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Biochemical and physiological improvement in a mouse model of Smith–Lemli–Opitz syndrome (SLOS) following gene transfer with AAV vectors

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ABSTRACT

Smith–Lemli–Opitz syndrome (SLOS) is an inborn error of cholesterol synthesis resulting from a defect in 7-dehydrocholesterol reductase (DHCR7), the enzyme that produces cholesterol from its immediate precursor 7-dehydrocholesterol. Current therapy employing dietary cholesterol is inadequate. As SLOS is caused by a defect in a single gene, restoring enzyme functionality through gene therapy may be a direct approach for treating this debilitating disorder. In the present study, we first packaged a human DHCR7 construct into adeno-associated virus (AAV) vectors having either type-2 (AAV2) or type-8 (AAV2/8) capsid, and administered treatment to juvenile mice. While a positive response (assessed by increases in serum and liver cholesterol) was seen in both groups, the improvement was greater in the AAV2/8–DHCR7 treated mice. Newborn mice were then treated with AAV2/8–DHCR7 and these mice, compared to mice treated as juveniles, showed higher DHCR7 mRNA expression in liver and a greater improvement in serum and liver cholesterol levels. Systemic treatment did not affect brain cholesterol in any of the experimental groups. Both juvenile and newborn treatments with AAV2/8–DHCR7 resulted in increased rates of weight gain indicating that gene transfer had a positive physiological effect.

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1. Introduction

Smith–Lemli–Opitz syndrome (SLOS, OMIM 270400. locus 11q13.4) was first described in 1964 [1] and was much later shown to be an inborn error of cholesterol (C) biosynthesis [2,3] that results in a broad range of physical dysmorphias and cognitive impairment [4]. The biochemical cause is deficient 3β-hydroxysterol-Δ^7-reductase (EC 1.3.1.21, 7-dehydrocholesterol reductase, DHCR7), which reduces the Δ^7 bond in 7-dehydrocholesterol (7DHC) to form cholesterol in the last step of the Kandutsch–Russell cholesterol synthetic pathway [5]. As a result, cholesterol production decreases, and amounts of dehydro precursors of cholesterol, such as 7DHC and 8-dehydrocholesterol (8DHC), increase in tissues and serum [2,3,6]. Comparing the concentration of 7DHC in plasma or serum to control levels, as measured by Gas Chromatography/Mass Spectrometry (GC/MS) [7], is used to diagnose SLOS. Prenatal diagnosis is also possible by measurement of the characteristic dehydrosterols in amniotic fluid or chorionic villus cells [7–9], and fetal-derived dehydrosteroid derivatives in maternal urine or serum [10].

SLOS predominantly presents in Caucasian populations, with the incidence reported to be between 1:10,000 and 1:70,000 in northern and central Europe [11–14], and likely less than 1:100,000 in North America [15,16]. The carrier frequency for the commonly found DHCR7 mutations predicts a much higher incidence rate, and it is possible that SLOS is under-diagnosed [15,17] because the milder cases may be missed [18,19], and the more severe cases may result in prenatal or neonatal death [13,20,21]. Currently, the only treatment is dietary cholesterol supplementation, and while this has been suggested to have positive biochemical and developmental benefits [22–25], pre-existing dysmorphias are irreversible and neurological outcomes are unchanged. In contrast to previous anecdotal evidence, a short-term, placebo controlled study [26] showed that supplemental exogenous cholesterol did not improve neurological outcome in SLOS children. Likely, this is because the blood–brain barrier prevents circulating cholesterol from altering the sterol composition in the brain [27]. Because fetal development is highly dependent on endogenous cholesterol synthesis [28–32], treatment would ideally be administered prenatally in order to moderate otherwise irreversible symptoms; however, any such treatment remains to be developed.

Gene therapy is a largely unexplored treatment for this disorder that could address many of the shortcomings of dietary cholesterol supplementation. By importing a functional DHCR7 gene, patients could increase cholesterol and concomitantly decrease 7DHC. This should be doubly beneficial, firstly because sufficient cholesterol is needed for its multiple roles in metabolism, structure and regulation, and secondly because the accumulation of 7DHC can change the physical properties of cell membranes [27] and has deleterious effects when metabolized to oxidized derivatives [33]. Therefore, gene therapy has the potential to improve systemic and brain cholesterol synthesis, and if administered in utero, may even decrease the prevalence of fetal loss and ameliorate early deleterious effects.

Here we focus on systemic treatment of newborn and juvenile mice. In a previous study, we showed "proof of concept" for gene therapy in a mouse model for SLOS. Using a recombinant type-2 adeno-associated virus (AAV2) vector carrying a human *DHCR7* cDNA and a heterologous promoter, we achieved the production of active DHCR7 enzyme and increased cholesterol synthesis, as demonstrated by lowered serum 7DHC/C ratio [34]. Because the biochemical improvement was modest and a complete normalization of cholesterol was not achieved, we hypothesized, and presently describe, that higher vector doses, a more efficient vector, and/or earlier treatment administration can yield a greater positive effect.

2. Materials and methods

2.1. Animal husbandry

Animal work conformed to NIH guidelines and was approved by the Institutional Animal Care and Use Committee. All animals were maintained in an AAALAC certified facility and were fed a normal, cholesterol-free chow (Teklad irradiated rodent diet 2918: Harlan, Madison, WI).

2.2. Generation of experimental animals

The use of mutant mice and their breeding protocol was as previously described [35]. In brief, study animals were generated by crossing two separate mouse models of SLOS: a null mutant containing a