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Omega-oxidation of very long-chain fatty acids in human liver microsomes: implications for X-linked adrenoleukodystrophy?

R.-J. SANDERS, R. OFMAN, M. DURAN, S. KEMP en R.J.A. WANDERS

In mammalian cells, fatty acid oxidation plays a major role in the production of energy particularly in the heart and skeletal muscle, and is the main energy source during periods of fasting. Mitochondria as well as peroxisomes are both capable of degrading fatty acids via β-oxidation. Mitochondria predominantly utilize short-, medium- and long-chain saturated fatty acids, whereas very-long-chain fatty acids (VLCFA, >22 carbons) are β-oxidized exclusively in peroxisomes (1).

X-linked adrenoleukodystrophy is the most common peroxisomal disorder, with an incidence of approximately 1 in 17,000. It is a progressive neurodegenerative disease that affects the cerebral white matter, spinal cord, peripheral nerves, adrenal cortex and testis (2). X-ALD is caused by mutations in the ABCD1 gene that encodes ALDP, an ATP-binding cassette transporter located in the peroxisomal membrane (3). Biochemically, X-ALD is characterized by elevated levels of saturated and mono-unsaturated VLCFAs in plasma and tissues, due to the impaired β-oxidation of VLCFAs in peroxisomes (Figure 1) (4-6). Since the pathogenesis of X-ALD is most likely due to the increased levels of VLCFAs, especially hexacosanoic acid (C26:0), correction of VLCFA levels is one of the primary objectives in therapeutic approaches. These include gene replacement therapy, lovastatin treatment, bone marrow transplantation, inhibition of VLCFA biosynthesis by mono-unsaturated fatty acids, notably oleic acid (C18:1ω9) and erucic acid (C22:1ω9) (Lorenzo's Oil), and induction of the expression of ALDP-related protein (ALDR) (see ref (4) for an overview). At present, there is currently no effective therapy for this disease.

An alternative route for the oxidation of fatty acids is via ω-oxidation in the endoplasmic reticulum. Under normal physiological conditions, ω-oxidation of fatty acids is a minor pathway that accounts for a small fraction of the total fatty acid oxidation in the liver. The first step in ω-oxidation of fatty acids involves the conversion of the ω-methyl group of the fatty acid into an ω-hydroxyl group. This reaction is catalyzed by one or more cytochrome P450 enzymes that mainly belong to the CYP4 family and requires NADPH and molecular oxygen (7). Subsequently, ω-hydroxy fatty acids may be oxidized further into ω-carboxylic acids, either via an NAD^+-dependent alcohol and aldehyde dehydrogenase system or via a cytochrome P450 mediated route (8). Finally, dicarboxylic acids can be β-oxidized in peroxisomes and/or mitochondria to shorter-chain dicarboxylic acids followed by excretion into the urine. Accumulation of dicarboxylic acids has not been detected in X-ALD patients, whereas in patients with a peroxisomal biogenesis disorder (PDB), elevated levels of medium- and long-chain dicarboxylic acids were found in urine (9). Furthermore, no abnormal β-oxidation of long-chain dicarboxylic acids was found in fibroblasts from X-ALD patients, whereas it was deficient in fibroblasts from PBD patients (10). These studies indicate that peroxisomes play an essential role in dicarboxylic acid degradation, but do not require ALDP. Although different fatty acids are known to undergo ω-oxidation, there is no data in the literature available with respect to the ω-oxidation of VLCFAs (7).

The objective of this study is to investigate if ω-oxidation of VLCFA may provide an alternative pathway for the breakdown of VLCFA. Until now, ω-oxidation of VLCFA has not been studied in humans and none of the enzymes potentially involved in the ω-oxidation system have been characterized. We have studied the ω-oxidation pathway for several saturated fatty acids known to be of relevance to X-ALD, which includes docosanoic acid (C22:0), tetracosanoic acid (C24:0) and hexacosanoic acid (C26:0).

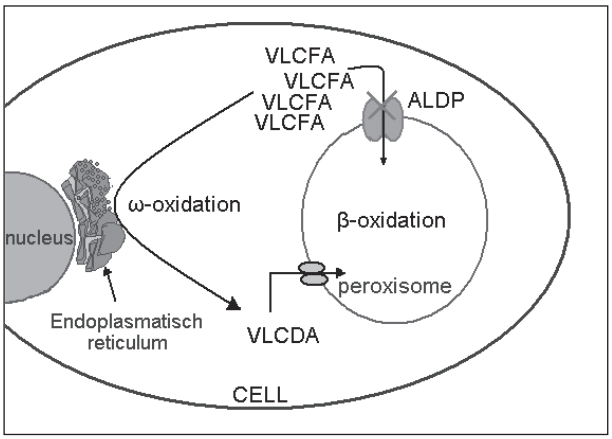

Figure 1. Alternative breakdown of VLCFAs via ω-oxidation. Since ALDP is not working properly in X-ALD, VLCFA accumulate in the cell. An alternative route for the degradation of VLCFAs would be the ω-oxidation pathway in the endoplasmic reticulum. Subsequently, the very long-chain dicarboxylic acids (VLCDA) could be transported across the peroxisomal membrane and undergo β-oxidation.

Methods

The experimental conditions used to study the hydroxylation of different VLCFAs were adapted from previous experiments with minor modifications (5, 8). Briefly, incubations were carried out for 30 min at 37 °C in a reaction mixture that contained Tris buffer pH 8.4 (100 mM), protein (50 µg), α-cyclodextrin (1 mg/ml) and NADPH (1 mM) in a total volume of 200 µl. The reaction was initiated by addition of the fatty acid at a final concentration of 200 µM and terminated by addition of 1 ml hydrochloric acid to a final concentration of 1.7 M. The reaction products were extracted with hexane and analyzed by electrospray ionization mass spectrometry.

Results

We have studied the ω-oxidation pathway for the VLCFA in human liver microsomes. Our results show that VLCFA are substrates for the human ω-oxidation system. The hydroxylation assay was optimized for the ω-oxidation of hexacosanoic acid (C26:0). Since humans have at least 57 cytochrome P450 enzymes, several specific inhibitors were used in order to identify the subfamily involved in VLCFA hydroxylation. 17-octadecynoic acid inhibited C26:0 hydroxylation in the nanomolar range. This indicates that cytochrome P450 enzymes belonging to the CYP4 family participate in VLCFA hydroxylation. Subsequent studies with human recombinant enzymes revealed that two P450 enzymes of the 4F subfamily, CYP4F2 and CYP4F3B, catalyze ω-oxidation of VLCFA. Kinetic studies show that both enzymes have a high affinity for VLCFAs (Table 1) .

Table 1. Kinetic parameters for VLCFA hydroxylation by CYP4F2 and CYP4F3B

	K_m µM	V_{max} pmol·min^{-1}·pmol P450^{-1}	V_{max}/K_m
CYP4F2			
C22:0	0.5	1.6	3.2
C24:0	1.1	1.6	1.5
C26:0	1.9	0.9	0.5
CYP4F3B			
C22:0	1.6	5.0	3.1
C24:0	3.8	9.8	2.6
C26:0	1.3	2.2	1.7

Conclusion

To summarize, VLCFAs are hydroxylated to very-long-chain ω-hydroxy and dicarboxylic acids in human liver microsomes. We have identified two cytochrome P450 enzymes that catalyze the ω-hydroxylation of VLCFAs. Both enzymes are members of the 4F subfamily, namely CYP4F2 and CYP4F3B. Both enzymes have a high affinity for saturated VLCFAs with Km values in the micromolar range and are therefore interesting from a physiological point of view.

Future work is aimed at the identification of the regulatory mechanisms involved in the expression of these enzymes. To generate new therapeutic options for patients with X-ALD, we will investigate whether the VLCFA ω-oxidation route can be induced in order to normalize the levels of VLCFAs in affected tissues.

Literature

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