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Cholesterol biosynthesis from birth to adulthood in a mouse model for 7-dehydrosterol reductase deficiency (Smith–Lemli–Opitz syndrome)

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ABSTRACT

Smith–Lemli–Opitz syndrome (SLOS) is caused by deficiency in the terminal step of cholesterol biosynthesis, which is catalyzed by 7-dehydrocholesterol reductase (DHCR7). The disorder exhibits several phenotypic traits including dysmorphia and mental retardation with a broad range of severity. Pathogenesis of SLOS is complex due to multiple roles of cholesterol and may be further complicated by unknown effects of aberrant metabolites that arise when 7-dehydrocholesterol (7-DHC), the substrate for DHCR7, accumulates. A viable mouse model for SLOS has recently been developed, and here we characterize cholesterol metabolism in this model with emphasis on changes during the first few weeks of postnatal development. Cholesterol and 7-DHC were measured in "SLOS" mice and compared with measurements in normal mice. SLOS mice had measurable levels of 7-DHC at all ages tested (up to 1 year), while 7-DHC was below the threshold for detection in normal mice. In perinatal to weaning age SLOS mice, cholesterol and 7-DHC levels changed dramatically. Changes in brain and liver were independent; in brain cholesterol increased several fold while 7-DHC remained relatively constant, but in liver cholesterol first increased then decreased again while 7-DHC first decreased then increased. In older SLOS animals the ratio of 7-DHC/cholesterol, which is an index of biochemical severity, tended to approach, but not reach, normal. While these mice provide the best available genetic animal model for the study of SLOS pathogenesis and treatment, they probably will be most useful at early ages when the metabolic effects of the mutations are most dramatic. To correlate any experimental treatment with improved sterol metabolism will require age-matched controls. Finally, determining the mechanism by which these "SLOS" mice tend to normalize may provide insight into the future development of therapy.

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1. Introduction

Smith–Lemli–Opitz syndrome (SLOS) is an inherited disorder resulting in dysmorphias and mental retardation. It is caused by deficient 3β-hydroxysterol-Δ^7-reductase (7-dehydrocholesterol reductase, DHCR7, EC 1.3.1.21), which catalyzes the last step in cholesterol synthesis. Thus, SLOS patients have elevated levels of dehydrocholesterol (DHC) and reduced ability to make cholesterol. The molecular and genetic basis of SLOS has been well described in Refs. [1–3]; however, due to the multiple and essential roles of cholesterol, the pathogenesis of SLOS is complex and not yet well understood. Among recessive inborn errors of metabolism, SLOS has a relatively high incidence varying from 1 in 10,000 to 1 in 60,000 in different regions of Europe and North America [4–10]. The carrier frequency for mutant alleles is estimated as high as 1 in 30 for Caucasian populations and lower for African and Asian populations [3,9,11–14]. It is likely that the condition is underdiagnosed because patients having mild disorder without distinctive phenotype may be missed and early fetal demise may be common in the most severely affected [15]. Biochemical diagnosis is based on plasma serum levels of DHC [16], and prenatal diagnosis is possible by measuring DHC in amniotic fluid [17] or chorionic villus cells [15]. More recently, it has been shown that noninvasive prenatal diagnosis of SLOS is possible by measurement of novel steroid metabolites in maternal urine [10,18].

Current treatment for SLOS is dietary cholesterol supplementation. Anecdotal reports show positive albeit limited effects of exogenous cholesterol on somatic growth and behavior, but developmental outcome does not appear to be altered [19–22]. An effect of dietary cholesterol on behavior is somewhat surprising as the brain is believed to be impervious to external cholesterol [23]. Simvastatin, which inhibits 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase and can cross the blood–brain barrier, is a promising treatment for lowering DHC levels in SLOS patients [1,24].

The recent development of animal models has made the understanding of SLOS and the possible development of new treatments more feasible. Two mouse models with null mutations in the *Dhcr7* gene have been created by homologous recombination [25,26]. For both of these knockout mutations, homozygous mutant progeny die within 1 day of birth, which limits experimental usefulness. A more recent "knock-in" mutation resulted in a single amino acid substitution of methionine for threonine in DHCR7 at the amino acid position equivalent to threonine 93 in the human protein. The resulting protein has greatly reduced enzymatic activity [27]. This T93M mutation mimics a known, relatively frequent human mutation, and provides mice with a mild SLOS phenotype [28]. Compound heterozygotes containing one null allele and one T93M allele are viable. Since these are the most severely affected viable SLOS animals available, they have been used in the present study. Although these mice mimic human SLOS at the molecular level, the dysmorphic phenotype is less severe. This may be due to efficient utilization of maternal cholesterol by the mouse fetus [29] in contrast to the human.

Clearly, effective cholesterol biosynthesis is especially critical at certain times during development and continues to be important throughout life. Cholesterol levels and the balance between endogenous and exogenous sources of cholesterol can be expected to change substantially with different stages of development, but patterns of change are remarkably similar among mammals [30]. Furthermore, because of the blood–brain barrier, cholesterol metabolism in the brain is independent of systemic (liver) cholesterol metabolism. In the mouse model for SLOS we can trace these changes with great sensitivity and accuracy by measuring sterol levels in different tissues at different developmental stages. This is important for understanding SLOS pathogenesis and for developing potential therapies. Here we have compared 7-DHC and cholesterol levels in SLOS mice and normal mice at different developmental ages from late fetus to mature adult. Our results confirm and extend previously reported results in Ref. [28] and show that there were dramatic changes in 7-DHC and total cholesterol concentrations with developmental age. Also, the pattern of change in liver was quite distinct from the pattern of change in brain.

2. Experimental

2.1. Reagents and chemicals

Cholesterol was from Sigma–Aldrich (St. Louis, MO, USA), 7-dehydrocholesterol was from Steraloids (Newport, RI, USA), and cholesterol-26,26,26,27,27,27-d_6 was from CDN Isotopes (Pointe-Claire, Canada). Ultrapure water with a resistivity of 18.2 MΩ cm was obtained using a MilliQ purification system (Millipore, Molsheim, France). *N*,*O*-bis(trimethylsilyl)-trifluoroacetamida (BSTFA) was from Pierce (Rockford, IL, USA). All other chemicals were of reagent grade and purchased from Merck (Darmstadt, Germany).

2.2. Preparation of standard solutions

Ethanolic stock solutions at 1 mg/ml of each sterol were prepared by dissolving 10 mg of each substance in 10 ml of ethanol in a volumetric flask. Working solutions of different concentrations were prepared by appropriate ethanolic dilution of stock solutions. All solutions were stored in the dark at −20 °C until used.

2.3. Mice

Two lines of mutant mice developed at NIH (by Porter and co-workers) were established at the CHORI Animal Facility. The first was a knockout mutation incorporating a deletion of coding exons 3, 4, and 5 ($\Delta^{3–5}$) into the *Dhcr7* gene [26]. Heterozygous progeny had a completely normal phenotype, which is consistent with the recessive nature of DHCR7 mutations in humans. Because homozygous $\Delta^{3–5}/\Delta^{3–5}$ mutants died shortly after birth, the $\Delta^{3–5}$ mutation was maintained by crossing heterozygous ($+/\Delta^{3–5}$, normal phenotype) with normal C57BL/6 (+/+) mice. Resulting progeny were screened for the $+/\Delta^{3–5}$ genotype by PCR [26]. Due to a minimum of six generations of backcrossing, mice carrying the $\Delta^{3–5}$ mutation had a predominantly C57BL/6 genetic background. The second mutant line contained a point mutation, T93M, in *Dhcr7* [28]. Homozygous (T93M/T93M) mice were viable and could be