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Human peroxisomal 3-oxoacyl-coenzyme A thiolase deficiency

(peroxisome/Zellweger syndrome)

ANDRÉ W. SCHRAM*, SIDNEY GOLDFISCHER†, CARLO W. T. VAN ROERMUND‡, ELISABETH M. BROUWER-KELDER*, JANNA COLLINS†, TAKASHI HASHIMOTO§, HUGO S. A. HEYMANS‡, HENK VAN DEN BOSCH¶, RUUD B. H. SCHUTGENS‡, JOSEPH M. TAGER*, AND RONALD J. A. WANDERS‡

*Laboratory of Biochemistry, University of Amsterdam, P.O. Box 20151, 1000 HD, The Netherlands; †Departments of Pathology and Pediatrics and the Marion Bessin Liver Research Centre, Albert Einstein College of Medicine, Bronx, NY 10461; ‡Department of Pediatrics, University Hospital Amsterdam, 1105 AZ Amsterdam, The Netherlands; §Shinshu University School of Medicine, Asahi, Matsumoto, 390 Japan; ¶Laboratory of Biochemistry, University of Utrecht, Padualaan 8, 3584 CH Utrecht, The Netherlands

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ABSTRACT **We investigated the peroxisomal β-oxidation system in liver from a patient with clinical features similar to those in the cerebrohepatorenal (Zellweger) syndrome and with elevated levels in body fluids of very-long-chain fatty acids and intermediates in the biosynthesis of bile acids. The peroxisomal β-oxidation of fatty acids, measured as the cyanide-insensitive formation of [^{14}C]acetyl units from [^{14}C]palmitoyl-CoA, was very low in the patient (<10% of the values in control subjects). Immunoblotting experiments using antibodies to peroxisomal β-oxidation enzymes indicated that peroxisomal 3-oxoacyl-CoA thiolase (acyl-CoA:acetyl-CoA *C*-acyltransferase, EC 2.3.1.16) was deficient. Addition of purified rat-liver peroxisomal 3-oxoacyl-CoA thiolase to a reaction mixture containing liver homogenate from the patient restored peroxisomal β-oxidation. We conclude that the deficiency of peroxisomal 3-oxoacyl-CoA thiolase is responsible for the very low peroxisomal β-oxidation activity and for the accumulation of very-long-chain fatty acids and intermediates in the biosynthesis of bile acids. Furthermore, the finding that both very-long-chain fatty acids and abnormal bile acids accumulate in this patient suggests that a single peroxisomal 3-oxoacyl-CoA thiolase is involved in the oxidative chain shortening of both very-long-chain fatty acids and the coprostanoic acids.**

Goldfischer *et al.* (1) recently described a patient with clinical, biochemical, and pathologic features similar in many respects to those seen in the cerebrohepatorenal (Zellweger) syndrome, a disease characterized by a profound deficiency of peroxisomes (2) and a generalized impairment of peroxisomal functions (3–6). In the patient the level of very-long-chain fatty acids in serum was elevated, there was an accumulation of trihydroxycoprostanoic acid in duodenal aspirate, and pipecolic acid concentrations in serum and urine were increased slightly. These metabolites accumulate in tissues and body fluids of patients with the Zellweger syndrome. However, examination of a liver biopsy sample from the patient indicated that peroxisomes were abundant in the parenchymal cells. Furthermore, the activity of the peroxisomal enzyme acyl-CoA:dihydroxyacetone-phosphate acyltransferase was normal in the patient; this membrane-associated enzyme is deficient in the Zellweger syndrome (7, 8). Finally, the peroxisomal oxidation of palmitoyl-CoA was reduced to about 15% of the control values (1).

These biochemical findings suggest that the lesion in the patient involves the peroxisomal β-oxidation system, which is responsible for the oxidation of very-long-chain fatty acids (9) and, probably, for the conversion of trihydroxycoprostanoic acid to cholic acid (10, 11). We have therefore carried out further studies on individual enzymes of the peroxisomal β-oxidation system to examine this possibility. The results described in this paper suggest that the basic defect in the patient is a deficiency of peroxisomal 3-oxoacyl-CoA thiolase (acyl-CoA:acetyl-CoA *C*-acyltransferase, EC 2.3.1.16).

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CASE REPORT

The patient, who was 11 months old at the time of her sudden death, is described in ref. 1. A liver biopsy was performed at 7 days of age with informed consent of the parents. A further liver sample was obtained about 10 hr postmortem.

METHODS

Peroxisomal β-oxidation in liver homogenates was estimated by measuring the rate of the cyanide-insensitive generation of [^{14}C]acetyl units from [^{14}C]palmitoyl-CoA, as described (12). Catalase, glutamate dehydrogenase, and D-amino acid oxidase activities in liver homogenates were measured as described (13).

Crossreactive immunological material in liver homogenates was estimated by an immunoblotting procedure (14), using rabbit antibodies to peroxisomal β-oxidation enzyme proteins isolated from rat liver. As shown previously (14), the antibodies to the rat liver enzyme proteins crossreact with the homologous human proteins.

RESULTS

Table 1 shows the activity of several enzymes in homogenates of liver obtained postmortem from the patient and two control subjects with unrelated disorders. Catalase was not deficient in the patient, in accordance with the results of the histochemical studies reported earlier (1). The activity of glutamate dehydrogenase, a mitochondrial enzyme, was lower in the patient than in the controls, but was within the normal range [1.1–2.4 nmol per min per mg of protein (n = 9); R.J.A.W. and C.W.T.v.R., unpublished observations]. In the patient the activity of D-amino acid oxidase measured in the absence of FAD was lower than that measured in the presence of the coenzyme. However, a similar result was obtained in the liver homogenates from the control subjects, indicating that an apparent deficiency of FAD-dependent oxidases such as that reported earlier (1) can be due to loss or dilution of FAD during preparation and/or storage of a homogenate.

Peroxisomal β-oxidation was measured with palmitoyl-CoA as substrate in the presence of cyanide (to inhibit the mitochondrial β-oxidation of fatty acids) and FAD. The formation of [^{14}C]acetyl units from [^{14}C]palmitoyl-CoA in homogenates of samples of the patient's liver obtained either

Table 1. Enzyme activities and peroxisomal β-oxidation in liver homogenates from the propositus and two control subjects

Enzyme (activity unit)	Enzyme activity per mg of protein		
	Propositus	Control 1	Control 2
Catalase (μmol of O_2 per min)	27	29	54
D-Amino acid oxidase (nmol/min)			
FAD absent	1.25	1.40	0.87
FAD present	6.90	3.90	2.94
Glutamate dehydrogenase (μmol/min)	0.99	1.74	2.44
Peroxisomal β-oxidation* (nmol/min)	0.10 ± 0.035†	1.56 ± 0.18†	1.33 ± 0.09†

Homogenates were prepared from postmortem livers stored at −20°C. Peroxisomal β-oxidation was estimated as the cyanide-insensitive formation of [^{14}C]acetyl units from [^{14}C]palmitoyl-CoA.
*FAD present.
†Mean ± SD (3 separate determinations).

postmortem (Table 1) or by biopsy (data not shown) was markedly reduced.

To determine what was responsible for the reduced ability of the patient's liver to oxidize palmitoyl-CoA, immunoblotting experiments were carried out using antibodies against the peroxisomal β-oxidation enzymes isolated from rat liver. The results obtained with autopsy material are shown in Fig. 1. In a control liver, crossreactive immunological material was present corresponding to the 72-kDa and 52-kDa components of acyl-CoA oxidase (see refs. 14 and 15), the 78-kDa bifunctional protein with enoyl-CoA hydratase and 3-hydroxyacyl-CoA dehydrogenase activities (see refs. 14 and 16), and the 41-kDa, mature form of 3-oxoacyl-CoA thiolase

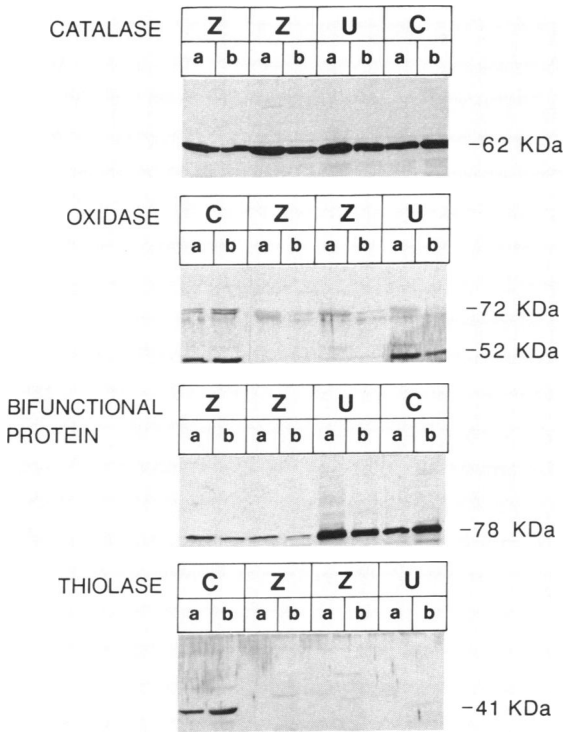

FIG. 1. Identification of crossreactive immunological material in liver homogenates from a control subject (C), two patients with the cerebrohepatorenal (Zellweger) syndrome (Z), and the propositus (patient U), by use of antibodies (described in ref. 14) against bovine catalase and rat liver peroxisomal β-oxidation enzyme proteins. Samples of liver were suspended in 25 mM potassium phosphate, pH 7.0/0.2% (wt/vol) Triton X-100, sonicated, and cleared by centrifugation as described (14). Two samples (a and b) of the cleared homogenate were analyzed by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate followed by immunoblotting exactly as described (14).

(see refs. 14, 17, and 18). The 52-kDa component of acyl-CoA oxidase, the 78-kDa bifunctional protein, and the 41-kDa form of 3-oxoacyl-CoA thiolase were deficient in liver from a patient with cerebrohepatorenal (Zellweger) syndrome, in agreement with earlier results (14, 19).

In our patient the polypeptides of catalase (62 kDa), acyl-CoA oxidase, and the bifunctional protein were present in normal amounts, but the 41-kDa band corresponding to the mature form of 3-oxoacyl-CoA thiolase was clearly deficient both in liver obtained postmortem (Fig. 1) and in biopsy material (data not shown).

The effect of rat liver 3-oxoacyl-CoA thiolase on peroxisomal β-oxidation activity in the human liver homogenates is shown in Fig. 2. The cyanide-insensitive conversion of palmitoyl-CoA to acetyl units in liver homogenate from a control subject was not affected by addition of the rat liver enzyme. In contrast, there was a dramatic increase in peroxisomal β-oxidation activity when rat liver 3-oxoacyl-CoA thiolase was added to the liver homogenate from the patient, the extent of the increase being dependent upon the amount of rat liver enzyme added.

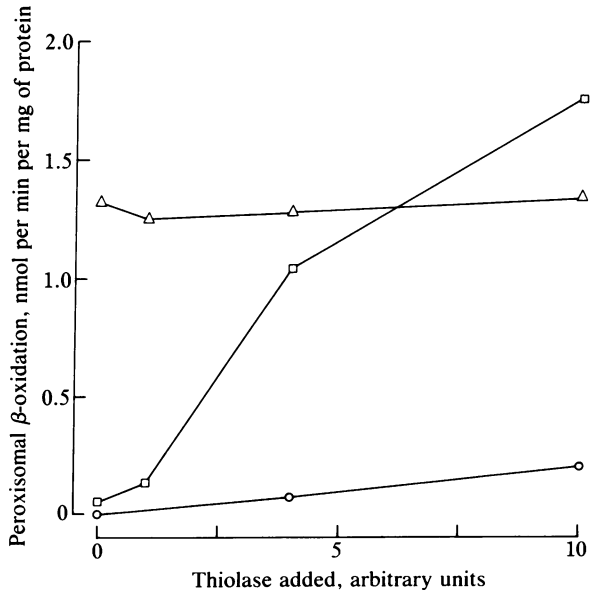

FIG. 2. Effect of addition of various amounts of rat liver 3-oxoacyl-CoA thiolase on peroxisomal β-oxidation activity in liver homogenates from a control subject (△) and from the propositus (□). Peroxisomal β-oxidation was estimated as the cyanide-insensitive formation of [^{14}C]acetyl units from [^{14}C]palmitoyl-CoA. Peroxisomal 3-oxoacyl-CoA thiolase was purified from rat liver as described (19). ○, No liver homogenate present.

DISCUSSION

This patient exhibited the clinical features of Zellweger syndrome and manifested many important biochemical features of that disorder, including an accumulation of very-long-chain fatty acids and intermediates in the biosynthesis of bile acids (1). The results described in this paper show that the peroxisomal β-oxidation system is impaired in the patient, thus explaining the accumulation of very-long-chain fatty acids (9). The increased level of intermediates in the biosynthesis of bile acids indicates that peroxisomal 3-oxoacyl-CoA thiolase participates in the oxidative cleavage of the side chain of the coprostanoic acids (10, 11). Although the antibody used in these experiments was to rat peroxisomal thiolase, the human enzyme shares antigenic components and is readily detected in control human liver. Restitution of peroxisomal β-oxidation was accomplished by addition of purified rat liver 3-oxoacyl-CoA thiolase, indicating that the decreased activity of the peroxisomal β-oxidation system is due to a deficiency of peroxisomal 3-oxoacyl-CoA thiolase. As far as we are aware this is the first identification of a genetic defect specifically involving a single β-oxidation enzyme. It remains to be determined whether the deficient protein is synthesized at all or whether it exists with altered antigenic and catalytic activity.

Homogenates of the patient's liver were unable to bring about the cyanide-insensitive conversion of [^{14}C]palmitoyl-CoA to [^{14}C]acetyl units, a measure of the peroxisomal β-oxidation system, despite the presence of mitochondrial thiolase. The reason for this is not known.

Comparison of the biochemical, pathological, and clinical features of patients with (*i*) a generalized impairment of peroxisomal functions [cerebrohepatorenal (Zellweger) syndrome, neonatal adrenoleukodystrophy, and infantile Refsum disease]; (*ii*) a deficiency of some, but not all, peroxisomal functions [rhizomelic chondrodysplasia punctata (20)]; and (*iii*) an impairment of a single peroxisomal function (X-linked adrenoleukodystrophy, 3-oxoacyl-CoA thiolase deficiency) will be of importance in elucidating the normal functions of peroxisomes as well as the pathogenesis of the clinical signs and symptoms in these diseases (3–6). Indeed, the similarity of distinct clinical and pathological features between this patient with a peroxisomal 3-oxoacyl-CoA thiolase deficiency and patients with the cerebrohepatorenal (Zellweger) syndrome (general deficiency of peroxisomes and impairment of peroxisomal structure and function) suggests that very-long-chain fatty acids and/or intermediates in the biosynthesis of bile acids may be of central significance in the development of the salient features of the syndrome—notably, defective neuronal migration and dysmyelination.

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