 1993
Accepted: 22 March 1994

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Abstract 3-Hydroxyacyl-CoA dehydrogenase deficiency is a newly recognised fatty acid oxidation disorder with a usually fatal outcome. We present a further patient who presented with hypoketotic hypoglycaemia, hepatopathy, secondary carnitine deficiency and increased plasma long-chain acylcarnitines. 3-Hydroxydicarboxylic aciduria was present and the diagnosis confirmed in cultured skin fibroblasts. Our patient is compared with those reported in the literature with respect to clinical symptoms, differential diagnosis and possible therapeutic regimens.

Key words Fatty acid oxidation
Cardiomyopathy · Hypoketotic hypoglycaemia · 3-Hydroxyacyl-CoA dehydrogenase deficiency

Abbreviations *CoA* co-enzyme A *LCAD* long-chain acyl-CoA dehydrogenase · *LCHAD* long-chain hydroxyacyl-CoA dehydrogenase *MCAD* medium-chain acyl-CoA dehydrogenase · *MCT* medium-chain triglycerides · *SCAD* short-chain acyl-CoA dehydrogenase · *VLCAD* very long-chain acyl-CoA dehydrogenase

Introduction

Inherited defects in mitochondrial fatty acid oxidation have become of increasing importance over the last few years. Since fatty acids are an essential metabolic fuel, particularly in times of stress or prolonged fasting, defective oxidation can lead to symptoms recognised as "sudden infant death syndrome", Reye-like episodes, hypoketotic hypoglycaemic coma, muscle weakness and profound cardiological dysfunction [10].

Fatty acids are initially activated to co-enzyme (CoA) esters and after carnitine-mediated transfer, are oxidised via acyl-CoA dehydrogenases, enoyl-CoA hydratase, 3-hydroxyacyl-CoA dehydrogenase and 3-ketoacyl-CoA thiolase (Fig. 1). There are four acyl-CoA dehydrogenases with different chain length specificities – short-chain (SCAD), medium-chain (MCAD), long-chain (LCAD) and very long-chain (VLCAD) – and defects in these enzymes have been recognised [11, 12, 25, 27, 35]. It may well be that most LCAD patients have VLCAD deficiency [35]. There are two 3-hydroxyacyl-CoA dehydrogenases having different substrate specificities [7]. The membrane-associated higher molecular weight enzyme has the greater activity toward long-chain substrates (LCHAD), whereas the enzyme with maximum activity for short-chain substrates appears to be in the mitochondrial matrix [7]. It was recently reported that LCHAD is part of a mitochondrial trifunctional protein containing long-chain enoyl-CoA hydratase and long-chain 3-oxo-

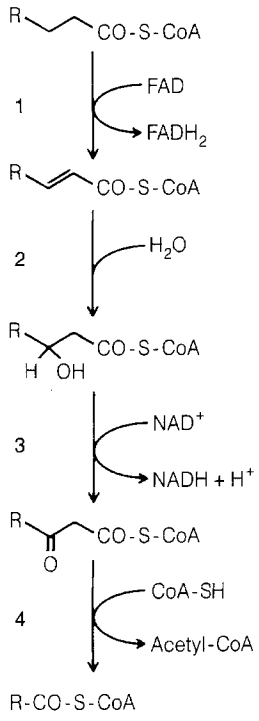

Fig. 1 Mitochondrial fatty acid oxidation. Acyl-CoA esters enter the spiral and are dehydrogenated by acyl-CoA dehydrogenases *(1)*. Enoyl-CoA hydratase *(2)* adds water to give a 3-hydroxy-CoA which is oxidised by 3-hydroxyacyl-CoA dehydrogenase *(3)*. In the presence of free CoA (CoA-SH), 3-ketoacyl-CoA thiolase *(4)* gives acetyl-CoA and a two-carbons shorter acyl-CoA fatty acid

acyl-CoA thiolase activity [2]. One patient with a deficiency of all three enzyme activities has been reported [33].

LCHAD deficiency has been shown to be associated with lethal cardiomyopathy in childhood [23] and to date over ten patients have been described [1, 5, 6, 13, 15, 16, 20, 21, 31, 32]. We describe a further new case in comparison to those previously published addressing the presenting clinical features and diagnostic possibilities together with appropriate treatment modalities.

Case report

The male patient was the first child of non-consanguineous, Caucasian parents. The father was treated for hypertension and had chronic hepatitis. The mother had a son by a previous marriage who had a ventrical septal defect. Pregnancy and delivery were uncomplicated. Birth weight was 3520 g, length 54 cm, head circumference 37 cm and Apgar was 10/10. The patient required phototherapy for hyperbilirubinaemia (14 mg/dl) for 4 days, whereon he lost weight and became lethargic with loss of appetite. After discharge, the patient was breast-fed but was referred to a general paediatrician because of weight loss and dehydration. Glucose i.v. rapidly reversed the situation. The child was thereafter bottle-fed.

At the age of 1 month the child was referred because of a borderline Guthrie screen for phenylalanine (4 mg/dl) which subsequently normalised (1.38 mg/dl). He had a slight gastro-oesophageal reflux but fed well and was otherwise healthy.

At 4 months of age the infant was re-admitted because of post-prandial vomiting which occurred ca. 2–3 h after feeding (ca. 600 ml Babyfit, Humana). During the previous 3 months, he had been found unresponsive in bed on two separate occasions but could be aroused. In the course of the previous 2–3 weeks he had slightly lost weight and appetite and was combatting a viral infection (influenza-like illness with laryngitis). Physical examination at this time was unremarkable (weight 7170 g, length 66 cm, head circumference 45.5 cm). Neurological examination showed a mild general muscular hypotonia. Cerebral ultrasound revealed slight dilatation of the lateral ventricles and the interhemispheric fissure, head circumference having just crossed the 97th percentile. The EEG (during quiet sleep) showed dysrhythmic slow waves over the temporal regions, predominantly over the right side. Serum transaminases were elevated (SGOT 129 U/ml; SGPT 190 U/ml) whereas other blood chemistries and haematological parameters were normal. Hepatopathy was suspected and further metabolic investigations instigated. A possible fructose intolerance was excluded by a normal fructose loading test.

During the next few days the patient's general condition deteriorated and he became increasingly apathic. The liver increased in size (9 cm under right costal margin) and he had a hypoglycaemic (blood glucose 23 mg/dl; 1.3 mmol/l) episode and disturbed coagulation parameters (decelerated Quick, increased PTT). Laboratory investigations at this time revealed increased transaminases (GOT 83 U/l, GPT 104 U/l), increased alkaline phosphatase (656 U/l), elevated free fatty acids (2.25 mmol/l) and slightly increased lactate (2.96 mmol/l). 3-Hydroxybutyrate was for technical reasons not determined. Total plasma carnitine was decreased (16.0 µmol/l; normal = 32–52) likewise plasma free carnitine (9.8 µmol/l; normal = 27–42). Plasma long-chain carnitine was increased (13.1 µmol/l; normal = < 7.0). A fatty acid oxidation defect was suspected and he was treated with parenteral glucose (15–17 mg/kg/day) and carnitine (100 mg/kg) which succeeded in normalising the plasma carnitine levels.

Muscular hypotonia was severe. He had a shrill cry, became increasingly lethargic, palid, and tachypnoeic; no muscle reflexes could be elicited. Heart rate was 100 beats/min. Chest X-ray showed cardiomegaly. He was transferred to intensive care where cardiac sonography revealed a dilatative cardiomyopathy with an enlarged left ventricle and mitral valve insufficiency. Increasing bradycardia together with the cardiomyopathy led to pulmonary oedema which did not respond to aggressive resuscitation. He died 48 h later due to acute cardiac decompensation before the diagnostic work up was completed and before a therapeutic attempt with MCT could be attempted.

Light microscopy of a liver biopsy obtained immediately post-mortem showed moderate portal fibrosis with

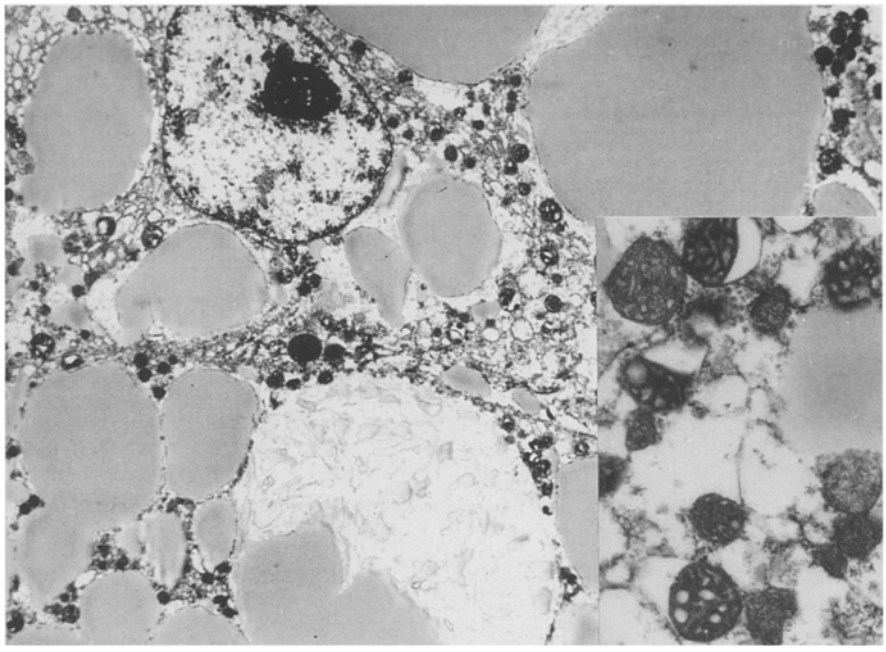

Fig. 2 Electron microscopy of liver tissue showing accumulated fat droplets (× 3.400). *Inset* shows somewhat enlarged mitochondria (× 16.000)

multiple micro- and macrovesicular steatosis. In addition to massive cytoplasmic fat droplets, electron microscopy revealed slightly enlarged mitochondria (Fig. 2).

Methods and results

Urinary organic acids were determined as trimethylsilyl derivatives by gas liquid chromatography-mass spectrometry as previously described [24]. The profile revealed increased lactate with diminished ketones (3-hydroxybutyric acid was not found and the labstix for ketones was negative), a saturated (adipic and suberic) and unsaturated (octenedioic, decendioic) dicarboxylic aciduria and excretion of 3-hydroxyadipic acid, 3-hydroxyadipic acid lactone, 3-hydroxysuberic acid and 3-hydroxydodecandioic acid suggesting a defect at the level of 3-hydroxyacyl-CoA dehydrogenase.

Fatty acid oxidation studies in cultured skin fibroblasts [29] gave normal results for octanoic acid (C8), but lowered rates for palmitic acid (C16). ^{3}H-Myristic acid oxidation [17] was also low (Table 1). The diagnosis was confirmed by enzyme analysis [31]. Although 3-hydroxyacyl-CoA dehydrogenase showed normal activity for acetoacetyl-CoA, the activity toward 3-ketohexadecanoyl-CoA was reduced giving a very low 3-ketohexadecanoyl-CoA/acetoacetyl-CoA activity ratio (Table 2). Thiolase and enoyl-CoA hydratase activities were normal excluding a trifunctional protein deficiency and giving results consistent with an isolated LCHAD defect.

Table 1 Fatty acid oxidation in intact skin fibroblasts (nmol/h/mg)

Substrate	Controls	Patient
1-^{14}C Octanoate	1.43 ± 0.72 (15)	1.11
1-^{14}C Palmitate	3.26 ± 0.98 (19)	0.90
Ratio Palmitate:Octanoate	2.66 ± 0.97 (15)	0.80
^{3}H Myristic acid	5.30 ± 1.86 (26)	1.23

Table 2 3-Hydroxyacyl-CoA dehydrogenase, enoyl-CoA hydratase and 3-ketoacyl-CoA thiolase activities in cultured skin fibroblasts from patient and control subjects (nmol/mg/min)

Enzyme	Substrate	Patient	Controls	
3-Hydroxyacyl-CoA dehydrogenase	Acetoacetyl-CoA (C4)	122	93 ± 28	(n = 56)
	3-Ketohexadecanoyl-CoA (C16)	22	81 ± 21	(n = 56)
	C16:C4 activity ratio	0.17	0.91 ± 0.21	(n = 53)
Enoyl-CoA hydratase	Acetoacetyl-CoA (C4)	418	356 ± 19	
	Dodecanoyl-CoA (C12)	89.9	84 ± 29	
	C12:C4 activity ratio	0.22	0.24 ± 0.04	
3-Ketoacyl-CoA thiolase	Acetoacetyl-CoA			
	$-K^{+}$	6.91	6.04 ± 2.18	(n = 32)
	$+K^{-}$	13.30	12.96 ± 4.21	(n = 32)

Discussion

Patients with inborn errors of fatty acid oxidation usually present with symptoms of fasting intolerance, hypotonia and hepatomegaly. Biochemical hallmarks include secondary carnitine deficiency and dicarboxylic aciduria of varying chain length depending on the site of the enzyme defect. Reports have recently appeared describing hydroxydicarboxylic aciduria in patients with progressive liver disease and cardiomyopathy who exhibited a rapidly fatal course [10, 19]. These symptoms were similar to those seen in LCAD deficiency although these patients do not excrete hydroxydicarboxylic acids, therefore a defect at the level of LCHAD was suspected [19]. To date at least ten patients with enzymatically confirmed LCHAD deficiency have been described and it seemed appropriate to compare our patient with those previously reported.

Our patient had a rapidly fatal course similar to those of Jackson et al. [15], Rocchioli et al. [23] and Poll-Thé et al. [20] (Table 3). All patients including ours had hypoketotic hypoglycaemia and hypotonia. Hepatopathy was present in all patients except that of Dionisi-Vici et al. [5], cardiomyopathy being a leading symptom in all patients except case two of Wanders et al. [32]. The three patients who were diagnosed at a later date appeared to do well despite having cardinal symptoms. Retinopathy was confined to the two cases of Poll-Thé et al. [20], that of Bertini et al. [1] and that of Ribes et al. [21], whereby the three former patients also showed peripheral neuropathy, the latter finding being more common in peroxisomal diseases. The patient of Dionisi-Vici et al. [5] had no hepatopathy but myoglobinuria, a symptom seen in carnitine-palmitoyl transferase deficiency [4].

Morphological findings in LCHAD deficiency include fatty infiltration and abnormal mitochondria, although these findings are common to several fatty acid oxidation disorders. Histological examination of a liver biopsy from our patient revealed such findings, confirming the observations in those patients in whom morphological studies were performed (Table 3). Laboratory results in LCHAD deficiency are characterised by increased saturated and unsaturated 3-hydroxydicarboxylic acids in urine without ketosis [23]. 3-Hydroxymonocarboxylic acids can be oxidised to completion by hepatic mitochondrial oxidation, whereas 3-hydroxydicarboxylic acids are only oxidised by skeletal/heart muscle mitochondria producing the 3-hydroxydicarboxylic aciduria present in LCHAD patients [34]. However, 3-hydroxysebacic and other 3-hydroxycarboxylic acids are also found in urine during ketoacidosis [9] and excessive 3-hydroxysebacic acid excretion has also been reported in peroxisomal disorders [22]. Moreover, 3-hydroxydicarboxylic acids have been associated with toxic reactions to acetaminophen and intrinsic liver disease [18], hence care must be taken in interpreting results of organic acid analysis when 3-hydroxydicarb-

Table 3 Clinical and biochemical parameters in patients with LCHAD deficiency (*LCC* long-chain carnitine, *3-OH-DCA* 3-hydroxydicarboxylic acids)

	Hale et al. [12]	Poll-The et al. [20] Wanders et al. [32]		Rocchioli et al. [23]	Jackson et al. [15]		Wanders et al. [31] Duran et al. [6]	Dionisi-Vici et al. [5]	Ribes et al. [21]	Bertini et al. [1]	Moore et al. [16]	Present case
		1	2		1	2						
Age at diagnosis	9 months	9 months	3 years	5 months	5 months	3.5 months	5 months	13 months	3.3 years	11 months	5 months	4 months
Age at death	?	Alive	Alive	9 months	5 months	Alive	Alive	Alive	4.5 years	11 months	Alive 2 years	4 months
Hypoketotic hypoglycaemia	+	+	+	+	+	+	+	+	+	+	+	+
Cardiomyopathy	+	–	–	+	+	+	+/–	+	+	+	+	+
Hypotonia	+	+	+	+	+	+	+	+	+	+	+	+
Hepatopathy	+	(+)	(+)	+	+	+	+	–	+	+	+	+
Retinopathy	–	+	+	–	–	–	–	–	+	+	–	–
Myoglobinuria	–	–	–	–	–	–	–	+	–	–	–	–
Peripheral neuropathy	–	+	+	–	–	–	–	–	–	+	–	–
Abnormal mitochondria	?	+	+	+	?	?	?	–	+	?	–	(+)
Lactic acidosis	(+)	?	?	(+)	+	+	+	–	?	+	+	+
Carnitine deficiency	+	?	?	+	?	+	+	+	+	?	+	+
Elevated LCC in plasma	+	?	?	?	?	+	+	?	?	?	+	+
Saturated 3-OH-DCA	+	+	+	+	+	+	+	+	+	+	+	+
Unsaturated 3-OH-DCA	+	+	+	+	+	+	+	+	+	?	+	+
Deficient LCHAD	+	+	+	+	+	+	+	+	+	+	+	+

oxylic aciduria is present. Lactic acidosis may also be an important hallmark of this disease since it does not normally occur in fatty acid oxidation defects. A secondary carnitine deficiency was present in most patients including ours, with increased plasma long-chain acylcarnitines (Table 3). In most cases, therapeutic attempts were unsuccessful. The patients of Jackson et al. [15], Duran et al. [6] and Moore et al. [16] responded to treatment with MCT and riboflavin. In those patients given carnitine, no effect on the eventual outcome was observed. Perhaps carnitine therapy in this disease is contra-indicated, since long-chain acylcarnitines are known to be cardiotoxic [3].

Enzymatic confirmation was provided in all cases using cultured skin fibroblasts. The assay can also be performed in leucocytes and cultured chorionic villus cells [30] thus providing the possibility of prenatal diagnosis. Recently molecular analysis has revealed a single mutation located in the dehydrogenase coding part of the mitochondrial trifunctional protein enabling prenatal diagnosis to be performed at the molecular level [14].

In conclusion, LCHAD deficiency appears to be a devastating fatty acid oxidation defect which, when detected early, may be treatable. It is important to underline the differential diagnoses which may mimic such a defect. In our patient, as well as in a patient of Pollitt et al. [18], the clinical and biochemical findings were suggestive of hereditary fructose intolerance which could be excluded by lack of improvement on a fructose-free diet and a normal i.v. fructose tolerance test. There are also clinical and biochemical similarities between LCHAD deficiency and other fatty acid oxidation defects such as LCAD deficiency [12], systemic carnitine deficiency [26] and carnitine-palmitoyl transferase deficiency [4]. Since heart muscle is virtually dependant upon fatty acid oxidation as a fuel supply, it is of no surprise that defects in the mitochondrial oxidation of long-chain fatty acids give rise to cardiological problems. In LCHAD and LCAD deficiencies the muscular and cardiac involvement is consistent with the accumulation of long-chain carnitines and long-chain dicarboxylic acids as toxic intermediates [28]. Recent studies in cultured human myocytes have demonstrated that long-chain carnitines directly activiate the calcium channel allowing an increased influx of calcium ions eventually producing arrythmia [8].

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Note added in proof We recently found that the patient described in this paper is homozygous for the common G 1528 C mutation in the dehydrogenase coding part of the α-subunit of multifunctional protein as recently identified (Wanders RJA, Ijlist L, Ushikubo S, Kamijo T, Hashimato T (1993) Molecular basis of long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency: identification of the major disease-causing mutation. Enzyme Prot 47: 173–174).