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A new peroxisomal β -oxidation disorder in twin neonates: Defective oxidation of both cerotic and pristanic acids

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Summary: Twin brothers were born with clinical symptoms indicating that they were suffering from Zellweger syndrome. However, instead of a generalized peroxisomal dysfunction, only very long-chain fatty acids and the pristanic acid/phytanic acid ratio were elevated in plasma and decreased oxidation of very long-chain fatty acids and pristanic acid was the only impairment found in fibroblasts. The other peroxisomal parameters tested were normal, including normal oxidation of phytanic acid and normal activity of dihydroxyacetonephosphate acyltransferase in fibroblasts as well as normal plasma bile acids. Although the biochemical results point to a defect in peroxisomal β -oxidation, the isolated finding of impaired oxidation of very long-chain fatty acids and pristanic acid has to our knowledge not been reported previously and is difficult to explain by a deficiency of a known peroxisomal β -oxidation enzyme.

Zellweger syndrome is an inherited disease caused by a generalized loss of peroxisomal functions. Clinically it is a distinct entity with many characteristic symptoms such as dysmorphic features, hypotonia, hepatomegaly and psychomotor retardation, and biochemically it is characterized by the absence of peroxisomes and undetectable or low activity of most of the enzymes normally present in the peroxisomes. It has now become clear that Zellweger syndrome can be caused by a defect in one of several genes. Complementation studies have shown the involvement of at least 9 different genes, each associated with impaired biogenesis of peroxisomes when mutated (Shimozawa et al 1993; Moser et al 1995). Only a few of these genes have been truly identified (Shimozawa et al 1992; Dodt et al 1995).

Patients with Zellweger-like appearance but with intact peroxisomes and only lacking the activity of one of the peroxisomal β -oxidation enzymes have also been described. Goldfischer and colleagues (1986) were the first to report such a patient showing all the clinical signs and symptoms of Zellweger syndrome. Pseudo-Zellweger syndrome was the name given to the entity shown to be due to a deficiency of peroxisomal thiolase (Goldfischer et al 1986; Schram et al 1987). As the name indicates, the clinical symptoms were very similar to those seen in classical Zellweger patients. Biochemically, however, peroxisomes were present and only the concentrations of very long-chain fatty acids (VLCFA), dihydroxycholestanoic acid (DHCA) and trihydroxycholestanoic acid (THCA) were elevated in plasma, which follows logically from the fact that peroxisomal 3-ketothiolase is involved in the β -oxidation of all these substrates (see Wanders et al 1995a). Patients lacking the peroxisomal bifunctional enzyme also display Zellweger-like symptoms and the same elevation of VLCFA, DHCA and THCA as in thiolase deficiency (Watkins et al 1989).

Pseudo-neonatal adrenoleukodystrophy is due to the deficiency of peroxisomal acyl-CoA oxidase (Poll-The et al 1988). As this enzyme is specific for the oxidation of VLCFA, only VLCFA are elevated. Besides the disorders of peroxisomal β -oxidation described above, several patients have been described with a defect of peroxisomal β -oxidation of unknown aetiology (see Wanders et al 1995a for discussion and references).

We report on twin brothers with clinical symptoms indicative of a Zellweger-like disease and highly elevated plasma VLCFA. In addition, an elevated pristanic acid/phytanic acid ratio was found while all other peroxisomal parameters were found to be normal, including normal plasma bile acids.

CASE REPORTS

Case 1 (Ho.O.): This boy was born to Moroccan parents who were first cousins. The pregnancy was complicated by severe anaemia. Caesarean section was performed at an estimated gestation period of 27 weeks owing to suspected intrauterine growth retardation. His birthweight was 2190g and length 49cm. The head circumference was 35cm. At birth he was very hypotonic. He never presented hypoglycaemia. His liver function tests were slightly elevated and remained so the rest of his life. On the second day he had generalized tonic-clonic seizures lasting for 5 minutes. EEG showed sharp waves and spikes, leading to antiepileptic treatment with phenobarbital and valproate. Only a moderate reduction in seizure activity was achieved. MRI showed defective neuronal migration, especially localized parietally. He had micrognathia and a high arched palate. Forehead and orbit were normal. Examination of the eyes was normal. Tube feeding with Profylac and Allomin was necessary owing to the hypotonia. He was thriving well on this regimen. His seizures persisted despite increased antiepileptic treatment. He died at 3 weeks of age owing to pneumonia and respiratory arrest. No fibroblast culture was established from this patient.

Case 2 (Ha.O.): This patient was the twin brother of case 1. His birth weight was 2365g and his length was 51cm. Head circumference was 33.5cm. Severe hypotonia was present from birth and on the second day he too presented with generalized seizures. EEG showed

paroxysmal activity, and treatment with phenobarbital and valproate was initiated. As in the twin brother, MRI showed a neuronal migration disorder. No cataract or fundus abnormalities were found on eye examination. Antiepileptic therapy was changed to oxcarbazepin because of increased seizure activity. He had a large forehead. In the neonatal period the liver was of normal size but, as with his brother, the liver function tests were slightly abnormal. At 5 months of age his liver had increased to 5cm below the costal margin. The spleen was just palpable. The severe hypotonia persisted, although he was able to suck part of his meals. He was thriving on Profylac and Allomin mainly supplied by tube feeding. At 7 months of age his clinical condition was unchanged. At 8 months of age he died owing to respiratory arrest. A skin fibroblast culture was established from this patient.

METHODS

Dihydroxyacetonephosphate acyltransferase (DHAPAT) activity was determined by a method slightly modified from the one published by Wanders and colleagues (1995b). The incubation time was lowered to 30min instead of 2h, into the range where activity was linear with time. The activity of phytanic acid oxidase was determined by measuring the release of $^{14}\text{CO}_2$ from $[1-^{14}\text{C}]$ phytanic acid by intact fibroblasts as described earlier (Brandt et al 1989). The activity of pristanic acid and cerotic acid (hexacosanoic acid) oxidation was measured by determination of $^{14}\text{CO}_2$ and ^{14}C -acid soluble material from $[1-^{14}\text{C}]$ pristanic acid and $[1-^{14}\text{C}]$ cerotic acid as described in detail (Wanders et al 1995c). Immunoblot analysis of peroxisomal β -oxidation enzyme proteins in fibroblasts was performed according to Wanders and colleagues (1995d). Pipecolic acid was determined by a capillary gas chromatographic method with electron-capture detection using $[^2\text{H}_{11}]$ pipecolic acid as internal standard (Zee et al 1992).

Very long-chain fatty acids (VLCFA) in plasma and cultured skin fibroblasts were measured by a gas chromatographic–mass spectrometric method using selected-ion monitoring (SIM) (Brandt et al 1989; Stellaard et al 1990). A similar method was used for the analysis of phytanic and pristanic acids (ten Brink et al 1992). Plasma bile acids were analysed according to the method of Stellaard and colleagues (1989).

RESULTS

Both twins had very elevated concentration of VLCFA and elevated ratios of $\text{C}_{26:0}/\text{C}_{22:0}$ and $\text{C}_{24:0}/\text{C}_{22:0}$ in the neonatal period (data not shown). In Table 1 are shown the concentrations and ratios of VLCFA in plasma taken from patient Ha.O. at the age of 4 months. Although the concentration of $\text{C}_{22:0}$ is subnormal and the concentration of $\text{C}_{24:0}$ is normal at that time, the rest of the VLCFA parameters are clearly abnormal. The concentration of phytanic acid was $0.37\mu\text{mol/L}$ (normal range $0.01\text{--}10.0$) and the concentration of pristanic acid was $0.95\mu\text{mol/L}$ (normal range $0.01\text{--}3.0$). Both these concentrations are well within the normal range. However, the ratio of pristanic acid/phytanic acid was 2.57 and this is elevated compared to the normal range of $0.05\text{--}0.40$. Similar elevated ratios are found in patients with defect in peroxisomal β -oxidation (ten Brink et al 1992).

The plasma bile acid profile was normal with normal concentrations of chenodeoxycholic acid (CDCA), cholic acid (CA), DHCA and THCA as well as normal

Table 1 Concentrations of very long-chain fatty acids in patient Ha.O.

<i>Fatty acid</i>	<i>Reference values</i>	
	<i>Fatty acid concentrations (μmol/L)</i>	
C _{22:1}	9.1	1.4–15.9
C _{22:0}	23	34–96
C _{24:1}	118	34–137
C _{24:0}	61	22–83
C _{26:1}	20.8	0.05–1.30
C _{26:0}	11.6	0.22–1.31
	<i>Fatty acid ratio</i>	
C _{24:1} /C _{22:0}	5.13	0.52–2.20
C _{24:0} /C _{22:0}	2.65	0.32–1.19
C _{26:1} /C _{22:0}	0.90	0.001–0.021
C _{26:0} /C _{22:0}	0.50	0.003–0.021

Table 2 Plasma bile acid concentrations in patient Ha.O

<i>Bile acids</i>	<i>Control values in newborns</i>	
	<i>Bile acid concentration (μmol/L)</i>	
CDCA	1.15	0.87–5.98
CA	3.15	0.17–7.42
THCA	0.029	0–0.035
DHCA	0.031	0–0.119
	<i>Bile acid ratio</i>	
THCA/CA	0.009	0–0.015
DHCA/CDCA	0.027	0.001–0.026

Table 3 Enzymatic activities in fibroblasts from patient Ha.O.

	<i>DHAPAT activity</i> (nmol/h per mg protein)	<i>Phytanic acid oxidase activity</i> (pmol/h per mg protein)
Normal controls	6.2 ± 1.0 (<i>n</i> = 10)	2.2 ± 2.8 (<i>n</i> = 18)
Zellweger patient	0.31	0.047
Case 2 (Ha.O.)	8.7	1.98

THCA/CA and DHCA/CDCA ratios (see Table 2). Also the concentration of plasma pipelicolic acid was normal (1.1 μmol/L; normal range 0.70–2.46).

The activities of DHAPAT and phytanic acid oxidase, which are decreased in Zellweger syndrome, were both normal when measured in cultured fibroblasts from one of the patients (see Table 3). In contrast, the oxidation of [1-¹⁴C]pristanic acid and [1-¹⁴C]cerotic acid was severely deficient (see Table 4).

DISCUSSION

The twin brothers had clinical symptoms that might indicate that they were suffering from Zellweger syndrome or one of the similar syndromes affecting peroxisomal metabolism.

Table 4 Fatty acid oxidation by intact fibroblasts from patient Ha.O.

	<i>Fatty acid oxidation</i> (pmol/min per mg protein)		
	<i>Myristic acid</i>	<i>Cerotic acid</i>	<i>Pristanic acid</i>
Normal range	3615 ± 768	961 ± 214	1404 ± 214
Case 2 (Ha.O.)	3897, 4825	4, 3	2, 8

Table 5 VLCFA and the bile acid intermediates in plasma from patients with different defects in peroxisomal β -oxidation

<i>Type of β-oxidation defect</i>	<i>VLCFA</i>	<i>Bile acid intermediates</i>
Acyl-CoA oxidase deficiency	+	N
Bifunctional protein deficiency	+	+
Peroxisomal thiolase deficiency	+	+
Our patients	+	N

N = normal; + = elevated

A generalized peroxisomal defect like 'classical Zellweger syndrome' is excluded by the finding of normal DHAPAT and phytanic acid oxidase activities.

The normal concentration of phytanic acid and pristanic acid could be due to there having been too short a time to accumulate these endogenous compounds, which are probably also found in low concentrations in the two feeding formulas used. However, the strongly elevated concentration of plasma VLCFA and the elevated pristanic acid/phytanic acid ratio are suggestive of a defect in peroxisomal β -oxidation, as directly demonstrated by the deficient oxidation of C_{26:0} and pristanic acid in cultured skin fibroblasts (Table 4). The normal profile of plasma bile acids (Table 2) suggests that bifunctional enzyme and peroxisomal thiolase are normally active, since patients with established deficiencies of these enzymes (Goldfischer et al 1986; Schram et al 1987; Watkins et al 1989) show at least slightly elevated concentrations of abnormal plasma bile acids (Table 5). Furthermore, an immunoblot analysis showed normal 41kDa thiolase, while the patient described by Goldfischer and colleagues (1986) showed absence of immunoreactive thiolase protein.

Although much remains to be learned about the pathway of peroxisomal β -oxidation in man, current thoughts are that the three peroxisomal metabolites C_{26:0}, pristanic acid and di- and trihydroxycholestanoic acid are activated by three distinct synthetases, whereas conversion of the subsequent acyl-CoA esters to their enoyl-CoA esters is catalysed by two distinct oxidases.

The first oxidase (straight-chain acyl-CoA oxidase) primarily accepts straight-chain acyl-CoA esters including the CoA-ester of C_{26:0}, whereas the second oxidase (branched-chain acyl-CoA oxidase) shows preference for branched-chain fatty acyl-CoA esters. This second oxidase (but not the first oxidase) reacts exclusively with pristanoyl-CoA and di- and trihydroxycholestanoyl-CoA (Vanhove et al 1993). In the two patients described in this paper both C_{26:0} and pristanic acid oxidation are impaired, which excludes (straight-chain) acyl-CoA deficiency. In patients with this defect there is accumulation of VLCFAs only with normal bile acid intermediates and normal pristanic acid and phytanic acid, both in absolute as well as relative terms (pristanic/phytanic acid ratio). This is observed in

patients in whom acyl-CoA oxidase is deficient owing to absence of the acyl-CoA oxidase protein (Poll-The et al 1988) but also in patients with acyl-CoA oxidase present but functionally inactive (Suzuki et al 1994). So far no patients have been described with a deficiency at the level of the second oxidase, the branched-chain acyl-CoA oxidase. Based on its substrate specificity one would expect accumulation of bile acid intermediates and pristanic acid. Furthermore, one would expect that oxidation of C_{26:0} would be normal in cultured fibroblasts of such patients, whereas oxidation of pristanic acid would be deficient. The findings in our patient are different from those for any of the patients described in the literature so far and it is difficult to think what the true enzymatic defect is. It may well be that the scheme of peroxisomal β -oxidation as used today is too simplistic and that more enzymes remain to be identified. In that respect, it is important to mention the recent results from Novikov and colleagues (1994), who reported the presence of multiple 3-hydroxyacyl-CoA dehydrogenases at least in rat liver.

Whatever the basic enzymatic defect is, it is important to stress that prenatal diagnosis is possible in such cases of undefined disorders. Indeed, prenatal diagnosis can be made reliably by measuring C_{26:0} and pristanic acid oxidation in cultured chorionic villus fibroblasts.

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