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Identification of the peroxisomal β-oxidation enzymes involved in the degradation of leukotrienes☆

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Abstract

Leukotrienes (LTs) are metabolically inactivated via ω-oxidation and subsequent β-oxidation from the ω-end. This β-oxidation process takes place in peroxisomes. In this study we investigated the role of different enzymes involved in peroxisomal β-oxidation in the degradation of LTs. We analyzed LTB_4, LTE_4, and their oxidation products in urine of patients with Infantile Refsum's disease (IRD), D-bifunctional protein (DBP) deficiency, Rhizomelic Chondrodysplasia Punctata (RCDP) type 1, and X-linked adrenoleukodystrophy (XALD). We found that patients with IRD and DBP deficiencies excrete increased amounts of LTB_4, LTE_4, ω-carboxy-LTB_4, and ω-carboxy-LTE_4 in their urine, whereas the β-oxidation products were not detectable. These results show that DBP plays an essential role in the degradation of LTs. In urine of patients with XALD and RCDP type 1 we found normal levels of LTB_4, LTE_4, and their oxidation products, indicating that the adrenoleukodystrophy protein and peroxisomal 3-ketoacyl-CoA thiolase are not involved in the metabolic inactivation of LTs.

Keywords: Leukotrienes; Peroxisomal β-oxidation; D-Bifunctional protein deficiency; RCDP type 1; X-linked adrenoleukodystrophy; Infantile Refsum's disease

Leukotrienes (LTs) are a group of potent lipid mediators synthesized from phospholipase-released arachidonic acid by the enzyme 5-lipoxygenase. They include LTB_4 and the cysteinyl LTs, LTC_4, LTD_4, and LTE_4. LTs are made predominantly by inflammatory cells like macrophages and mast cells, and they act at nanomolar concentrations in intercellular communication, signal transduction, and in host defense. LTB_4 mainly functions as a chemotactic agent for inflammatory cells, whereas the cysteinyl LTs can cause a variety of biological effects, e.g., smooth muscle contraction and increase of microvascular permeability (see for review [1,2]).

The metabolic inactivation and degradation of LTs are of major importance because of their potent biological activities. In the blood circulation LTC_4 is rapidly converted via LTD_4 to the less active LTE_4. LTB_4 and LTE_4 are inactivated via ω-oxidation and subsequent β-oxidation from the ω-end (Fig. 1). The β-oxidation of the LTs from the ω-end occurs in the peroxisome. This was shown both by in vitro studies with isolated peroxisomes [3] and studies in patients with a peroxisome biogenesis disorder [4–6]. In urine from patients with Zellweger syndrome, who lack functional peroxisomes, there was a significantly increased excretion of LTB_4 and LTE_4 and their ω-oxidation products, ω-carboxy-LTB_4 and ω-carboxy-LTE_4, respectively, while the β-oxidation products ω-carboxy-tetranor-LTB_3 and ω-carboxy-tetranor-LTE_3 were not detectable in urine [4,6].

☆ *Abbreviations*: ALDP, adrenoleukodystrophy protein; BCOX, branched-chain acyl-CoA oxidase; DBP, D-bifunctional protein; HPLC, high performance liquid chromatography; IRD, Infantile Refsum's disease; LBP, L-bifunctional protein; LT, leukotriene; RCDP, Rhizomelic Chondrodysplasia Punctata; SCOX, straight-chain acyl-CoA oxidase; SCPx, sterol carrier protein X; XALD, X-linked adrenoleukodystrophy.

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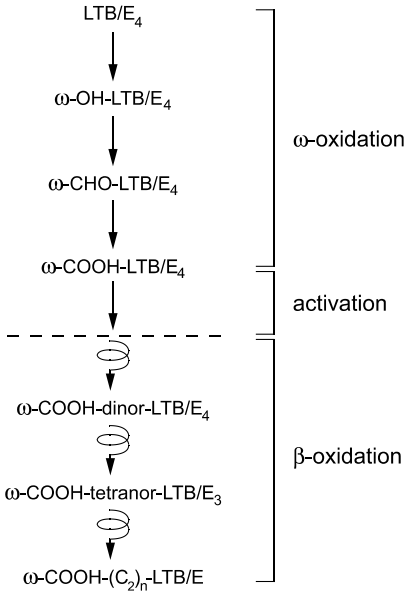

Fig. 1. Catabolism of LTs. LTB_4 and LTE_4 are degraded via analogous reactions. LTB_4 and LTE_4 are first ω-oxidized yielding ω-hydroxy-LT, ω-aldehyde-LT, and ω-carboxy-LT. After activation at the ω-end by an acyl-CoA synthetase, several peroxisomal β-oxidation cycles lead to a stepwise degradation of the LT from the ω-end via ω-carboxy-dinor-LT, ω-carboxy-tetranor-LT, and more polar β-oxidation products.

Peroxisomes are the sites of β-oxidation of a range of fatty acids and fatty acid derivatives that cannot be broken down in mitochondria. Substrates of the peroxisomal β-oxidation system include both straight-chain fatty acids like C26:0 and C24:0 and 2-methyl-branched-chain fatty acids like pristanic acid and the bile acid intermediates di- and trihydroxycholestanoic acids (DHCA and THCA) (reviewed in [7]). Fig. 2 shows a schematic representation of the peroxisomal β-oxidation system. There are two complete sets of β-oxidation enzymes present in the peroxisome. Straight-chain acyl-CoA oxidase (SCOX) is responsible for the initial oxidation of very long-chain fatty acyl-CoAs, while branched-chain acyl-CoA oxidase (BCOX) oxidizes branched-chain fatty acyl-CoAs. The enoyl-CoA esters of both straight- and branched-chain fatty acids are then hydrated and subsequently dehydrogenated by the same enzyme: D-bifunctional protein (DBP). The function of the second multifunctional protein present in the peroxisome, L-bifunctional protein (LBP), is still unknown. The last step of the β-oxidation process, the thiolytical cleavage, is performed by sterol carrier protein X (SCPx) in case of the branched-chain substrates, while straight-chain substrates most likely are handled by both SCPx and the classical 3-ketoacyl-CoA thiolase (Fig. 2).

Until now, the only true deficiencies of single peroxisomal β-oxidation enzymes that have been identified are deficiencies of SCOX and DBP [8–12]. In addition, patients suffering from Rhizomelic Chondrodysplasia Punctata (RCDP) type 1 lack 3-ketoacyl-CoA thiolase in their peroxisomes. This is, however, not the only deficiency in these patients. Due to a defect in *PEX7* [13–15], the gene encoding the peroxisome targeting signal 2 (PTS2) receptor, their peroxisomes lack all proteins imported via this receptor, including 3-ketoacyl-CoA thiolase and, in addition, alkyl-dihydroxyacetonephosphate synthase, an enzyme of the plasmalogen biosynthetic pathway, and phytanoyl-CoA hydroxylase, the first enzyme of the peroxisomal α-oxidation pathway. X-linked adrenoleukodystrophy (XALD) is the most common peroxisomal fatty acid β-oxidation disorder. Patients with XALD accumulate very long-chain fatty acids because of an impaired peroxisomal β-oxidation of these fatty acids. However, this is not caused by a deficiency of one of the enzymes of the β-oxidation system, but by a defect of the peroxisomal membrane protein adrenoleukodystrophy protein (ALDP) [16,17],

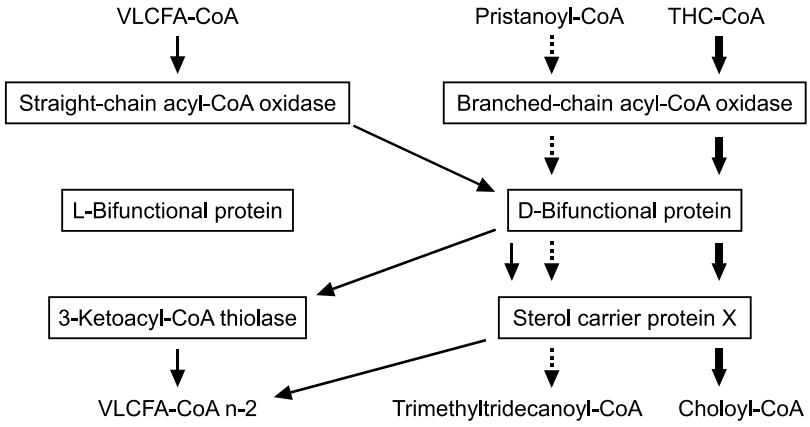

Fig. 2. Schematic representation of the fatty acid β-oxidation machinery in human peroxisomes catalyzing the oxidation of very long-chain fatty acyl-CoAs (VLCFA-CoA) and branched-chain fatty acyl-CoAs (pristanoyl-CoA and THC-CoA). Oxidation of VLCFA-CoAs (C24:0 and C26:0) involves straight-chain acyl-CoA oxidase, D-bifunctional protein (DBP), and both 3-ketoacyl-CoA thiolase and sterol carrier protein X (SCPx), while oxidation of branched-chain fatty acyl-CoAs involves branched-chain acyl-CoA oxidase, DBP, and SCPx (see [7] for review).

which is believed to be involved in the transport of very long-chain fatty acids into the peroxisome.

To elucidate the role of various enzymes involved in peroxisomal β-oxidation in degradation of LTs, we analyzed in this study the excretion of LTB_4 and LTE_4 and their metabolites in urines of patients with a deficiency of DBP, RCDP type 1, XALD as well as Infantile Refsum's disease (IRD), the latter representing a milder variant of Zellweger syndrome, characterized by a less severe peroxisomal deficiency.

Materials and Methods

Excretion of LTB_4, LTE_4, and their metabolites was studied in urine samples of patients with IRD ($n = 2$), patients with a deficiency of DBP ($n = 4$), a patient with RCDP type 1 ($n = 1$), and patients with XALD ($n = 4$). All patients with IRD had the clinical and biochemical abnormalities described for patients with a peroxisome biogenesis disorder, including deficient C26:0, pristanic acid β-oxidation, and phytanic acid α-oxidation [18]. The DBP-deficient patients all had mutations in the encoding gene and no enzyme acitivity could be measured in cultured skin fibroblasts of these patients [9–12]. The patient with RCDP type 1 we studied had a mutation in the *PEX7* gene encoding the PTS2 receptor and as a consequence had no 3-ketoacyl-CoA thiolase located in his peroxisomes. Immunoblot studies performed with an antibody raised against 3-ketoacyl-CoA thiolase revealed that only the unprocessed protein of 44 kDa is present in fibroblast homogenates. It is known that 3-ketoacyl-CoA thiolase is synthesized as a precursor protein and proteolytically cleaved to its mature form of 41 kDa inside the peroxisome [19]. The XALD patients studied all had mutations in the gene encoding the peroxisomal membrane protein ALDP [16,17], which results in an impaired C26:0 β-oxidation as studied in fibroblasts of these patients.

Urine was obtained either from spontaneous micturition or was collected from a catheter introduced into the urinary bladder and stored at −20 °C until analysis. Urinary LTB_4 and LTE_4 and their ω- and β-oxidation metabolites were separated by reversed-phase high performance liquid chromatography (HPLC) and subsequently quantified by immunoassays and gas chromatography–mass spectrometry using [^{18}O]-labeled LTs as internal standards [4].

Results and discussion

The concentrations of LTB_4 and LTE_4 and their oxidation products in the urines from patients with different peroxisomal-oxidation defects and from healthy controls are summarized in Table 1. LTE_4 and its ω-oxidation product ω-carboxy-LTE_4 were not only significantly increased in the urines of IRD patients, which is in agreement with the findings of previous studies in urines of patients with a peroxisome biogenesis disorder [4], but also in the urines of DBP-deficient patients. In contrast, analyses of urine samples from patients with RCDP type 1 as well as XALD revealed normal levels of LTE_4 and ω-carboxy-LTE_4 compared to control subjects. The β-oxidation product ω-carboxy-tetranor-LTE_3 was not detectable in the urines of patients with IRD and DBP deficiencies, whereas control values were measured in the urines of patients with XALD and RCDP type 1.

Similar results were obtained for the LTB_4 series. LTB_4 and ω-carboxy-LTB_4 levels were present and significantly increased in the urines of patients with IRD and patients with a deficiency of DBP, while these compounds were undetectable in the urines of control subjects (Table 1 and [6]) and patients with XALD or RCDP type 1. The β-oxidation product ω-carboxy-tetranor-LTB_3 was not detectable in any of the urine samples studied, neither from patients nor from control subjects.

The results obtained in this study show that in patients with IRD, which is a mild variant of a peroxisome biogenesis disorder characterized by a relatively mild peroxisomal deficiency [18], there is an impaired degradation of LTs comparable to the deficiency reported in patients with Zellweger syndrome [4,6]. In addition, we showed that DBP is the enzyme responsible for the second and third steps of the β-oxidation process of both ω-carboxy-LTB_4 and ω-carboxy-LTE_4, because patients with a deficiency of DBP excrete in their urine increased amounts of both LTB_4 and LTE_4 as well as their ω-oxidation products. It has been proposed that the other peroxisomal bifunctional protein, LBP, might be involved in the β-oxidation of prostaglandins and LTs, since at the moment the functional significance of this enzyme is still unclear [20]. However, based on the results of the analyses in urines of DBP-deficient patients described in this paper, it is evident that LBP is *not* involved in the degradation of LTs and that for the time being the physiological role of LBP remains elusive. The impaired degradation and inactivation of LTs in patients with a DBP deficiency lead to increased levels of these biologically very potent mediators and this might be of pathological significance in this disorder. LTB_4 promotes neutrophil chemotaxis and adhesion to vascular endothelium [2]. The cysteinyl LTs cause plasma leakage from postcapillary venules and enhance mucus secretion and smooth muscle contraction [2]. In addition, LTB_4 is an activator of the transcription factor PPARα (peroxisome proliferator-activated receptor α) [2]. In brain, LTs may have neuromodulatory and neuroendocrine functions [21]. Increased levels of these LTs could therefore very well be involved in the clinical symptomatology in patients with DBP deficiency [22].

In case of the last step of the β-oxidation of ω-carboxy-LTs, which involves thiolytic cleavage, our results obtained in urine of an RCDP type 1 patient suggest that the classical 3-ketoacyl-CoA thiolase is not essential for normal inactivation of LTs. This means that either the other peroxisomal thiolase, SCPx, is involved in the degradation of LTs or that both thiolases are able to perform the last step of the β-oxidation of ω-carboxy-LTs and can take over each other's function in case of a deficiency of one of the enzymes. This has also been

Table 1
Concentrations of LTE_4, ω-carboxy-LTE_4, ω-carboxy-tetranor-LTE_3, LTB_4, ω-carboxy-LTB_4, and ω-carboxy-tetranor-LTB_3 in urines of patients with IRD, DBP deficiency, RCDP type 1, and XALD

Metabolite (nmol/mol creatinine)	Controls ($n = 20$)	IRD ($n = 2$)	DBP ($n = 4$)	RCDP ($n = 1$)	XALD ($n = 4$)
LTE_4	27[a]	238	194	23	36
	10–51[b]	307	418		47
			331		22
			226		19
ω-Carboxy-LTE_4	30[a]	1625	1598	15	28
	11–43[b]				
		2035	2628		16
			2355		31
			1986		24
ω-Carboxy-tetranor-LTE_3	52[a]	<5	<5	27	42
	24–108[b]				
		<5	<5		34
			<5		68
			<5		53
LTB_4	<5[c]	46	37	<5	<5
		69	126		<5
			85		<5
			74		<5
ω-Carboxy-LTB_4	<5[c]	283	192	<5	<5
		317	583		<5
			266		<5
			471		<5
ω-Carboxy-tetranor-LTB_3	<5[c]	<5	<5	<5	<5
		<5	<5		<5
			<5		<5
			<5		<5

[a] Mean.
[b] Range.
[c] 5 nmol/mol creatinine is the limit of detection calculated from urines with up to 10 mmol/L creatinine; IRD, patients with Infantile Refsum's disease; DBP, patients with a deficiency of D-bifunctional protein; RCDP, patient with Rhizomelic Chondrodysplasia Punctata type 1; XALD, patients with X-linked adrenoleukodystrophy.

observed for C26:0 and C24:6*n*-3 β-oxidation, the last step of docosahexaenoic acid (C22:6*n*−3) formation, which is normal both in patients with RCDP type 1 and in SCPx knockout mice [23,24]. Future studies in mice lacking SCPx will have to elucidate the role of SCPx in the degradation of LTs. Our results obtained in urines of XALD patients have shown that ALDP, which is mutated in patients with XALD resulting in impaired β-oxidation of very long-chain fatty acids including C26:0 and C24:0, is not involved in β-oxidation of ω-carboxy-LTs.

In conclusion, the results presented in this paper underline the essential role of peroxisomes in the degradation of LTB_4 and the cysteinyl LTs. We have shown that in urines of patients with a deficiency of DBP there are increased concentrations of LTB_4, LTE_4, and their ω-oxidation products, indicating that DBP activity is necessary for the inactivation of LTs. Furthermore, we have found that in urines of patients with RCDP type 1 and XALD there are normal concentrations of LTs, suggesting that peroxisomal 3-ketoacyl-CoA thiolase and ALDP, both involved in peroxisomal β-oxidation of very long-chain fatty acids, are not involved in peroxisomal β-oxidation of ω-carboxy-LTB_4 and ω-carboxy-LTE_4 or are at least not essential for this process.

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