Peroxisomal disorders affecting phytanic acid α-oxidation: a review

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Abstract

Peroxisomes are involved in the synthesis and degradation of complex fatty acids. They contain enzymes involved in the α-, β- and ω-oxidation pathways for fatty acids. Investigation of these pathways and the diseases associated with mutations in enzymes involved in the degradation of phytanic acid have led to the clarification of the pathophysiology of Refsum’s disease, rhizomelic chondrodysplasia and AMACR (α-methylacyl-CoA racemase) deficiency. This has highlighted the role of an Fe(II)- and 2-oxoglutarate-dependent oxygenases (PhyH (phytanoyl-CoA 2-hydroxylase), also known as PAHX), thiamin-dependent lyases (phytanoyl-CoA β-lyase) and CYP (cytochrome P450) family 4A in fatty acid metabolism. The differential regulation and biology of these pathways is suggesting novel ways to treat the neuro-ophthalmological sequelae of Refsum’s disease. More recently, the discovery that AMACR and other peroxisomal β-oxidation pathway enzymes are highly expressed in prostate and renal cell cancers has prompted active investigation into the role of these oxidation pathways and the peroxisome in the progression of obesity- and insulin resistance-related cancers.

Introduction

Peroxisomes are cellular organelles involved in biosynthetic and degradative functions [1], which, like mitochondria, may have originated from ancient commensal bacteria. Anabolic functions of peroxisomes include the biosynthesis of cholesterol and plasmalogens for cell and neuronal membranes [2]. They degrade unsaturated and aromatic fatty acids including bile acids. Peroxisomes also contain an analogous fatty acid α-oxidation pathway to that found in mitochondria and also specific enzymes for the α-oxidation of 3-methyl branched fatty acids including PA (phytanic acid) [3].

Peroxisomes

Peroxisomes are able to catabolize a wide variety of lipids, including long-chain (C12–C18), very-long-chain (C20–C26) and ultra-long-chain (C28–C38) fatty acids, unsaturated fatty acids, bile acid intermediates, 2-methyl fatty acids (e.g. PA) and diethyl ether phospholipids [3]. The primary catabolic route for peroxisomal lipids is the β-oxidation pathway. Although different enzymes are used, it seems that β-oxidation in peroxisomes is roughly analogous to that occurring in mitochondria. Human peroxisomes apparently contain two β-oxidation systems: an inducible pathway that metabolizes long-chain fatty acids and a constitutive pathway that oxidizes pristanic and bile acids (2-methyl branched acids). In humans, carnitine derivatives of ethanoic (acetic) acid and propanoic acid, as well as short-chain fatty acids (typically C6–C11), are exported from peroxisomes to mitochondria for further oxidation.

Refsum’s disease, a disorder of fatty acid α-oxidation

ARD (adult Refsum’s disease) (OMIM register of inherited human genetic disease 266510), also called heredopathia atactica polyneuritiformis and hereditary sensory motor neuropathy type IV, was first described in 1945, but only recognized as a syndrome by Refsum in 1946 [4]. He described a constellation of signs comprising of retinitis pigmentosa, anosmia, deafness, ataxia and polynuereopathy allied with raised levels of CSF (cerebrospinal fluid) protein. The biochemical defect was identified in 1963 when PA was noted in the plasma of affected patients [5] and defective α-oxidation was later suggested as the cause of ARD [6].

Clinical signs and symptoms

In contrast with Zellweger’s syndrome (OMIM 214100), neonatal adrenoleukodystrophy (OMIM 202370), IRD (infantile Refsum’s disease; OMIM 266500) and rhizomelic chondrodysplasia [RCDP (rhizomelic chondrodysplasia punctata type 1)] (OMIM 601757), ARD usually presents in late childhood with progressive deterioration of night vision, the occurrence of progressive retinitis pigmentosa and anosmia (Figure 1) [7]. Anosmia, contrary to early reports, is a constant feature of ARD [8]. After 10–15 years, deafness, ataxia, polynuereopathy, ichthyosis and cardiac arrhythmias can occur. Short metacarpals or metatarsals are found in approx. 30% of patients. Rare findings include psychiatric
Figure 1 | Cumulative incidence of clinical features on presentation of 15 patients with Refsum's disease

disturbance and proteinuria. Premature death can occur in acute cases from the arrhythmias.

The pathognomonic finding of ARD is a highly raised plasma PA level (≥ 200 μmol/l; normal <30 μmol/l), in contrast with other peroxisomal disorders where levels are usually lower and other metabolic abnormalities are also present. Unlike in RCDP or the peroxisomal biogenesis disorders, no intellectual defects are seen, bone abnormalities are mild (if at all present) and there is no defect in plasmalogen synthesis. In IRD, which is a mild clinical variant of the peroxisomal biogenesis disorder encompassing Zellweger’s disease as its most severe form, numerous subtle peroxisomal defects are present and the condition presents from birth [2]. The age at which symptoms first present in ARD can be variable although most cases present in adolescence.

Clinical enzymology
PA [(3R,5S,7R,11R,15)-tetramethylhexadecanoic acid] is an isoprenoid lipid derived from the phytol side chain of chlorophylls by bacterial degradation in ruminants, invertebrates or pelagic fish (Figure 2). Phytol can be oxidized to an unsaturated fatty acid, phytanic acid, and this is saturated by a pathway involving FALDH-10 (fatty aldehyde dehydrogenase-10) in microsomes to PA [9]. The significance of this pathway in human is unclear although high PA levels have been described in some patients deficient in FALDH-10 with the Sjogren–Larsson syndrome [10]. Most PA is ingested from the adipose tissue and muscle of herbivores or pelagic fish that absorb PA as a result of bacterial degradation of chlorophyll. The average human daily dietary intake of PA in Western societies is 50–100 mg of which approx. 50% is absorbed and metabolized [11].

PA is transported in plasma allied to VLDL (very-low-density lipoprotein) and later LDL (low-density lipoprotein) [12], with its elimination from tissue stores occurring by mechanisms associated with reverse cholesterol transport [HDL (high-density lipoprotein)]. PA is preferentially taken up by the liver and may account for up to 50% of the non-esterified fatty acid pool in hepatocytes. This pool is labile and can be acutely mobilized by stress, infection or starvation, resulting in rapid PA release [13]. Plasma PA levels are <10% of the levels found in adipose tissue and neurons, which accumulate PA because of its hydrophobicity. The elimination half-life of total body PA is usually 1–2 years [14].

Most fatty acids are metabolized by the β-oxidation pathways in peroxisomes and mitochondria. PA cannot be metabolized by this route due to the presence of a β-methyl group. Instead, PA is metabolized either by α-oxidation to pristanic acid, or by ω-oxidation from the other end of the molecule. Using radiolabelled [14C]PA as a substrate, an enzyme activity responsible for the α-oxidation of PA in cell lysates was described in 1967 [15]. This activity was eventually localized within peroxisomes, and after 30 years the pathway responsible for α-oxidation has been clarified [7,16,17].

α-Oxidation of PA
Most metabolism of PA occurs in the liver and kidney by α-oxidation although skin fibroblasts are used for clinical diagnostic purposes. PA from plasma enters the peroxisome in association with the SCP2 (sterol carrier protein-2) and is metabolized by a four-step initial α-oxidation pathway [7,16,17]. Unusually, it appears that this pathway can equally well metabolize two stereoisomers of its substrate. One carbon atom is then removed from the latter in a lyase reaction to give pristanal and formyl-CoA. Pristanal is then oxidized to pristanic acid, which is thioesterified using CoA to give a racemic mixture. The action of an AMACR (α-methylacyl-CoA racemase) converts the (2R)-epimer to the (2S)-epimer. Further degradation of (2S)-pristanic acid by the stereospecific β-oxidation pathway then occurs, with the release of propionyl and acetyl-CoA units [18]. Further β-oxidation reactions (including epimerization) are required to generate the dimethylundecanolic and dimethylnonanoic and methyl-heptanoic acid derivatives, which are finally exported for mitochondrial β-oxidation [19].

Molecular genetics
The defect in ARD was soon identified as being due to lack of an α-oxidase [6]. It took 30 years for the enzyme responsible, PhyH (phytanoyl-CoA 2-hydroxylase), to be identified. Two groups identified the gene for PhyH simultaneously in 1997. One group employed molecular cloning using N-terminal sequence information from the purified native enzyme [20]. The other identified PhyH via a homologous cloning strategy for enzymes with a PST-2 recognition site similar to that found in 3-ketoacyl-CoA thiolase [21]. The PhyH gene includes nine exons and codes for a 338-amino-acid protein including the 30-amino-acid ‘signal’ domain, which is cleaved on entry into the peroxisome [22]. Like all PTS-2 (type 2
peroxisomal targeting signal)-containing proteins, PhyH is transported into peroxisomes by the protein transporter Pex 7 (peroxin 7). Deficiency in this transporter is responsible for RCDP [23]. PhyH is an Fe(II)- and 2-oxoglutarate-dependent oxygenase with little overall sequence similarity to other human oxygenases [24]. Numerous mutations in the PhyH have now been described in ARD patients, many of which affect 2-oxoglutarate conversion [25,26].

Genetic mapping studies have shown that most but not all cases with classical ARD map to chromosome 10 [27].
The locus for the second form of ARD comprising approx. 10% of cases was localized to chromosome 6q22-24 and biochemical studies of fibroblasts from patients with ARD established that these patients have subtle deficiencies of PTS-2-dependent enzyme functions (plasmalogen synthesis) consistent with mild variants of RCDP [28]. Ironically, one of the original patients described with ARD turns out to have the RCDP variant [29]. Refsum’s ‘disease’ is actually a syndrome with mutations to more than one gene responsible for producing the clinical phenotypes. The description of the ‘Refsum-like’ AMACR deficiency (OMIM 604489), whose symptoms include adult onset retinitis pigmentosa, but predominantly a sensory neuropathy with a minor elevation in PA levels but gross pristanic acidemia, also favours this hypothesis [18] and there may well be other genes involved in ARD as patients exist in whom the causal mutation has not been found in any candidate gene surveyed.

Clinical aspects of ω-oxidation

Patients with ARD are unable to detoxify PA by ω-oxidation, and so the ω-oxidation pathway is the only metabolic pathway available for PA degradation. This pathway produces 3-MAA (3-methyladipic acid) as the final metabolite, which is excreted in the urine. Thus 3-MAA levels can be used as a clinical index of the molar activity of the ω-oxidation pathway. After ingestion of a test load of PA, 3-MAA is detected in the urine of healthy controls and ARD heterozygotes, showing that ω-oxidation plays a significant role in postprandial metabolism of PA in humans [13]. Activity of the ω-oxidation pathway is approximately doubled in ARD patients compared with normal levels but this microsomal pathway has considerable reserve capacity, which is best observed on initial presentation of ARD [13]. In one case study, in a newly presented patient of ARD, levels of PA fell from 2000 to 200 µM on a low-PA diet resulting in a gradual fall in plasma and tissue PA levels with a plasma half-life of 22.4 days; PA clearance through ω-oxidation was initially 32% of total PA metabolism but rose to 100% by 40 days, indicating that the balance of PA intake and ω-oxidation is likely to determine long-term PA levels. Patients with ARD often clinically relapse secondary to illness or drastic weight loss. Fasting induces ketosis and lipolysis and acute mobilization of PA in hepatocyte and adipocyte fatty acid pools. This process can induce the release of 5000 mg (14.8 mmol)/day of PA (50-fold normal). In experimental ketosis following acute starvation, PA doubled in 29 h in patients with ARD and an 80% rise was seen in urinary 3-MAA levels, indicating that ω-oxidation was buffering part of this rise [13]. PA levels can exceed the capacity of the residual α- and ω-oxidation pathways. Excess PA is excreted by low-affinity pathways. PA can be glucuronidated and it can also be lost non-specifically in the urine as nephropathy is a feature of ARD.

The enzymology of the ω-oxidation pathway in ARD has been clarified and occurs through the microsomal CYP4A (cytochrome P450 family 4A) system as well as the peroxisome [30]. The capacity of the ω-oxidation pathway has been measured by excretion of 2,6-DMOA (2,6-dimethyloctanedioic acid; the C₁₂ ω-2-methyl thioster derivative of PA) at 30 mg of PA (89 µmol)/day. However, other studies measuring 3-MAA excretion showed a far lower capacity of 6.9 mg (20.4 µmol)/day [13]. These differences in activity may reflect the metabolic fates of the respective markers. 2,6-DMOA and 3-hexanedioic acid are products of the initial steps of ω-oxidation and may be dependent on carnitine ester formation for activation for further metabolism. Phytanoyl-carnitines which occur in ARD [31] may impair the activation reaction through competition and lead to urinary excretion of excess 2,6-DMOA and 3-methylhexanedioic acid so that the initial steps of ω-oxidation may seem to have a greater capacity than that of the whole pathway when measured by the final product 3-MAA.

Molecular toxicology of Refsum’s disease

The exact mechanism of the toxicity of PA to neuronal, cardiac and bone tissue is gradually being clarified. Some studies indicated that PA is directly toxic to ciliary ganglion cells and induces calcium-driven apoptosis in Purkinje cells [32]. Structural homology between PA and vitamin A (11-cis-retinol), vitamin E (α-tocopherol), GPP (geranyl pyrophosphate) and FPP (farnesyl pyrophosphate) have been noted [14] and it has been suggested that PA may have a role in the regulation of isoprenoid metabolism and protein prenylation. More recent studies have focused on the role of PA as a direct toxin to mitochondria and it has been found that PA has a rotenone-like action in uncoupling complex I in the oxidative phosphorylation chain in the mitochondrial inner membrane with subsequent likely production of reactive oxygen species [33–35]. This metabolic toxicity may explain why neuronal or allied retinal pigment tissues rich in mitochondria are the prime tissues affected in ARD.

Treatment of Refsum’s disease

Long-term prospects for treatment (at least some forms) of ARD are good as it is one of the few inherited disorders of metabolism with an exogenous precipitating cause. The disease is treated symptomatically by restriction of PA intake in the diet or its elimination by plasmapheresis or apheresis [36]. These regimes reduce plasma PA levels by 50–70%, to values typically around 100–300 µmol/l, and can eliminate PA completely from fat stores in some patients. Treatment successfully resolves symptoms of ichthyosis, sensory neuropathy and ataxia in approximately that order. However, it has uncertain effects on the progression of the retinitis pigmentosa, anosmia or deafness although it does seem to stabilize these signs. One of the problems with this approach is the late diagnosis of ARD after retinitis pigmentosa has been noted with delays of 6–10 years (Figure 1) such that the disease has progressed further before treatment is instituted. As ARD is the only exogenously caused retinitis pigmentosa syndrome and a simple cheap biomarker exists (PA), there may be a case for screening of all patients with retinitis pigmentosa for ARD [37].
Reduction of dietary PA is already successful in ameliorating some symptoms but newer more efficacious therapies are still required to fully reverse the progression of this disease. The signalling pathways that regulate α-oxidation in human are unclear. In contrast with rodents where the RXR-β (retinoid X receptor-β) [38] and PPAR-α (peroxisome-proliferator-activated receptor-α) [39] pathways do control α-oxidation and thus fibrate (PPAR-α agonist) therapy increases activity, this does not seem to be true in humans [40]. As ω-oxidation is capable of large increases in activity and is most mediated through CYP enzymes, it forms a good candidate for therapeutic interventions to induce enzyme activity and reduce PA levels in ARD. However, at the moment, no drug therapy trials of compounds capable of modulating either the α- or ω-oxidation pathways have been conducted in humans.

The wider significance of PA metabolism

α-Oxidation disorders have seemed to be an obscure area involved in the metabolism of complicated fatty acids and that PPARs and retinoids seem to be involved in their control, little notice was taken of α-oxidation. Occasionally, a hypothesis would surface link α-oxidation to PPAR metabolism and thus to atherogenic dyslipidaemias and diabetes [41,42] but no evidence has been found to substantiate this. However, this has changed since the serendipitous discovery that AMACR is highly up-regulated in prostatic adenocarcinoma [43–45]. In parallel, up-regulation occurs of the enzymes involved in peroxisomal β-oxidation, indicating that it is likely to be the peroxisomal function of AMACR rather than the mitochondrial that is relevant to oncological progression [46]. AMACR is used in the immunohistological staining of the mitochondrial that is relevant to oncological progression but no evidence has been found to substantiate this. However, as with many inherited errors, it turns out that the peroxisomal oxidation of complicated fatty acids and now the oncological role of the peroxisome is the prime focus of investigation.

References


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