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Enzymatic characterization of ELOVL1, a key enzyme in very long-chain fatty acid synthesis

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ABSTRACT

X-linked adrenoleukodystrophy (X-ALD) is a neurometabolic disease that is caused by mutations in the *ABCD1* gene. ABCD1 protein deficiency impairs peroxisomal very long-chain fatty acid (VLCFA) degradation resulting in increased cytosolic VLCFA-CoA levels, which are further elongated by the VLCFA-specific elongase, ELOVL1. In adulthood, X-ALD most commonly manifests as a gradually progressive myelopathy (adrenomyeloneuropathy; AMN) without any curative or disease modifying treatments. We recently showed that bezafibrate reduces VLCFA accumulation in X-ALD fibroblasts by inhibiting ELOVL1. Although, in a clinical trial, bezafibrate was unable to lower VLCFA levels in plasma or lymphocytes in X-ALD patients, inhibition of ELOVL1 remains an attractive therapeutic option.
In this study, we investigated the kinetic characteristics of ELOVL1 using X-ALD fibroblasts and microsomal fractions from ELOVL1 over-expressing HEK293 cell lines and analyzed the inhibition kinetics of a series of fibrates. Our data show that the CoA esters of bezafibrate and gemfibrozil reduce chain elongation by specifically inhibiting ELOVL1. These fibrates can therefore serve as lead compounds for the development of more potent and more specific inhibitors for ELOVL1.

1. Introduction

X-linked adrenoleukodystrophy (X-ALD) is a progressive neurodegenerative disease caused by mutations in the *ABCD1* gene [1] and characterized by the accumulation of very long-chain fatty acids (VLCFA; ≥C22:0) in plasma and tissues [2]. Clinically, the disease ranges from the rapidly progressive cerebral form of X-ALD (cerebral-ALD), to the more slowly progressive adult form adrenomyeloneuropathy (AMN) and primary adrenocortical insufficiency [3]. In addition, over 80% of women with X-ALD develop AMN [4]. At present, treatment options are very limited. For boys with early stage cerebral-ALD, a bone-marrow transplantation is curative [5]. However, for AMN, which represents 85% of X-ALD cases (males and females combined), no disease modifying therapy is available [6].

The *ABCD1* gene encodes a protein (ALDP) that transports VLCFA into peroxisomes [7,8]. ALDP deficiency has two major consequences, including: 1) the impaired beta-oxidation of VLCFA in peroxisomes [9,10], and 2) raised cytosolic VLCFacyl-CoA levels which serve as substrates for further elongation to even longer fatty acids by ELOVL1, the VLCFA-specific elongase [11,12].

In males, unambiguous diagnosis of X-ALD can be achieved by demonstration of elevated VLCFA levels in plasma [2]. The strongest increase in VLCFA has been reported in complex lipids in the central nervous system, in particular in myelin, which is predominantly (>70%) composed of lipids [13,14]. While only a small part of VLCFA is taken up from the diet, the majority of VLCFA are synthesized endogenously from long-chain fatty acids, mainly C16:0, via chain elongation [15]. Fatty acid elongation consists of four enzymatic reactions (condensation, reduction, dehydration and reduction) [16]. The first step involves the malonyl-CoA driven formation of a 3-ketoacyl-CoA catalyzed by one of seven elongases (ELOVL1-7). In the second reaction, the 3-ketoacyl-CoA is reduced to 3-hydroxyacyl-CoA. This reaction requires NADPH and is carried out by a single enzyme named, 3-ketoacyl-CoA reductase, encoded by *HSD17B12*. In the third step, 3-hydroxyacyl-CoA is dehydrated to trans-2,3-enoyl-CoA by 3-hydroxyacyl-CoA dehydratase (HACD). Finally, trans-2,3-enoyl-CoA is reduced to a fatty acyl-CoA by trans-2,3-enoyl-CoA reductase (TECR), which also requires NADPH. The result is a fatty acyl-CoA ester that is extended with 2 carbon atoms, which can be elongated further during subsequent elongation cycles.

Previously, we identified ELOVL1 as the elongase responsible for the elongation of C22:0 to C24:0 and C26:0 [12] and demonstrated that knockdown of ELOVL1 lowers C26:0 synthesis and C26:0 levels in X-ALD fibroblasts [12], which provided proof-of-concept for

Abbreviations: ALDP, adrenoleukodystrophy protein; AMN, adrenomyeloneuropathy; ELOVL, elongation of very long-chain fatty acids; PPAR, peroxisome proliferator-activated receptor; VLCFA, very long-chain fatty acids

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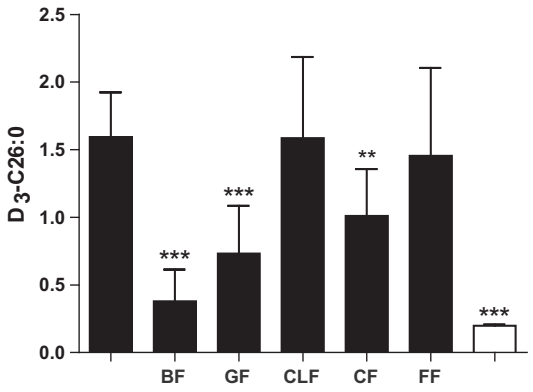

Fig. 1. Effect of fibrates on *de novo* VLCFA synthesis in intact cells. D_3-C26:0 synthesis from D_3-C22:0 was measured in 3 X-ALD (black bars) and 6 control (white bars) cell lines. To test the effect on the synthesis of D_3-C26:0, fibrates were supplemented to the culture medium at the following final concentrations: 400 μM BF, 400 μM GF, 400 μM CLF, 400 μM CF or 100 μM FF. Fatty acid levels are in nmol/mg protein. We obtained p values using one-way ANOVA followed by Dunnett's post-hoc test using untreated X-ALD cell lines as control (** = $p < 0.01$ and *** = $p < 0.001$).

ELOVL1 inhibition as a therapeutic option for X-ALD. Importantly, ELOVL1 is ubiquitously expressed in all tissues including the adrenal gland, brain and testis [17], the tissues which are primarily affected in X-ALD [3].

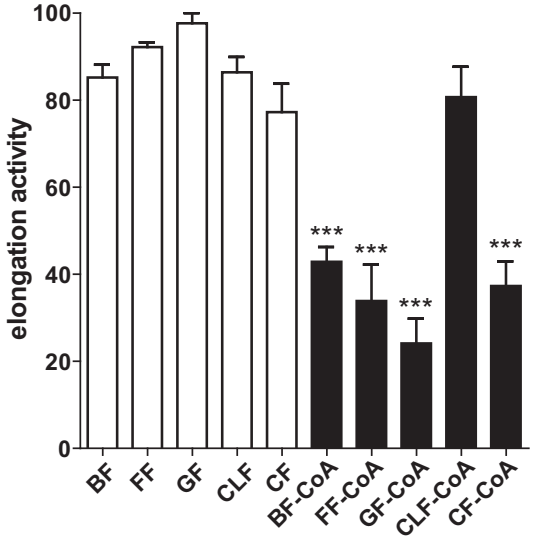

Fig. 3. Only fibroyl-CoA esters inhibit VLCFA elongation activity. Microsomes were isolated from HEK293 ELOVL1 overexpressing cells and used to analyze the effect of fibrates (white bars) and fibroyl-CoA esters (black bars) on C22:0-CoA (20 μM) elongation. Final concentration of each fibrate and its corresponding CoA-ester was 100 μM. Reactions were started with the addition of 50 μg protein. The enzyme activity is expressed relative to the activity measured in the absence of inhibitor (set at 100%). Error bars indicate the variation between duplicate measurements.

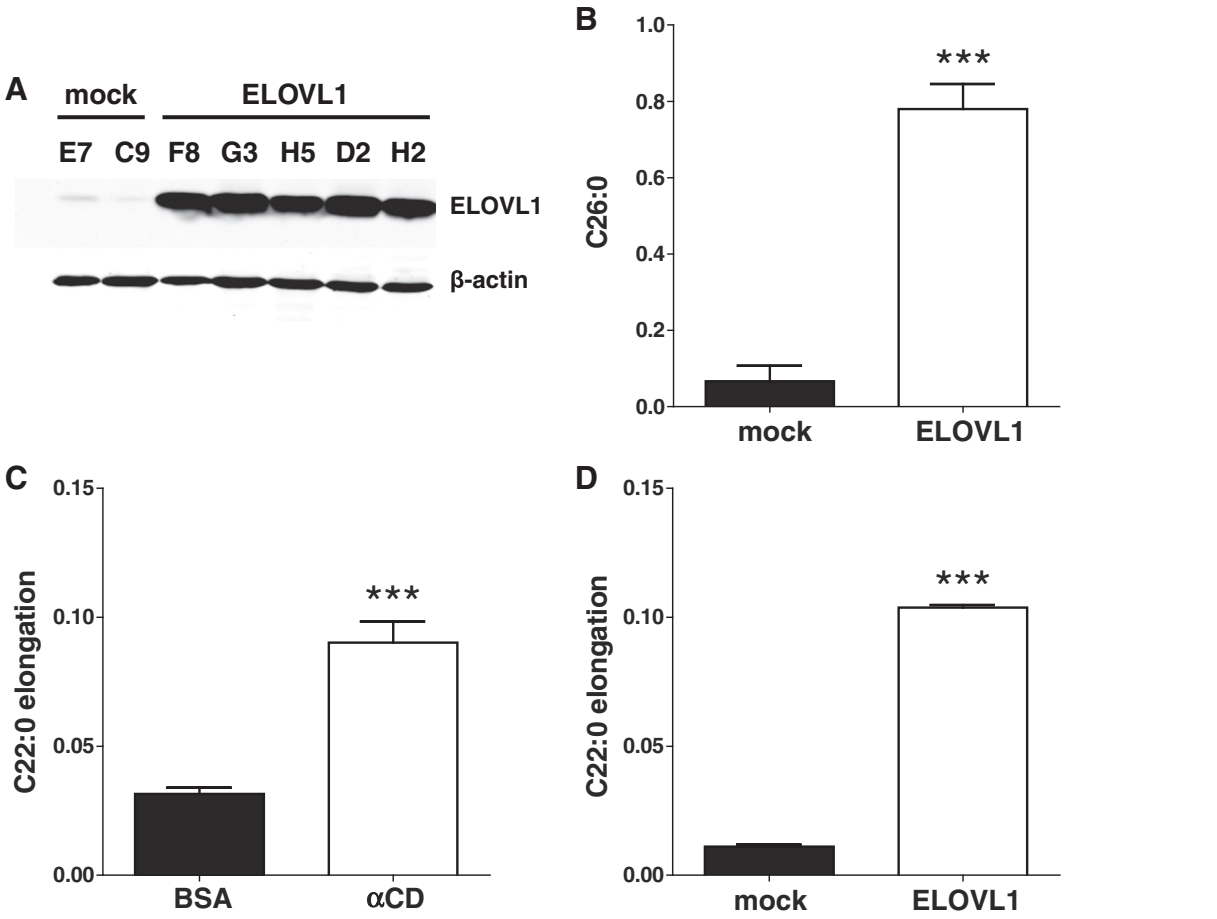

Fig. 2. Over-expression of ELOVL1 results in increased VLCFA synthesis. Independent clones of stable transfected HEK293 Flp-In cell lines were characterized for the expression of ELOVL1, VLCFA levels and VLCFA elongation activity. (A) Immunoblot analysis of ELOVL1 in mock transfected and five ELOVL1 overexpressing clones. (B) C26:0 levels in two mock transfected (black bar) and five ELOVL1 overexpressing HEK293 clones (white bar). (C) Microsomes isolated from the HEK293 ELOVL1 overexpressing clone (H2) were used to measure fatty acid elongation activity with either BSA (black bar) or α-cyclodextrin (αCD, white bar). Malonyl-CoA and C22:0 concentrations were kept constant at 60 μM and 20 μM, respectively. (D) Microsomal fractions, isolated from a mock transfected and ELOVL1 overexpressing HEK293 clone were used for the fatty acid elongation assay with C22:0-CoA as substrate. Fatty acid levels are in nmol/mg protein. Elongation activities are in nmol/min/mg protein. Data are mean ± SD. *** = $p < 0.001$ by unpaired Student's *t*-test.