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Review

Regulation of the adrenoleukodystrophy-related gene (*ABCD2*): Focus on oxysterols and LXR antagonists

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ABSTRACT

The regulation of the *ABCD2* gene is recognized as a possible therapeutic target for X-linked adrenoleukodystrophy, a rare neurodegenerative disease caused by mutations in the *ABCD1* gene. Up-regulation of *ABCD2* expression has indeed been demonstrated to compensate for *ABCD1* deficiency, restoring peroxisomal β-oxidation of very-long-chain fatty acids. Besides the known inducers of the *ABCD2* gene (phenylbutyrate and histone deacetylase inhibitors, fibrates, dehydroepiandrosterone, thyroid hormone and thyromimetics), this review will focus on LXR antagonists and 22S-hydroxycholesterol, recently described as inducers of *ABCD2* expression. Several LXR antagonists have been identified and their possible indication for neurodegenerative disorders will be discussed.

Contents

1. Introduction

Childhood cerebral adrenoleukodystrophy (CCALD) and the adult form called adrenomyeloneuropathy (AMN) are the main clinical phenotypes of X-linked adrenoleukodystrophy (X-ALD, OMIM 300100) [1]. CCALD is characterized by inflammatory demyelination of the central nervous system. AMN is characterized by a non-inflammatory distal axonopathy mainly affecting spinal cord and peripheral nerves. In spite of the success of a gene therapy trial, this fatal disorder is still in search for an efficient therapy that could represent a good alternative to the hematopoietic stem cell transplantation, the unique therapy available for the patients with the cerebral form. From a biochemical point of view, X-ALD is characterized by the accumulation of very-long-chain fatty acids (VLCFA) resulting from a β-oxidation defect caused by mutations in the *ABCD1* gene. *ABCD1* encodes for a peroxisomal half ABC transporter participating in the entry of VLCFA-CoA into the peroxisome, the unique site of their β-oxidation [2]. Overexpression of the *ABCD2* gene [3], the closest homolog of *ABCD1*, has been shown to compensate for *ABCD1* deficiency in X-ALD skin fibroblasts [4,5]. Functional redundancy is also recognized *in vivo* since reversion of the adrenomyeloneuropathy-like phenotype has been observed in *Abcd1* null mice overexpressing *Abcd2* [6]. Therefore, pharmacological induction of *ABCD2* could represent an alternative therapy for X-ALD.

Numerous investigations focused on the transcriptional regulation of *ABCD2* to discover endogenous or synthetic inducers and/or novel molecular regulation pathways have been initiated. These studies faced with different concerns. To limit side effects, it is necessary to find out regulators with an elevated specificity for *ABCD2*. Obviously, a pleiotropic effect may be considered as positive if such inducers lead to an increase in peroxisomal β-oxidation, a decreased elongation of fatty acids, an anti-inflammatory effect or a decrease in the level of oxidative stress. Moreover, pharmacological induction of *ABCD2* should a priori occur in the key tissues

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affected by the disease (adrenals, testis, brain) and specific cell types such as glial (especially oligodendrocytes) or microglial cells. At first glance, it would be necessary that pharmacological inducers were able to enter into the brain. However, it cannot be excluded that pharmacological induction of *ABCD2* in circulating cells would be sufficient to trigger beneficial effects in central nervous system of X-ALD patients. Finally, the ability to overexpress *ABCD2* in a tissue in which expression level is already elevated might be difficult.

2. Regulators of *ABCD2* expression

In 1998, sodium phenylbutyrate (4-PBA), a histone deacetylase (HDAC) inhibitor, was shown to normalize VLCFA levels in human and murine X-ALD fibroblasts by increasing their peroxisomal β-oxidation [4]. In 4-PBA-treated *Abcd1* null mice, VLCFA levels were decreased both in adrenals and brain. Induction of peroxisome proliferation and/or *Abcd2* in brain was hypothesized to be responsible for this observation. In rats and different cell types, 4-PBA was shown to induce peroxisome proliferation and increase peroxisomal β-oxidation by inducing the *Abcd2* gene through its minimal promoter (Fig. 1) [7]. However, while induction of *Abcd2* was observed *in vitro* in glial cells and in the liver of treated-rats, there was no evidence of induction in the brain. A clinical trial in adrenomyeloneuropathy patients failed to demonstrate efficiency probably because of the very short half-life of 4-PBA *in vivo* [8]. Since the proof of concept that molecules involved in chromatin remodeling could be indicated for X-ALD, several studies have been initiated on analog compounds. Valproic acid (a non-specific HDAC inhibitor used in epilepsy) was shown to induce *ABCD2* expression in X-ALD fibroblasts but apparently without decrease of saturated VLCFA levels [9]. Suberoylanilide hydroxamic acid (SAHA) was able to normalize VLCFA (peroxisomal β-oxidation and *ABCD2*/*ABCD3* expression levels are increased while the elongase *ELOVL1* expression is decreased) in X-ALD fibroblasts and to down-regulate the expression of proinflammatory cytokines in *Abcd1*/*2*-silenced mouse astrocytes [10]. Similar results were recently obtained with caffeic acid phenethyl ester [11].

Fibrates are hypolipidemic drugs that induce peroxisomal proliferation and peroxisomal β-oxidation in rodents through PPARα activation. Early studies demonstrated inducibility of *Abcd2* in the liver but not in the brain of rodents [5,12,13]. Up-regulation of *Abcd2* was shown to be dependent on PPARα although no functional peroxisome proliferator response element has been identified in the promoter of *Abcd2* [12]. Indirect regulation involving SREBP2 has been suspected [14] but there is currently no direct proof in favor of this hypothesis. Other potent PPARα activators (GW6867, GW7647) were shown to induce *Abcd2* expression in the liver but not in brain. Similar results were obtained with tetradecylthioacetic acid, another PPARα agonist known to cross the blood–brain barrier [14]. A clinical trial was recently initiated on bezafibrate, a drug shown to reduce VLCFA accumulation in X-ALD fibroblasts contrary to other PPAR ligands but failed to demonstrate induction of *ABCD2* and improvements in adrenomyeloneuropathy patients [15].

Dehydroepiandrosterone (DHEA) and its sulfate ester are the most abundant steroids in humans. DHEA is involved in neuroprotection and neurite growth and exerts anti-oxidant and anti-inflammatory effects through several nuclear receptors including PPARα. *Abcd2* was induced in the liver of male rodents treated with DHEA or its 17β-reduced metabolite ADIOL (5-androstene-3β,17β-diol) independently of PPARα [16]. A clinical study on X-ALD patients showed that DHEA supplementation for 3 months did not lower plasma levels of VLCFA [17]. Other testosterone metabolites were shown to increase peroxisomal β-oxidation and reduce the accumulation of VLCFA in X-ALD fibroblasts [18].

The *ABCD2* promoter contains a DR-4 motif, which is recognized by the thyroid hormone receptors TRα and TRβ and serves as a thyroid hormone response element [19,20] (Fig. 1). Treatment of rodents with thyroid hormone (T3) resulted in *Abcd2* and *Abcd3* induction in the liver but not in the brain [19]. *Abcd2* induction was also observed in T3-treated oligodendrocyte cells but not in astrocytes. In human X-ALD fibroblasts, T3-dependent induction of *ABCD2* was transitory and correlated with an increase of peroxisomal β-oxidation and a decrease of VLCFA levels [19]. GC-1 or CGS23425, two thyromimetics specific of TRβ (avoiding cardiac side effects due to TRα activation), were shown to stably up-regulate *ABCD2* and *ABCD3* genes in human HepG2 cells and X-ALD skin fibroblasts [21]. Further studies in animals would be required to test their therapeutic potentialities.

The DR-4 motif is also a response element for the liver X receptor (LXR), the nuclear receptor for oxysterols [22] and overlaps with a sterol regulatory element (SRE), which binds SREBP [23,24]. This promoter region is therefore a convergence point for a complex cross-talk involving key players of the lipid metabolism (TR, LXR, SREBP) which are closely associated and whose expression levels and effects are mutually controlled (Fig. 1). Deiodinase 2, which converts T4 to T3, is repressed by natural endogenous LXR ligand 22(R)-hydroxycholesterol (22R-HC) [25]. Unliganded TRβ represses LXRα transactivation [26]. TRβ and LXRα modulate the expression of SREBP [27]. The *ABCD2* gene was found to be up-regulated by cholesterol depletion *via* SREBP in X-ALD fibroblasts [23]. Lovastatin treatment was reported to reduce cholesterol and saturated VLCFA levels in X-ALD patients [28] but failed to demonstrate clinical improvement [29]. Interestingly, cholesterol excess known to induce SREBP1c expression and to activate LXRα, resulted in a reduced hepatic expression of *Abcd2* in mice [24]. LXRα activation with the synthetic agonist T0901317 or 22R-HC was shown to interfere with SREBP1c-mediated activation of the *Abcd2* promoter [24]. Besides, polyunsaturated fatty acids (PUFA) were found to modulate the *Abcd2* hepatic expression in rodents [30]. PUFA are known activators of PPARα and modulators of cholesterol levels acting through SREBP inhibition and by antagonizing the activation of LXRα by oxysterols [31].

From these different results, it became clear that molecules capable of inhibiting the LXR pathway would have an interest in the context of X-ALD. The LXR antagonist GSK(17) (GSK1440233A, compound 17) was identified from a screening of GlaxoSmithKline compound collection [32]. Another LXR antagonist, 22(S)-hydroxycholesterol (22S-HC), was described to reduce lipogenesis and free cholesterol [33,34]. Both molecules were shown to moderately induce the expression of *ABCD2* in HepG2 hepatoma cells and X-ALD skin fibroblasts [35]. This study confirmed that 22R-HC down-regulates *ABCD2* expression in both cell types. Interestingly, both *ABCD3* and *CTNNB1* genes (the gene encoding for β-catenin) were induced by LXR antagonists. β-catenin and TCF-4, important components of the Wnt/β-catenin signaling pathway, were recently described as inducers of the *ABCD2* expression [36]. A functional TCF binding element (TBE) was indeed characterized in the promoter of *ABCD2*. Since the Wnt components are repressed by oxysterols [37,38], the induction of *ABCD2* upon treatment with LXR antagonists could be a direct consequence of LXR antagonization on the promoter of *ABCD2* and/or the result of the indirect activation of β-catenin expression (Fig. 1). Besides, LXR antagonists were shown to decrease oxidative stress in X-ALD fibroblasts while 22R-HC treatment resulted in increased reactive oxygen species production [35]. Oxidative stress changes may result from the moderated alterations observed in monounsaturated fatty acid levels as a direct consequence of the induction of *ABCD2* and stearoyl-CoA desaturase-1 expression. Activation of the Wnt/β-catenin pathway whose role in liver protection against oxidative stress has previously been demonstrated [39] may be considered as an alternative