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Sterols and oxysterols in plasma from Smith-Lemli-Opitz syndrome patients

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ABSTRACT

Smith-Lemli-Opitz syndrome (SLOS) is a severe autosomal recessive disorder resulting from defects in the cholesterol synthesising enzyme 7-dehydrocholesterol reductase (Δ^7-sterol reductase, DHCR7, EC 1.3.1.21) leading to a build-up of the cholesterol precursor 7-dehydrocholesterol (7-DHC) in tissues and blood plasma. Although the underling enzyme deficiency associated with SLOS is clear there are likely to be multiple mechanisms responsible for SLOS pathology. In an effort to learn more of the aetiology of SLOS we have analysed plasma from SLOS patients to search for metabolites derived from 7-DHC which may be responsible for some of the pathology. We have identified a novel hydroxy-8-dehydrocholesterol, which is either 24- or 25-hydroxy-8-dehydrocholesterol and also the known metabolites 26-hydroxy-8-dehydrocholesterol, 4-hydroxy-7-dehydrocholesterol, 3β,5α-dihydroxycholest-7-en-6-one and 7α,8α-epoxycholesterol. None of these metabolites are detected in control plasma at quantifiable levels (0.5 ng/mL).

Contents

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1. Introduction

Smith-Lemli-Opitz syndrome (SLOS, MIM no. 270400) was first described in 1964 [1]. It is an autosomal recessive disorder resulting from deficiency of the enzyme 7-dehydrocholesterol reductase (DHCR7, EC 1.3.1.21, 3β-hydroxysterol Δ^7-reductase) [2]. DHCR7 reduces the Δ^7-double bond in 7-dehydrodesmosterol (7-DHD, cholesta-5,7,24-trien-3β-ol) and in 7-dehydrocholesterol (7-DHC, cholesta-5,7-dien-3β-ol) leading to the formation of desmosterol (cholesta-5,24-dien-3β-ol) and cholesterol (cholest-5-en-3β-ol) via the Bloch and Kandutsch-Russel pathways, respectively (Fig. 1A) [3]. SLOS patients show decreased levels of cholesterol and increased levels of 7-DHC and its isomer 8-dehydrocholesterol (8-DHC, cholesta-5,8(9)-dien-3β-ol) in serum and tissues [4]. SLOS was the first human syndrome discovered due to an inborn error of sterol synthesis [2]. The phenotypic spectrum of SLOS is extremely broad; while severe cases may die *in utero*, mild cases show only minor physical, learning and behavioural problems [5]. Limb abnormalities are common in SLOS, and patients often show a distinctive cognitive and behavioural phenotype, although normal intelligence is also possible [6].

The *DHCR7* gene is encoded by nine exons, and over 100 mutations have been identified in SLOS patients [7]. Genotype-phenotype correlations are poor, although many missense mutations result in residual enzyme activity which is associated with a less severe phenotype [7]. SLOS has a high carrier frequency in Caucasians. In European populations the combined carrier frequency of two of the most common mutations c.964-1G>C (IVS8-1G>C) and p.W151X ranges from 1 to 2.3% [8]. Considering these numbers, the clinical incidence of SLOS (1:10,000–1:70,000 in Northern and Central European populations, 1:50,000 in the USA) is much lower than that predicted [5]. This is most likely due to several factors, including under-diagnosis of mild cases, and early prenatal pregnancy loss in severe cases. It is tempting to speculate that the high carrier frequency, particularly in populations from Northern and Central Europe, conveys a heterogeneous advantage [5]. 7-DHC is a precursor of vitamin D_3 (Fig. 1B), and increased vitamin D_3 levels in the skin could protect against vitamin D deficiency.

SLOS can be diagnosed biochemically based on increased 7-DHC in serum and tissues [9]. 7-DHC levels are typically more than 50-fold elevated in SLOS cases, although there are equivocal cases of SLOS with serum 7-DHC levels just above normal levels [5]. Gene sequencing of *DHCR7* is an alternative to biochemical analysis, but is limited by known pathogenic mutations.

Dietary supplementation with cholesterol to reduce *de novo* synthesis of 7-DHC and increase cholesterol levels is a standard treatment for SLOS. Dietary cholesterol supplementation is reported to improve behaviour [10], but as cholesterol does not pass the blood brain barrier (BBB), this improvement may be mediated by cholesterol metabolites, *e.g.* oxysterols, which can cross the BBB. Theoretically, statin therapy should also reduce 7-DHC biosynthesis and also tissue levels [11].

Although the underlying enzymatic defect in SLOS is well established there are likely to be multiple mechanisms responsible for SLOS pathology. For instance, cholesterol has numerous biological functions and substitution of 7-DHC for cholesterol, and 7-DHD for desmosterol, may alter physiochemical properties and function of membranes. Also 7-DHC, its isomer 8-DHC, their metabolites and 7-DHD analogues may have a direct toxic effect on cells [12]. Cholesterol is the precursor of steroid hormones and bile acids and dehydrocholesterol analogues of pregnenolone, pregnanetriol, dehydroepiandrosterone and androstenediol have been reported [13]. 7-DHC derived bile acid precursors have been reported to be formed in liver mitochondrial incubations from a rat model of SLOS, including 26-hydroxy-7-dehydrocholesterol (26-OH-7-DHC, cholesta-5,7-diene-3β,26-diol) and 26-hydroxy-8-dehydrocholesterol (26-OH-8-DHC, cholesta-5,8(9)-dien-3β,26-diol) (Fig. 1C) [14]. Note, we use here the systematic nomenclature where a hydroxy group introduced to the terminal carbon of the sterol side-chain is at carbon-26 [15]. Unless stated otherwise, this is assumed to introduce R stereochemistry at carbon-25. Further metabolism remains to be fully elucidated, although Natowicz and Evans reported unusual bile acids in the urine of SLOS patients [16]. These results have not been confirmed by others. 26-OH-7-DHC and 26-OH-8-DHC have been reported to be present in plasma from SLOS patients at levels of 0.04–0.51 μM (16–204 ng/mL), the Δ^7 isomer being an inhibitor of sterol synthesis and a ligand to the liver X receptor α [17]. The mitochondrial enzyme, cytochrome P450 (CYP) 27A1, oxidises cholesterol to 26-hydroxycholesterol (26-OHC, cholest-5-en-3β,(25R) 26-diol) and it is likely that this is the mitochondrial enzyme which also oxidises 7- and 8-DHC to Δ^7 and Δ^8 analogues of 26-OHC (Fig. 1C) [18]. In a study of infants with SLOS, Björkhem et al. found reduced plasma levels of 24S-hydroxycholesterol (24S-OHC, cholest-5-ene-3β,24S-diol), but increased levels of 26-OHC [19]. The reduced level of brain derived 24S-OHC was not surprising in light of the reduced abundance of its precursor, cholesterol, but the elevated level of 26-OHC was less easy to explain [19].

In a more recent study Liu et al. have identified 4α- and 4β-hydroxy-7-dehydrocholesterol (4α- and 4β-OH-7-DHC, cholesta-5,7-diene-3β,4α/β-diol) in plasma of SLOS patients [20]. The 4β-hydroxy compound could be formed enzymatically via a CYP3A4 catalysed reaction analogous to that which forms 4β-hydroxycholesterol from cholesterol. Liu et al. also found elevated levels of 7-oxocholesterol (7-OC, 3β-hydroxycholest-5-en-7-one) in SLOS plasma which correlated positively with SLOS severity scores [20]. Interestingly, 7-OC, is a product of the CYP7A1 oxidation of 7-DHC (Fig. 1E) [21]. Goyal et al. have also found 7-DHC to be a substrate for other CYP enzymes [22]. They found that CYP46A1 can oxidise 7-DHC to 24-hydroxy-7-dehydrocholesterol (24-OH-7-DHC, cholesta-5,7-dien-3β,24-diol) and to 25-hydroxy-7-dehydrocholesterol (25-OH-7-DHC, cholesta-5,7-dien-3β,25-diol, Fig. 1E) [22]. Endo-Umeda et al. have shown that 7-DHC can also be metabolised by CYP27A1 to 25-OH-7-DHC and that this oxysterol and 26-OH-7-DHC are present in SLOS plasma at levels of 4 ng/mL and 33 ng/mL, respectively [18]. 24-OH-7-DHC, 4α- and 4β-OH-7-DHC, 7-OC and also 3β,5α-dihydroxycholest-7-en-6-one (DHCEO) have been identified in tissues and fluids from a rat model of SLOS [23,24] and/or *Dhcr7*-null mouse embryos [25,26]. DHCEO is formed non-enzymatically via free radical oxidation of 7-DHC (Fig. 1E) [26]. This reaction occurs *in vivo*, at least in *Dhcr7*-deficient Neuro2a cells and SLOS fibroblasts [27], but the propensity of 7-DHC to undergo free radical oxidation reactions highlights the importance of sample handling procedures to avoid the *ex vivo* formation of 7-DHC oxidation products. In regard of the free radical oxidation of 7-DHC, Porter and