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Short Communication – SSIEM Award

Phytanoyl-CoA hydroxylase is not only deficient in classical Refsum disease but also in rhizomelic chondrodysplasia punctata

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Phytanic acid (3,7,11,15-tetramethylhexadecanoic acid) is a branched-chain fatty acid which has long been known to accumulate in a variety of inherited disorders including classical Refsum disease (McKusick 26650), rhizomelic chondrodysplasia punctata (RCDP; McKusick 215100) and the disorders of peroxisome biogenesis (ten Brink et al 1992). It has been shown that in all these disorders this accumulation results from a defect in the degradation of phytanic acid (Skjeldal et al 1987). The presence of a methyl group at the carbon-3 position prohibits direct β -oxidation of phytanic acid. Instead, phytanic acid first undergoes α -oxidation, resulting in pristanic acid as decarboxylation product. Recent studies (Watkins et al 1994; Mihalik et al 1995) have shed new light on the pathway of phytanic acid α -oxidation. Indeed, it is now clear that α -oxidation of phytanic acid first requires activation to its CoA-ester followed by hydroxylation of phytanoyl-CoA to 2-hydroxyphytanoyl-CoA. This reaction is catalysed by phytanoyl-CoA hydroxylase, a dioxygenase with a reaction sequence comparable to that of prolyl 4-hydroxylase (EC 1.14.11.2). It is not known how 2-hydroxyphytanoyl-CoA is further converted into pristanic acid (or pristanoyl-CoA).

We have recently found that phytanoyl-CoA hydroxylase is deficient in liver from patients with Zellweger syndrome and classical Refsum disease (heredopathia actastica polyneuritiformis) (Jansen et al 1996, 1997). We have now extended these studies to rhizomelic chondrodysplasia punctata, a peroxisomal disorder characterized by a tetrad of biochemical abnormalities including a deficiency of dihydroxyacetonephosphate acyltransferase (DHAPAT, EC 2.3.1.42), alkylldihydroxyacetonephosphate synthase

Table 1 Phytanoyl-CoA hydroxylase activity (nmol/h per mg protein) in human liver homogenates from controls, a patient with Refsum disease and patients with rhizomelic chondrodysplasia punctata

<i>Phytanoyl-CoA hydroxylase activity</i>	
Patients with RCDP	
1	≤0.05
2	≤0.05
Patient with Refsum disease	≤0.05
Controls (mean ± SD)	2.45 ± 0.88 (n=11) (range 0.80–3.83)

(alkylDHAP synthase, EC 2.5.1.26), peroxisomal thiolase (EC 2.3.1.16) and phytanic acid α -oxidation (see Wanders et al 1995).

MATERIALS AND METHODS

Phytanoyl-CoA hydroxylase activity measurements: Human liver specimens were kept at -80°C until the day of the experiment and homogenates were prepared in a buffer containing 100mmol/L KCl plus 50mmol/L Tris-HCl (pH 7.5). Incubations were subsequently done using a medium of the following composition: 25mmol/L Tris-HCl, 10mmol/L ATP, 5mmol/L MgCl_2 , 0.2mmol/L coenzyme A, 0.5mmol/L $(\text{NH}_4)_2\text{Fe}^{\text{II}}(\text{SO}_4)_2$, 1mmol/L 2-oxoglutarate, 1mmol/L ascorbate, 0.25mmol/L dithiothreitol and 25 $\mu\text{mol/L}$ $[1-^{14}\text{C}]$ phytanoyl-CoA, final pH 7.5. Incubations were carried out for 30min at 37°C , followed by HPLC analysis as described in detail elsewhere (Mihalik et al 1995).

Patients: The patients whose liver specimens were used in the present study showed all the clinical and biochemical characteristics described for rhizomelic chondrodysplasia punctata (RCDP). In all cases extensive studies were done in fibroblasts (*de novo* plasmalogen synthesis, $\text{C}_{26:0}$, pristanic and phytanic acid oxidation, DHAPAT-activity, alkyl-DHAP synthase activity, immunoblot analysis of acyl-CoA oxidase and thiolase, catalase immunofluorescence, very long-chain fatty acid analysis).

RESULTS

We have recently set up a method allowing measurement of phytanoyl-CoA hydroxylase in human liver homogenates. The method is based upon incubation of a human liver homogenate in a medium containing phytanoyl-CoA as substrate plus 2-oxoglutarate, Fe^{2+} and ascorbate as necessary components for the phytanoyl-CoA hydroxylase reaction, followed by HPLC analysis of the acyl-CoA derivatives formed (Jansen et al 1996). Table 1 shows that phytanoyl-CoA hydroxylase is not only deficient in liver from a Refsum patient but also in liver from two rhizomelic chondrodysplasia punctata patients.

DISCUSSION

Rhizomelic chondrodysplasia punctata is a peroxisomal disorder characterized by a combination of four biochemical abnormalities (see Wanders et al 1995). The most likely explanation for this peculiar set of peroxisomal enzyme deficiencies is that the primary defect in RCDP is at the level of a component of the peroxisome biogenesis machinery involved in correctly directing these proteins to peroxisomes. This was recently tested by Motley and coworkers (Motley et al 1994). These authors presented convincing evidence that there is a differential defect in the uptake machinery of peroxisomal proteins in RCDP. So far, two peroxisomal targeting signals (PTS) have been identified. The first PTS (PTS1) comprises a serine-lysine-leucine (SKL) sequence or one of its conservative variants located at the extreme carboxy-terminal end of the protein. The other PTS (PTS2) is less well defined, but available evidence suggests that the targeting information is contained within a stretch of 9 amino acids located near the amino-terminus of the polypeptide, with the amino acids at positions 1, 2, 8 and 9 being most important (Braverman et al 1995).

Motley and coworkers (1994) have shown that the uptake of PTS1-containing proteins into peroxisomes is fully normal in RCDP, in contrast to the import of PTS2-containing proteins. This leads to the conclusion that phytanoyl-CoA hydroxylase belongs to the group of peroxisomal proteins containing a PTS2 signal. So far, only one such peroxisomal protein has been identified in higher eukaryotes (peroxisomal 3-oxoacyl-CoA thiolase), although several PTS2-containing proteins have been identified in yeast species (see Elgersma and Tabak 1996).

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