Journal of Steroid Biochemistry and Molecular Biology 254 (2025) 106822

Contents lists available at ScienceDirect

Journal of Steroid Biochemistry and Molecular Biology

journal homepage: www.elsevier.com/locate/jsbmb

The role of cholesterol biosynthesis and metabolism causing medical complexity in patients with Smith-Lemli-Opitz Syndrome (SLOS)

Ellen Roy Elias

Pediatrics and Genetics, University of Colorado School of Medicine, Special Care Clinic, Children's Hospital Colorado, 13123 E 16th Ave, Aurora, CO 80045, United States

A R T I C L E I N F O

Keywords:
Smith-Lemli-Opitz Syndrome (SLOS)
Cholesterol
7-dehydrocholesterol (7-DHC)
8-dehydrocholesterol (8-DHC)
Oxysterols
Bile acids
Retinal disease

A B S T R A C T

Smith-Lemli-Opitz syndrome (SLOS) is an autosomal recessive genetic disorder associated with complex anatomic abnormalities, accompanied by medical, developmental and behavioral challenges. It was the first human disorder identified to be caused by an error in the complex cholesterol biosynthetic pathway, more than thirty years ago. This review will cover the clinical and developmental phenotype of patients with SLOS, and the understanding of how cholesterol deficiency, accumulation of the cholesterol precursors 7- and 8-dehydrocholesterol (7-DHC and 8-DHC), and the oxidation of these precursors into toxic oxysterols, are now known to cause this complex phenotype. There is a wide range of severity in patients with SLOS. The most severely affected babies may be miscarried or die in the newborn period due to lethal congenital anomalies. The most mildly impacted patients may show few anatomic abnormalities other than 2–3 toe syndactyly, but still display cognitive and behavioral challenges along the autism spectrum. The review will also cover the medical evaluation and interventions which are recommended in caring for patients with SLOS. There is no cure for this devastating disease, but certain interventions can lead to an improved quality of life, and stabilization of progressive problems for these complex patients.

1. Introduction

1.1. Smith-Lemli-Opitz syndrome – description and history

Smith-Lemli-Opitz Syndrome is an autosomal recessive genetic disorder caused by homozygous or compound heterozygous variants in the enzyme DHCR7, the final step in cholesterol biosynthesis. SLOS was the first human disorder identified in the cholesterol biosynthetic pathway. There are now at least a half dozen other disorders described in this pathway, but SLOS is by far the most common [1,2]. The clinical disorder was first described in 1963 [3] by the three geneticists for whom it is named. The underlying biochemical cause was not discovered until 30 years later (1993–1994), by the discovery of very low plasma cholesterol levels and high levels of cholesterol precursors in patients with SLOS [4,5]. The causative gene variants were subsequently identified [6], and the diagnosis is currently most often confirmed with molecular testing (whole exome sequencing or whole genome sequencing), following suspicion based on clinical and developmental features, and biochemical abnormalities (low cholesterol levels plus elevated precursors 7- and 8-DHC).

Smith-Lemli-Opitz syndrome is one of the more common autosomal recessive disorders in humans. The carrier frequency in Caucasians in North America is felt to be approximately 1 %, and the carrier frequency is higher in populations from Eastern Europe. The actual incidence of Smith-Lemli-Opitz is less than would be expected from such a high carrier rate, estimated at 1:10,000 to 1:70,000 depending on the underlying population. It is felt that loss of severely affected fetuses, as well as lack of a correct diagnosis in more mildly affected individuals who do not have birth defects or obvious facial features, may be the cause of this lower incidence than expected [2].

The clinical phenotype of a patient with SLOS is typically that of a child with dysmorphic facial features (including microcephaly, micrognathia, abnormal pinnae, ptosis, and palatal clefting) growth failure, developmental disabilities, behavioral challenges along the autism spectrum, and multiple congenital anomalies including brain, cardiac, renal and limb deformities (2–3 toe syndactyly and polydactyly) [4,5]. There is a spectrum of severity ranging from complex issues so severe that the fetus may be miscarried prior to birth, or the child might die in early infancy, to features so mild that the child may have no severe birth defects and only mild developmental/behavioral concerns [7].

Biochemically, the major metabolic findings include hypocholesterolemia, and elevated levels of the precursors 7-dehydrocholesterol (7-

E-mail address: ellen.elias@childrenscolorado.org.

https://doi.org/10.1016/j.jsbmb.2025.106822
Received 16 January 2025; Received in revised form 24 June 2025; Accepted 27 June 2025
Available online 1 July 2025

DHC) and its isomer 8-dehydrocholesterol (8-DHC). An important note for clinicians trying to diagnose this condition is that to determine an accurate cholesterol level and to measure precursors, the testing must be done in a special laboratory, as a routine cholesterol level in a local hospital cannot differentiate between cholesterol and its precursors.

The medical consequences of the cholesterol biosynthetic error seen in patients with SLOS are related to the important physiological roles of cholesterol in the human body. There have been significant advances in the understanding of how severe cholesterol deficiency impacts physiological function of multiple organs in the past thirty years since this metabolic error was first reported. Other medical consequences are related to the detrimental effects of cholesterol precursor accumulation, and the toxic effects of oxysterol production, caused by oxidation of cholesterol precursors.

The first section of this paper will address the medical consequences of cholesterol deficiency, which begin in early fetal life and continue throughout the lifespan of patients with SLOS.

2. Part 1

2.1. Embryologic consequences of cholesterol deficiency

Within less than 5 years of the discovery of the inborn error of cholesterol metabolism as the cause of SLOS, the embryologic importance of cholesterol was further clarified [8–11]. Since the initial discovery of the cause of SLOS, other genetic disorders in the post-squalene cholesterol biosynthetic pathway causing human disease have been identified [2]. These include the very rare autosomal recessive conditions lathosterolosis, desmosterolosis and sterol-C-4 methyloxidase-like deficiency as well as two X-linked disorders, CHILD syndrome (Congenital hemidysplasia with ichthyosiform erythroderma) and X-linked dominant Chondrodysplasia punctata. These disorders are caused by enzymatic defects which may result in prenatal cholesterol deficiency, as well as accumulation of pre-cholesterol metabolites different from the 7- and 8-dehydrocholesterol seen in Smith-Lemli-Opitz syndrome. Although these disorders share some common features with SLOS, including short stature, microcephaly, growth failure, skeletal abnormalities (including chondrodysplasia punctata), developmental disabilities and autism, each of these conditions have unique presentations which are distinctive from SLOS. It is felt that the phenotypic differences may be due not only to prenatal cholesterol deficiency but rather to the impact of different accumulating precursors arising from enzymatic errors in this complex biosynthetic pathway.

Fetal cholesterol levels are dependent on endogenous fetal cholesterol production. The existence of both placental and blood-brain barriers prevent the maternal metabolism from compensating for the fetal metabolic block in cholesterol production. Thus, metabolic errors in cholesterol production are different than other classes of inborn errors of metabolism, which often do not present with dysmorphic features and birth defects. In fact, Smith-Lemli-Opitz syndrome was the first inborn error of metabolism identified in humans which is associated with malformations of the central nervous system, cardiac defects, renal anomalies and skeletal abnormalities. At first it was difficult to understand how cholesterol deficiency in the fetus could lead to these different birth defects.

Advances in understanding the important embryologic pathway starting with the Sonic Hedgehog gene, and the roles of the genes Patched and Smoothened allowed the understanding of how fetal cholesterol deficiency could lead to birth defects [8–11]. The critical discovery that the activation of the Sonic hedgehog gene pathway was dependent on post-translational binding to cholesterol, led to the understanding of the importance of this pathway on the formation of the brain, heart, kidneys, genitalia and limbs.

Prenatal testing of a fetus suspected of having SLOS has been available since the late 1990's [12]. An important note for clinicians performing prenatal testing to rule out SLOS is that performing only a karyotype or microarray on fetal testing will not give the diagnosis, as SLOS is not caused by a chromosome abnormality. Rather, sending biochemical testing to look for abnormal elevation of the precursors, or sending Whole Exome Sequencing (WES) on fetal testing would detect the gene variants in DHCR7 associated with SLOS (see below).

Evaluation and Testing: Fetal suspicion of an affected embryo with SLOS can first be made on ultrasound by the detection of multiple congenital anomalies, and poor fetal growth. Further suspicion can be raised by the detection of low Estriol levels in the prenatal "Quad screen", a maternal blood test drawn in the early 2nd trimester of pregnancy. Cholesterol is the precursor for Estriol, so a low Estriol level on the Quad screen coupled with intrauterine growth failure and birth defects should prompt further workup for possible SLOS.

The diagnosis can be suspected by measuring abnormally elevated levels of cholesterol precursors in amniotic fluid, and more definitively confirmed by finding molecular (DNA) variants in the DHCR7 gene via prenatal techniques including chorionic villus sampling (CVS) done at 11–13 weeks, and amniocentesis (done at approximately 15–16 weeks). Prenatal testing via periumbilical blood sampling (PUBS) has been done much less frequently than CVS or amniocentesis, but is a procedure which could potentially allow the introduction of a high-cholesterol containing fluid such as fresh-frozen plasma to the embryo [13].

Cholesterol can cross the placenta from the mother to the fetus, but the availability of cholesterol during pregnancy and its transport across the placenta to the fetus is a complex process which is poorly understood [14]. Placental conditions change during pregnancy: for example maternal cholesterol levels rise in the 3rd trimester, while fetal cholesterol levels, which are high at the end of the 2nd trimester, decrease towards term [14]. While much progress has been made in understanding the transport across the placenta of maternal cholesterol to the fetus [15], how this functions in pregnancies where the fetus has SLOS is still not well understood [16].

2.2. Postnatal consequences of cholesterol deficiency

Cholesterol plays critical roles in human physiology after birth. Cholesterol serves as the substrate for steroid hormones such as cortisol and sex hormones, and for the production of bile acids which are necessary for intestinal absorption of certain nutrients. Cholesterol is particularly critical for the function and stability of cell membranes. The lipid composition of cell membranes, particularly the ratio of cholesterol to phosphatidylcholine, is an important determinant of cell membrane function, affecting the ability of cell membranes to transport substances, and for the embedded membrane enzymes and receptors to function properly [17].

To better understand how cholesterol deficiency impacts the cell membranes of multiple human organ systems, particularly the role of cholesterol deficiency on organs which undergo rapid cell turnover are addressed below:

2.2.1. The skin

The skin is an essential body organ whose function maintains homeostasis of body fluids and body temperature. Skin cells usually undergo rapid turnover to replace damaged tissue in order to maintain an adequate protective barrier for the body. Insufficient cholesterol in skin cell membranes leads to an inability to replace damaged skin cells in a timely way, with resultant fragility of the skin barrier. Patients with severe SLOS can manifest this skin cell fragility by displaying eczema, poor wound healing, dehiscence of surgical wounds, frequent rashes and skin infections. There are not references to support the poor healing and wound dehiscence in the literature, but this has been seen by this author on many occasions. Reference to photosensitivity and eczema are made in GeneReviews [18].

A different insight into skin pathology in patients with SLOS might be found in steroid biochemical research published by Kim et al. in 2009,

describing the presence of 5,7-diences in patients with SLOS. These compounds are produced from the precursor 7-dehydrocholesterol, and are elevated in patients with SLOS. One particular substance, (17-COOH-7DA) is biologically active in skin cells, and can inhibit the normal proliferation of keratinocytes. The accumulation of 5,7-dienes in SLOS might impact normal skin production and function, and possibly contribute to the abnormal skin findings seen in these patients. However, there are no more recent publications documenting the clinical effect of 5,7-dienes on skin in patients with SLOS [19].

Photosensitivity will be further addressed in the following section on the impact of precursor accumulation and oxidation.

Evaluation and treatment: ensuring optimal nutrition status and cholesterol levels with caloric and cholesterol supplementation can improve skin function. Protection from the sun with clothing, wearing of hats, and sunscreen which protects from UVA are also important.

2.2.2. The eye

The Retina is one of the areas in the body which undergoes quite rapid cell turnover. The Retina is made up of photoreceptors, including rods and cones which are essential for normal vision. In human retinae, there are many more rods than cones. Retinal studies of patients with SLOS show that although rods and cones may be impacted, rods are far more affected than cones [20].

Photoreceptor cells are composed of cell membranes with important chemicals such as rhodopsin, necessary for light absorption. Photoreceptors also contain biconcave discs within the cell membranes, made up of lipids with cholesterol being an important component. Cholesterol deficiency can cause severe impact on retinal function, leading to vision loss in patients with SLOS. Vision loss can be progressive over time, which can be a devastating situation for patients who may also experience cognitive, behavioral and hearing disabilities [20]. Other chemical reactions involving precursors and oxysterols may further damage retinal function (see below). Other ocular abnormalities found in patients with SLOS include progressive pigmentary retinopathy and cataracts. Please see additional discussion of the retina in the section on oxysterols below. Interestingly, the lens of the eye typically has a very high content of cholesterol in its cell membranes, higher than in any other part of the body. Whether the decreased cholesterol in the lens of patients with SLOS can lead to cataracts is unknown [17].

Evaluation and Treatment: Ophthalmologic serial evaluation can determine the extent of retinal disease and impact on vision. Retinal photos can document the extent of pigmentary changes and Electroretinograms can objectively document rod and cone function [20–24]. (See section below on oxysterols for further discussion of antioxidant treatment.

Red blood cells are necessary for carrying oxygen and nutrients throughout the body, undergoing rapid turnover and replacement every few months. The function of red blood cells is dependent on their ability to squeeze into tiny capillaries throughout the body. This in turn, is dependent on red cell membranes which must be flexible and able to assume different shapes [25]. Normally, red blood cells contain a relatively high level of cholesterol in the cell membranes, up to 50 % versus other cells which may have only 10–30 % cholesterol in the cholesterol to phosphatidylcholine ratio [17]. In patients with SLOS and very low cholesterol levels, the low cholesterol impacts the membranes of RBC's, which can be abnormal in their function, and in shape (poikilocytosis, acanthocytosis). Abnormalities of red cell shape can easily be seen under the microscope in patients with SLOS and reported in their CBC's (seen by author on multiple occasions).

The spleen is the organ whose job is to filter out abnormally shaped red cells. The spleen can become enlarged in more severe patients with SLOS. This is called *hypersplenism* and may result in the spleen filtering out other types of blood cells in addition to abnormally shaped RBC's. This can lead to thrombocytopenia (low platelets) and subsequent bleeding.

Evaluation and Treatment: involvement and consultation by a Hematology specialist may become necessary if the patient develops severe issues with anemia or thrombocytopenia, including transfusions of RBC's and platelets. Splenomegaly may be appreciated on physical exam, which should prompt a further evaluation for possible hypersplenism.

2.2.3. Other blood cells

White blood cell function, critical for fighting infections, may be impaired in patients with severe cholesterol deficiency due to altered phagocytic function of neutrophils. Please see paper by Chattopadhyay and Sharma which details membrane function abnormalities in patients with SLOS [26]. This was demonstrated in patients with AIDS and hypocholesterolemia during the early days of the HIV epidemic, prior to the development of antiviral treatment [27].

Mast cells, which are important cells in response to allergies, may not function properly as mast cell function is dependent on the presence of lipid rafts, made from cholesterol [28]. This may explain why many patients with SLOS show severe food allergies which may impact feeding and growth.

The GI tract is often impacted in patients with SLOS. There are many different types of cells in the GI tract important for normal nutritional status. Insufficient cholesterol available for production of cells in the GI tract can lead to difficulties throughout the GI system from the esophagus down to the small and large intestines. Symptoms in patients with a delicate and friable GI tract may include feeding intolerance with subsequent failure to thrive, gastritis with GI bleeding, and diarrhea with malabsorption of nutrients.

Evaluation and Treatment: Many patients with more severe SLOS are dependent on nutritional supplements including high calorie formulas, tube feeds with surgical placement of tubes either into the stomach (feeding gastrostomy) or tubes directly into the duodenal or jejunum. There are specialized growth curves for patients with SLOS [29].

Cholesterol levels in the blood can certainly be increased by taking more cholesterol in the diet. However, most pediatric formulas used for tube feedings are relatively low in cholesterol, with inadequate cholesterol content to raise a low cholesterol level due to abnormal biosynthesis. For that reason, patients with severe cholesterol deficiency may require cholesterol supplementation in addition to that which can be gotten from foods. This may be accomplished through taking egg yolk in liquid or powdered form which can be purchased on line (one egg yolk = 200 mg cholesterol, approximately), or taking one of the special cholesterol medications which have been developed for patients with SLOS, including a soy -based suspension which is 200 mg/ml (the equivalent of 5 eggs per teaspoon), or an aqueous suspension which is 150 mg/ml. Severe food allergies are common in patients with SLOS as mentioned above, which can limit the ability to take eggs.

See role of cholesterol as substrate for bile acid production below, for further discussion about the need for bile acid supplementation in addition to cholesterol.

2.2.4. Special note about neonates with severe SLOS

caring for the neonate and young infant with severe SLOS can be quite a challenge due to multiple factors. The infants may have complex and severe birth defects including holoprosencephaly, complex congenital heart disease, ambiguous genitalia and renal dysplasia. The baby has often experienced severe intrauterine growth failure. Very ill newborns may not be able to take in nutrition enterally for many days to weeks after birth, and thus giving any form of medication enterally such as cholesterol supplementation or bile acids may not be possible or tolerated.

Evaluation and Treatment in the Neonate with SLOS: Blood work should be obtained to determine the severity of the cholesterol deficiency and how high the precursor levels are (sent to a special outside lab). Following kidney and liver function is important. Following blood counts is necessary to determine level and function of blood cells including RBC's, WBC's and platelets. Obtaining ultrasounds should be

done of the head to rule out brain anomalies, and of the heart and kidneys to assess structural defects. Measurement of cortisol is important to know if the baby requires daily or stress steroids.

It is not possible to give pure cholesterol as an IV medication – it does not exist. However, a cholesterol-rich product called Fresh Frozen Plasma (FFP) has been used successfully in critically ill newborns to raise the cholesterol levels up sufficiently to provide substrate for adrenal hormones, and to stabilize membranes dependent on cholesterol levels. It has been given at a dose of 10 ml/kg, to help stabilize a critically ill newborn with SLOS.

2.2.5. The kidneys

Kidneys may be dysplastic and dysfunctional related to prenatal influence of cholesterol deficiency on their formation. Postnatally, renal function is highly dependent on the normal function of renal cell membranes to filter ions and chemicals properly. Common symptoms of this problem in patients with SLOS include electrolyte deficiencies (hyponatremia is a common finding necessitating sodium supplementation), which can be problematic as hypertension is also common in patients with SLOS and may be refractory to treatment. FFP has also been successfully used in older patients with SLOS who are critically ill and not able to take anything orally, to raise a very low cholesterol level associated with severe disease.

2.2.6. Role of cholesterol as precursor for steroid hormones

Cholesterol is the main precursor for steroid hormones including cortisol produced by the adrenal glands, and sex steroids produced by the testes and ovaries. The role of insufficient sex hormones was at first presumed to be associated with abnormalities in the reproductive organs which may be seen in patients with SLOS, but prenatally this is more likely to be secondary to the abnormally functioning embryological pathway originating with Sonic Hedgehog.

Female patients with SLOS can have a delay in pubertal development and onset of menses as well as hypoplastic labia on exam. Males may also display delay in acquiring secondary sexual characteristics.

The biggest hormonal concern, however, is adrenal insufficiency which can be severe and even lethal in those patients with very severe disease. Adrenal insufficiency can most often be manifested by the inability to produce stress steroids, which can happen with major surgical procedures, or severe illness including sepsis [30].

Evaluation and Treatment: all patients with severe cholesterol deficiency (ie $<$ 50 mg/dL) should be tested for adrenal insufficiency with the consultation of a pediatric endocrinologist. Some patients need daily steroid treatment, while others may just need stress dosing in the event of a severe infection or major surgical procedure.

2.2.7. Role of cholesterol as a substrate for bile acid production

Bile acids are made from cholesterol in the liver and are essential for nutrient absorption in the gut. Patients with SLOS and severe cholesterol deficiency are not able to make sufficient bile acids to maintain adequate nutritional status. The outcome is often growth failure which may be quite severe. Historically, bile acids have been used to treat patients with severe SLOS since the early 1990's when the cholesterol deficiency was first described. Bile acids including Ursodeoxycholic acid, Chenodeoxycholic acid, and Cholic acid have all been tried. Cholic acid, which was available in the 1990's and seemed to be beneficial, was dropped from production due to cost, and did not become available again until the past few years.

Recently, a small pilot study was performed to determine the safety and efficacy of using Cholic acid in patients with SLOS in addition to cholesterol supplementation. Cholic acid was shown to be safe and well tolerated [31]. Cholesterol levels increased in 11/12 patients treated. There was not a statistically significant drop in precursor levels, but the treatment only lasted 2 months. Further studies are needed to determine if there is true benefit to using cholic acid in patients with SLOS.

Evaluation and Treatment: careful nutritional evaluation and nutritional supplementation via special formulas is often necessary to help growth. Laboratory monitoring of liver function may also be necessary. Prescription of bile acids may help improve absorption of nutrients as well as supplemental cholesterol to raise cholesterol levels. This in turn will decrease hepatic production of the precursors through feedback inhibition. As mentioned earlier, following cholesterol and precursor levels requires sending blood specimens to a special laboratory, such as the Kennedy-Krieger lab in Baltimore, MD. Routine hospital labs cannot distinguish between true cholesterol, 7-DHC and 8-DHC. Patients with lower cholesterol levels may benefit from bile acid supplementation [31]. Cholic acid is FDA approved for use in patients with SLOS, and may prove to be of benefit in severely affected infants with very low cholesterol levels, although this has not yet been studied. Because Cholic acid is an expensive prescription medication, it should be prescribed by a physician with knowledge of how to dose and follow its use.

2.2.8. Role of cholesterol in liver disease

As the organ which is responsible for cholesterol biosynthesis, the liver may be very impacted in patients with severe SLOS. Cholestatic liver disease may be seen in severely affected newborns and may lead to severe liver failure and even death. Bile acids have long played a role in treating cholestatic liver disease and may help stabilize hepatic function and allow some growth in these very ill children. Mild elevations in transaminases are frequently seen in patients with severe and moderate SLOS.

Evaluation and Treatment: consultation with a pediatric gastroenterologist is often required in those patients with moderate and severe liver disease. Serial measurements of hepatic transaminases can be helpful in following progression of the liver disease.

2.2.9. Problems with fighting infection

Young patients with SLOS often suffered recurrent, severe infections. This can be attributed to a variety of causes, including severe malnutrition. The role of abnormal WBC function due to membrane abnormalities and abnormal phagocytosis is also felt to play a role [26,27].

Evaluation and Treatment: recurrent infections are often seen in patients with SLOS who exhibit failure to thrive and nutritional deficiency. Frequent ear infections can also be seen in patients with palatal clefts due to abnormal eustachian tube function. Patients with urological abnormalities are more prone to urinary tract infections which may be severe and lead to urosepsis. Careful attention to febrile episodes and evaluation to determine the underlying cause is important for patients with SLOS. Antibiotic treatment is often required and stress steroid courses may be necessary in those patients with severe illness who are not able to mount a normal adrenal response.

3. Part 2: role of elevated precursors –7-dehydrocholesterol (7-DHC) and 8-dehydrocholesterol (8-DHC) in human disease

Since the metabolic error in cholesterol biosynthesis causing SLOS was first discovered in 1993–4, new discoveries were made in terms of the role of cholesterol deficiency in embryologic, hepatic, nutritional and neurological disorders. However, it also soon became clear that cholesterol deficiency alone was not the only culprit. Marked elevation in the cholesterol precursors, 7- and 8-DHC were also found to play a role in human disease.

The first important clue was that the precursors do NOT function interchangeably with cholesterol, and even though the levels are quite high, the important roles of cholesterol were not being met. Secondly, the precursor accumulation was found to have toxic effects on many different organ systems including the skin, the liver, and the eye (see below). Thirdly, the precursors are oxidized approximately 200x more readily than cholesterol into toxic substances called oxysterols, which are particularly damaging to neurons and retinal cells.

3.1. Role of 7-DHC and 8-DHC in skin disease

One of the most interesting clinical problems identified in patients with SLOS was that of photosensitivity. This is *easy sunburning* which has been quite extreme in some patients to the point that it can limit the ability of the patient to be outside, even for very brief periods.

Studies done in the 1990's showed that solar UVA radiation stimulates formation of reactive oxygen species (ROS) and prostaglandin E(2) (PGE(2)), which are involved in skin photosensitivity and tumor promotion. High levels of 7-dehydrocholesterol (7-DHC), the precursor to cholesterol, cause exaggerated photosensitivity to UVA in patients with Smith-Lemli-Opitz syndrome (SLOS) [32]. Although only a subset of patients with SLOS display photosensitivity to this severe degree, dermatologic studies to quantify this phenomenon showed that all patients had some degree of sun sensitivity, which was predominantly to UV light in the UVA range. In Vitro studies showed that the higher the level of 7-DHC in fibroblasts, the greater the level of sun sensitivity [32,33]

Evaluation and Treatment: Patients with SLOS should be careful to avoid exposure to light for long periods of time. Special ultraviolet-blocking clothing can be purchased on-line. When buying sunscreen, it is important to ensure that the sunscreen protects against both UVB (the most common form of sunscreen protection) as well as UVA, which is what patients with SLOS are most sensitive to.

3.2. Role of 7-DHC and 8-DHC in eye disease

The role of low cholesterol in rod and cone cell function has been previously mentioned. In addition to this, animal studies by Dr S Fleisler in a rat model, indicated that there was more to the progressive loss of vision and pigmentary retinopathy than could be attributed to cholesterol deficiency alone. Accumulation of the precursors 7- and 8-DHC appear to cause worsening retinal function on ERG as well as anatomic distortion of the retina in the rat model. Part of this may be due to the presence of toxic oxysterols, made from chemical oxidation of the cholesterol precursors, which cause severe retinal disease in the animal model (see further discussion below) [21–23,34].

4. Part 3: role of toxic oxysterol accumulation in patients with SLOS

4.1. Neuronal damage

The brain is clearly a target organ for symptoms in patients with SLOS. The great majority of patients function in the intellectually disabled range. Behavioral challenges are also common, with a subset of patients with SLOS displaying features consistent with Autism Spectrum Disorder (ASD). It was initially thought that well more than half of patients with SLOS displayed features of autism, but with evolution of the definition of Autism Spectrum Disorder over time, this percentage has dropped to 25–33 % of patients. Other behavioral issues including Attention Deficit Hyperactivity disorder (ADHD) and Anxiety also play a role in the behavioral challenges seen in patients with SLOS. What may complicate medical management of behavioral disorders in patients with SLOS is that psychiatric medications commonly used to treat these types of behavioral challenges have been shown to disrupt normal cholesterol biosynthesis [35–40], so the choice of medications which can be used in patients with SLOS needs to be thoughtfully and carefully made.

Cholesterol biosynthesis and metabolism in the brain is different than that in the rest of the body. Cholesterol found in the brain is made in the brain (not the liver), and cholesterol which is consumed and absorbed in the GI tract is not able to pass into the brain due to the blood/brain barrier. Additionally, medications such as statins, developed to lower cholesterol production can in fact cross the blood/brain barrier, and could therefore be potentially harmful to patients with SLOS.

As neuronal cells try to synthesize cholesterol, but instead make increased levels of the precursors 7- and 8-DHC, research has been devoted to better understanding the role of these precursors on brain function. A major breakthrough in this understanding was made by Libin Xu in understanding the role of increased oxidation of cholesterol precursors (200x easier than oxidation of cholesterol itself) into toxic oxysterols, and the damage caused by these toxic substances throughout the body [23,36,37]. Important research has been published by Korade et al. to help better understand the role that oxysterols play in neuronal apoptosis and brain function [35,36,41].

Developmental/Behavioral problems are common in patients with SLOS including intellectual disabilities in the vast majority, and Autism Spectrum disorder in at least 25-30 %. Many patients display challenging behavioral concerns including self-injurious behaviors, aggression and sleep problems. Furthermore, psychiatric medications which are commonly used in other patients with these types of behavioral problems may actually be contraindicated in patients with SLOS, as some medications including Haldol and Aripiprazole may actually interfere with the enzyme DHCR7, leading to elevated levels of 7-DHC. This is also true for the medication Trazodone, which is commonly prescribed for sleep in patients with autism and intellectual disabilities. This has been shown both in humans and in a mouse model. Furthermore, using a combination of these medications (ie Aripiprazole + Trazodone) can be even more concerning as the combination can lead to an even more profound effect on inhibiting DHCR7 and raising the level of 7-DHC [38–40].

4.2. Retinal deterioration

The role of oxysterol accumulation causing visual complications is best understood from the work of Fleisler and Xu, in an animal model. Oxysterols cause disruption of retinal anatomy and function, leading to abnormalities seen on electroretinogram and under the microscope in the rat [20–23]. In humans with SLOS, deterioration of vision had been reported over time, as well as progression of pigmentary retinopathy [20].

Since 2008, an ongoing research study has been performed by Elias to assess retinal function in patients with SLOS. The purpose of this study was to assess whether antioxidant medication might prevent retinal deterioration seen in human patients with SLOS, following the reports of retinal improvement in an animal SLOS retinal model by Fleisler [34].

Patients with SLOS have been treated with cholesterol supplementation and the addition of an antioxidant preparation which was originally developed to treat pediatric patients with Cystic Fibrosis and associated vitamin deficiency. The antioxidant preparation contains several different vitamins including Vitamin E, beta-carotene, Co-enzyme Q10, selenium, B vitamins, and Vitamin D. Vitamin D levels are carefully followed to ensure that the patients do not develop elevated levels of vitamin D while on treatment.

The retinal function and appearance of patients with SLOS who are being treated with antioxidants is studied by serial evaluations under anesthesia which include an ophthalmologic evaluation with retinal photos, and electroretinography (ERG). There are two objective electroretinogram findings which can be followed over time. The first is called the implicit time, which is the time it takes for light stimulation to cause the formation of a b-wave. The second finding is the height or amplitude of the wave. Findings seen in patients with SLOS include a prolongation of the implicit time and diminution in the amplitude of the wave. In patients with SLOS, this type of study requires general anesthesia due to the intellectual disabilities and behavioral challenges seen in this patient population. Therefore, studies cannot be conducted that frequently (no more often than annually, for patient safety concerns). The COVID pandemic also impacted the frequency with which these studies could be scheduled.

Twenty-four patients with Smith-Lemli-Opitz syndrome have been

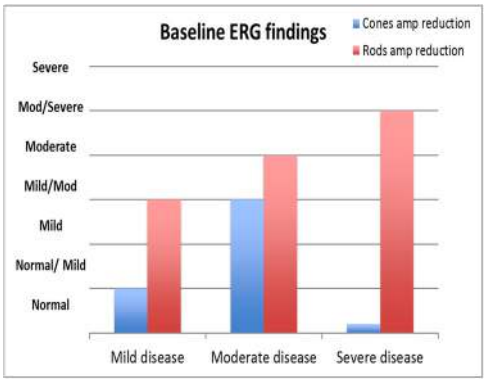

Fig. 1. Baseline ERG findings in patients with SLOS being treated with antioxidants.

followed over time and all demonstrated abnormalities of retinal function detected on Electroretinogram (ERG). Nine of these patients were able to be followed serially while treated with antioxidants. The abnormalities included prolonged implicit times in all patients, regardless of severity of disease. Also noted were decreased wave amplitudes in Rod cells in all patients, with a greater change found as disease severity worsened. Rod cell amplitudes were affected more than cone cells in mild and moderately affected patients. Cone cell amplitudes were close to normal in severely affected patients for unclear reasons (Fig. 1).

On antioxidant treatment, prolonged implicit times and decreased b-wave amplitude improved in all mild patients and two thirds of the moderate and severe patients. The other patients remained stable, and no patient showed deterioration of retinal function on treatment.

In terms of the retinal changes seen on ophthalmology exam under anesthesia, while pigmentary retinopathy was known to worsen over time in patients treated with cholesterol alone (historical finding), on antioxidant treatment, pigmentary retinopathy improved or remained stable. Pigmentary retinopathy was only seen in a subset of patients. Pigmentary retinopathy was present in the periphery (where the rods are most prevalent) and when subtle, required a good exam under anesthesia to document it.

While under anesthesia, patients also underwent a hearing test called auditory brainstem response (ABR) in addition to the electroretinograms. Patients were found to have prolongation of the time from Wave I to Wave V on this hearing test, which is analogous to the prolonged implicit time seen on ERG. However, the ABR is not a sensitive enough study to determine a change or improvement in hearing in these patients. The abnormal Wave I-V interval was also not associated with whether hearing loss was present. Hearing loss is associated with SLOS and seen in a subset of patients.

Treatment with antioxidants, in both animals and human patients with SLOS can help protect the retina from ongoing damage, and even prevent progressive visual loss. In the animal model it is possible to determine that retinal oxysterol levels can be reduced by antioxidant treatment. This is clearly not possible to do in children, but as retinal changes are at least stable or improved on antioxidant treatment, the hope is that toxic oxysterol accumulation is being better controlled.

5. Summary

Smith-Lemli-Opitz syndrome is a complex genetic disorder, associated with multiple birth defects, unusual facial features, growth failure, cognitive disabilities and challenging behaviors along the autism spectrum. Since its first description in the early 1960's, and the discovery of the error in cholesterol biosynthesis thirty years later, much has now been clarified regarding the role of cholesterol in embryologic mechanisms, in physiologic pathways affecting multiple organ systems, and in nutritional, and endocrine functions.

Much still remains to be learned about this disease. While we know that cholesterol levels can be increased by food choices and medications, there is still not a "cure" for patients with SLOS which corrects their intellectual and behavioral disabilities. Cholesterol does not cross the blood/brain barrier and thus effects on development and behavior of treating with cholesterol supplementation, or medications which can impact cholesterol synthesis in the brain are still not well understood [34].

While we know that accumulation of the precursors 7- and 8-dehydrocholesterol can also impact disease in patients with SLOS, and that precursors cannot be substituted for cholesterol, there is still not proven treatment which reduces precursor levels. While giving bile acids such as cholic acid can increase cholesterol absorption and raise levels, there was not a statistically significant drop in precursor levels on this treatment. More research is needed to show not just biochemical benefit, but other medical and developmental benefit of treatment such as cholesterol and bile acid supplementation.

The role of oxysterol damage to organs such as the eye and the brain is now more clearly understood. Antioxidant medications have been shown to decrease this damage in an animal model, and preliminary studies have shown benefit in human retinal disease, but it is still not clear which antioxidants are most beneficial, at what dose and at what age should they be started. More research is clearly needed to answer these questions, so that there can be hope for a better quality of life and improved health for complex patients with SLOS in the future (Table 1).

CRediT authorship contribution statement

Ellen Roy Elias: Writing – review & editing, Writing – original draft, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence

Table 1
Roles of hypocholesterolemia, precursor accumulation and oxysterols in disease in patients with SLOS.

Role of cholesterol in the body
Embryologic consequences of Chol deficiency on SHH pathway
Role in cell membranes – skin, blood cells, kidney function, retina
Role as a precursor for steroid hormones
Role as substrate for bile acid production
Liver disease
Role in fighting infection
Role of elevated precursors – 7-Dehydrocholesterol (7-DHC) and its isomer, 8-Dehydrocholesterol (8-DHC)
Can't substitute for cholesterol
Increased photosensitivity
Oxidized 200x more easily than cholesterol into toxic oxysterols
Role of Oxysterols
Neuronal damage
Retinal deterioration

the work reported in this paper.

Data availability

Data will be made available on request.

References

[1] G.E. Herman, Disorders of cholesterol biosynthesis: prototypic metabolic malformation syndromes, Hum. Mol. Genet. 12 (R1) (2003) R75–R88.
[2] F.B. Porter, G.E. Herman, Malformation syndromes caused by disorders of cholesterol synthesis, J. Lipid Res. 52 (2011) 6–34.
[3] D.W. Smith, L. Lemli, J.M. Opitz, A newly recognized syndrome of multiple congenital anomalies, J. Pediatr. 64 (1964) 210–217.
[4] M. Irons, E.R. Elias, G. Salen, G.S. Tint, A.K. Batta, Defective cholesterol biosynthesis in Smith-Lemli-Opitz syndrome, Lancet 341 (1993) 1414.
[5] G.S. Tint, M. Irons, E.R. Elias, A.K. Batta, R. Frieden, T.S. Chen, G. Salen, Defective cholesterol biosynthesis associated with the Smith-Lemli-Opitz syndrome, N. Engl. J. Med. 330 (1994) 107–113.
[6] C.A. Wassif, et al., Mutations in the human sterol delta7-reductase gene at 11q12-13 cause Smith-Lemli-Opitz Syndrome, Am. J. Hum. Genet. 63 (1998) 55–62.
[7] F.D. Porter, RSH/Smith-Lemli-Opitz Syndrome: a multiple congenital anomaly/mental retardation syndrome due to an inborn error of cholesterol biosynthesis, Mol. Genet. Metab. 71 (2000) 163–174.
[8] J.A. Porter, K.E. Young, P.A. Beachy, Cholesterol modification of hedgehog signaling proteins in animal development, Science 274 (1996) 255–259.
[9] M.K. Cooper, C.A. Wassif, P.A. Krakowiak, J. Taipale, R. Gong, R.I. Kelley, F. D. Porter, P.A. Beachy, A defective response to hedgehog signaling in disorders of cholesterol biosynthesis, Nat. Genet. 33 (2003) 508–513.
[10] J. Taipale, M.K. Cooper, T. Maiti, P.A. Beachy, Patched acts catalytically to suppress the activity of Smoothened, Nature 418 (6900) (2002) 892–897.
[11] T. Koide, et al., Negative regulation of Hedgehog signalizing by the cholesterologenic enzyme 7-dehydrocholesterol reductase, Development 133 (2006) 2395–2405.
[12] L.E. Kratz, R.I. Kelley, Prenatal diagnosis of the RSH/Smith-Lemli-Opitz syndrome, Am. J. Med. Genet. 82 (1999) 376–381, 500.
[13] M.B. Irons, et al., Antenatal therapy of Smith-Lemli-Opitz syndrome, Pediatr. Res. 43 (1998) 125.
[14] M. Yanez, A. Leiva, Human placental intracellular cholesterol transport: a focus on lysosomal and mitochondrial dysfunction and oxidative stress, Antioxidants 11 (3) (2022) 500–502. Mar 4.
[15] W. Palinski, Maternal-fetal cholesterol transport in the placenta: good, bad and target for modulation, Circ. Res. 104 (5) (2009) 569–571.
[16] A. Woollett, Review: transport of maternal cholesterol to the fetal circulation, Placenta 32 (5) (2011) 218–221 (Mar).
[17] W.F. Subczynski, et al., High cholesterol/low cholesterol: effects in biologic membranes: a review, Cell Biochem. Biophys. 17 (2017) 369–389.
[18] M. Nowaczyk, C.A. Wassif, SLOS, GeneReviews, 2020, ⟨https//www.ncbi.nim.nib.gov/books/NBK1143/⟩.
[19] T.-K. Kim, et al., A new steroidal 5,7-diene derivative 3B-hydroxyandrosta-5, 7-diene-17B-carboxylic acid, shows potent anti-proliferative activity, Steroids 75 (3) (2009) 230–239. Dec 16.
[20] E.R. Elias, R.M. Hansen, M. Irons, N.B. Quinn, A.B. Fulton, Rod photoreceptor responses in children with Smith-Lemli-Opitz syndrome, Arch. Ophthalmol. 121 (2003) 1738–1743.
[21] S.J. Fleisler, et al., Retinal degeneration in a rodent model of Smith-Lemli-Opitz syndrome: electrophysiologic, biochemical and morphologic features, Arch. Ophthalmol. 122 (2004) 1190–1200.
[22] K. Boesze-Battaglia, et al., Alteration of rod outer segment membrane fluidity in a rat model of Smilth-Lemli-Opitz syndrome, J. Lipid Res. 49 (2008) 1488–1499.
[23] L. Xu, L.G. Sheflin, N.A. Porter, S.J. Fliesler, 7-Dehydrocholesterol derived oxysterols and retinal degeneration in a rat model of Smith-Lemli-Opitz syndrome, Biochim. Biophys. Acta 1821 (6) (2012) 877–883 (Jun).
[24] R.K. Keller, et al., Formation of 7-dehydrocholesterol-containing membrane rafts in vitro and in vivo, with relevance to Smith-Lemli-Opitz Syndrome, J. Lipid Res. 45 (2004) 347–355.
[25] E.J. Niesor, et al., Red blood cell membrane cholesterol may be a key regulator of sickle cell disease microvascular complications, Membrane 12 (11) (2022) 1134. Nov 11.
[26] A. Chattopadhyay, A. Shwarma, Smith-Lemli-Opitz syndrome: a pathophysiological manifestation of the bloch hypothesis, Front. Mol. Biosci. 10 (2023) 1120373. Jan 12.
[27] G. Coodley, M.K. Coodley, Hypocholesterolemia and malabsorption in HIV infection, West J. Med. 154 (6) (1991) 735. PMID: 1877219; PMCID: PMC1002890.
[28] A.M. Silveira E Souza, V.M. Mazucato, M.C. Jamur, C. Oliver, Lipid rafts in mast cell biology, J. Lipids (2011), https://doi.org/10.1155/2011/752906. Epub 2011 Feb 10. PMID: 21490812; PMCID: PMC3066649.
[29] R.W. Lee, J. McGready, S.K. Conley, N.M. Yanjanin, M.J. Nowaczyk, F.D. Porter, Growth charts for individuals with Smith-Lemli-Opitz syndrome, Am. J. Med. Genet. A 158A (11) (2012) 2707–2713, https://doi.org/10.1002/ajmg.a.35376. Epub 2012 May 21. PMID: 22615010; PMCID: PMC3427420.
[30] M.J.M. Nowaczyk, V.M. Siu, P.A. Krakowiak, F.D. Porter, Adrenal insufficiency and hypertension in a newborn infant with Smith-Lemli-Opitz syndrome, Am. J. Med. Genet. 103 (2001) 223–225.
[31] Ellen R. Elias, Lucas E. Orth, Amy Li, Libin Xu, Sara M. Jones, William B. Rizzo, Cholic acid increases plasma cholesterol in Smith-Lemli-Opitz syndrome: a pilot study, Mol. Genet. Metab. Rep. 38 (2024) 101030.
[32] A. Valencia, A. Rajadurai, A.B. Carle, I.E. Kochevar, 7-Dehydrocholesterol enhances ultraviolet A-induced oxidative stress in keratinocytes: roles of NADPH oxidase, mitochondria, and lipid rafts, Free Radic. Biol. Med. 41 (11) (2006 Dec 1) 1704–1718, https://doi.org/10.1016/j.freeradbiomed.2006.09.006. Epub 2006 Sep 9. PMID: 17145559; PMCID: PMC1880892.
[33] C.R. Charmanet, Photosensitivity associated with the Smith–Lemli–Opitz syndrome, Br. J. Dermatol. 138 (5) (1998) 885–888.
[34] S.J. Fliesler, Antioxidants: the missing key to improved therapeutic intervention in the Smith-Lemli-Opitz syndrome? Hered. Genet. 2 (2) (2013) 119–124.
[35] Z. Korade, et al., The effects of small molecules in sterol homeostasis: measuring 7-DHC in dfcr7-deficient neuro2a cells and human fibroblasts, J. Med. Chem. 59 (2016) 1102–1115.
[36] Z. Korade, L. Xu, R. Shelton, N.A. Porter, Biological activities of 7-dehydrocholesterol-derived oxysterols: implications for Smith-Lemli-Opitz syndrome, J. Lipid Res. 51 (2010) 3259–3269.
[37] Hideaki Tomita, Kelly M. Hines†, Josi M. Herron, Amy Li, David W. Baggett‡, Libin Xu, 7-Dehydrocholesterol-derived oxysterols cause neurogenic defects in Smith-Lemli-Opitz syndrome, eLife 11 (2022) e67141.
[38] Z. Korade, et al., Medication effects on developmental sterol biosynthesis, Mol. Psychiatry 27 (2022) 490–501.
[39] M. Balog, A. Anderson, T.C. Genaro-Mattos, Z. Korade, K. Mirnics, Individual and simultaneous treatment with antipsychotic aripiprazole and antidepressant trazodone inhibit sterol biosynthesis in the adult brain, J. Lipid Res. 63 (2022) 100249.
[40] Z. Korade, et al., Trazodone effects on the developing brain, Transl. Psychiatry 11 (2021) 85.
[41] Z. Korade, L. Xu, F.E. Harrison, R. Ahsen, S.E. Hart, M.F. Oakleigh, K. Mirnics, N. A. Porter, Antioxidant supplementation ameliorates molecular deficits in Smith-Lemli-Opitz syndrome, Biol. Psychiatry 75 (2014) 215–222.