

Cone ERG responses in patients with Smith-Lemli-Opitz Syndrome (SLOS)

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Received: 7 August 2009 / Accepted: 15 April 2010
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Abstract

Purpose To evaluate cone and cone-driven retinal function in patients with Smith-Lemli-Opitz syndrome (SLOS), a condition characterized by low cholesterol. Rod and rod-driven function in patients with SLOS are known to be abnormal.

Methods Electroretinographic (ERG) responses to full-field stimuli presented on a steady, rod suppressing background were recorded in 13 patients who had

received long-term cholesterol supplementation. Cone photoreponse sensitivity (S_{CONE}) and saturated amplitude (R_{CONE}) parameters were estimated using a model of the activation of phototransduction, and post-receptor b-wave and 30 Hz flicker responses were analyzed. The responses of the patients were compared to those of control subjects ($N = 13$).

Results Although average values of both S_{CONE} and R_{CONE} were lower than in controls, the differences were not statistically significant. Post-receptor b-wave amplitude and implicit time and flicker responses were normal.

Conclusions The normal cone function contrasts with the significant abnormalities in rod function that were found previously in these same patients. Possibly, cholesterol supplementation has a greater protective effect on cones than on rods as has been demonstrated in the rat model of SLOS.

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Keywords Smith-Lemli-Opitz syndrome ·
Electroretinogram · Cone function ·
Cholesterol · Cholesterol precursors

Introduction

Smith-Lemli-Opitz syndrome (Mendelian Inheritance in Man [OMIM] 270400) is an autosomal recessive disorder that is characterized by low cholesterol levels and elevated levels of the cholesterol precursors 7- and 8-dehydrocholesterol (7-DHC and 8-DHC) [1–4] due

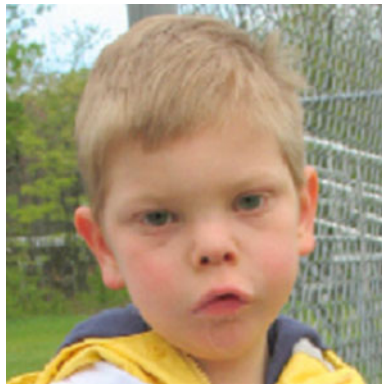


Fig. 1 A boy with Smith-Lemli-Opitz Syndrome. There is mild facial dysmorphism with turned up nose, anteverted nares, and small chin

to a defect in 3-beta-hydroxysterol-delta7-reductase, the final enzyme in the cholesterol biosynthetic pathway [2]. The clinical features of SLOS include a characteristic facial appearance (Fig. 1), major structural anomalies, growth retardation, and intellectual disability. Based on recognition of the clinical phenotype, the incidence of SLOS has been estimated to range from 1 in 10,000 to 1 in 60,000 in those of northern and central European ancestry and appears to be less common in those of African and Asian ancestry [5]. Ocular features include ptosis, cataracts, and mild

but statistically significant compromise of rod photoreceptor function [5, 6]. In patients with SLOS, the kinetics of activation and deactivation of rod phototransduction, as derived from the electroretinographic (ERG) a-wave, are slow [6]. In the rat model of SLOS, both rod and cone structure and function are affected [7]. Herein, we report cone and cone-driven retinal responses in patients with SLOS.

Patients and methods

Subjects

Thirteen patients (Table 1) had biochemical confirmation of SLOS based on elevation of the cholesterol precursors 7-DHC and 8-DHC. Three sibling pairs (patients 2 and 3, 4 and 5, and 11 and 12) were included. The median spherical equivalent was +1.00 (range, -0.50 to +3.5) diopters. Patients 1 and 3 had tiny (<2 mm diameter) nuclear cataracts in both eyes. Patients 3, 11, and 12 had ptosis.

All patients had been treated long-term with dietary cholesterol supplementation therapy (a suspension of purified crystalline cholesterol in soy oil). Cholesterol levels (Table 1) of the patients with SLOS ranged from 57 to 181 mg/dL; cholesterol levels in healthy,

Table 1 Characteristics of patients with Smith-Lemli-Opitz Syndrome

Pt. #	Age (year)	Sex	Chol level (mg/dL)	7-DHC ^a (mg/dL)	8-DHC ^b (mg/dL)	Ratio ^c	Genetic defect
1	9	F	79	13.0	12.0	0.76	N/A
2	9	F	110	4.8	5.5	0.91	N/A
3	2	F	59	6.5	6.0	0.83	N/A
4	3	M	95	5.3	5.6	0.90	IVS8-1G->C/T2891
5	4	M	124	3.1	3.5	0.95	IVS8-1G->C/T2891
6	13	M	134	1.5	0.8	0.98	IVS8-1G->C/S169L
7	4	F	181	0.6	0.6	0.99	IVS8-1G->C/?
8	19	M	100	10.0	9.1	0.84	IVS8-1G->C/T93M
9	2	F	169	1.9	2.6	0.97	F284L/V326L
10	4	M	57	7.0	6.9	0.80	G303R/G303R
11	15	F	118	10.0	6.0	0.88	IVS8-1G->C/R352W
12	20	F	157	20.0	12.0	0.83	IVS8-1G->C/R352W
13	2	M	103	0.2	Undetectable	1.00	No mutation detected

^a Normal 7-DHC value: 0.036 ± 0.020 mg/dL

^b Normal 8-DHC value: 0.061 ± 0.033 mg/dL

^c Ratio = cholesterol/total sterol

typically developing children and adolescents range from 60 to 200 mg/dL. Despite cholesterol supplementation, the precursors 7-DHC and 8-DHC remained elevated in nearly all patients. A genetic diagnosis (Table 1) was established in nine of the patients.

The patients were aged 2 to 20 years at the time of ERG testing. ERG responses mature by age 1 year [8, 9]. The patients' ERG results were compared to previously reported data from 13 control subjects aged 8 to 40 [9]. The study conformed to the principles outlined by the Declaration of Helsinki and was approved by the Children's Hospital Committee on Clinical Investigation. Informed consent was obtained from parents prior to the patient's participation in the study.

ERG procedure

Testing was done in conjunction with other diagnostic procedures under inhalation anesthesia (≤ 1 MAC, minimum alveolar concentration) which does not alter ERG responses [10]. The pupils were dilated with cyclopentolate 1% and the eyes dark-adapted from room light for 20 min under light tight patches. Then, in dim red light, 0.5% proparacaine was instilled, and a bipolar Burian-Allen electrode was placed on the left cornea. A ground electrode was placed on the skin over the left mastoid. Testing proceeded in the dark.

Responses were differentially amplified (bandpass 1–1,000 Hz; gain 1,000), displayed on an oscilloscope, digitized, and stored on disc for analysis later. An adjustable voltage window was used to reject

records contaminated by artifacts. Two to 16 responses were averaged in each stimulus condition.

After rod responses had been recorded as previously described [6], cone responses were recorded to a 1.8 log unit range (+1.4 to +3.2 log phot td s) of full-field, red (Wratten 29 $\lambda > 610$ nm) strobe stimuli (Novatron, Dallas, TX) presented on a steady, rod-saturating background. This approach has been used to isolate cone responses [11]. The stimuli were incremented in 0.3 log unit steps. On records such as those shown in Fig. 2, cone photoresponse parameters were derived from the a-wave. The trough to peak amplitude and implicit time of the b-wave were measured and examined as a function of log flash intensity. Cone-driven function was also tested using 30 Hz flickering white light (+2.1 log phot td s) in 11 of the patients.

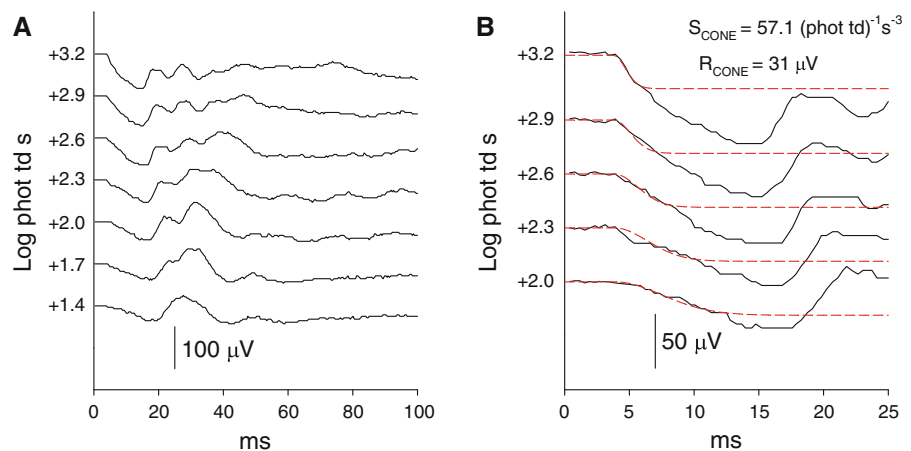
Cone photoresponse analysis

The Hood & Birch [11, 12] modification of the Lamb & Pugh [13, 14] model of the activation of phototransduction was fit to the a-wave [15–19]. The modification incorporates a cascaded RC filter that models the capacitance of the cone membrane [11, 20] by numerical convolution of the filter output with the delayed Gaussian function used to model the rod response [13, 14]. The equation [11] is:

$$R(i, t) = \left[\left(1 - \exp\{-0.5 I S_{\text{CONE}}(t - t_d)^2\} \right) R_{\text{CONE}} \right] * \exp(-t/\tau) \quad (1)$$

where R_{CONE} is the saturated response amplitude (μV), S_{CONE} the gain parameter [$(\text{phot td})^{-1} \text{s}^{-3}$], t_d a brief delay (ms), and τ the time constant of the RC

Fig. 2 Sample records. **a** Cone-mediated ERG records from 15-year-old patient 11. **b** Fit of Eq. 1 (dashed lines) to the a-wave. The calculated values of S_{CONE} and R_{CONE} are shown



filter (ms). The symbol * represents the convolution operation. For calculation of S_{CONE} and R_{CONE} , τ was fixed at 1.8 ms and t_d at 3 ms [9]. To reduce the effect of early negative post-receptor components, the model was fit to the first 5.4 ms of the a-wave [21]. The model was fit to the ensemble of responses to the five highest intensity stimuli.

Calibrations

The unattenuated flash and steady background were measured with a detector (IL 1700, International Light, Newburyport, MA) placed at the position of the subject's cornea. The maximum intensity red light produced a retinal illuminance of approximately +3.2 log phot td s. The background light produced approximately +3.0 log phot td.

Analysis

S_{CONE} and R_{CONE} of patients with SLOS and controls were compared using *t*-tests. The relation of cholesterol and its precursors to the ERG parameters was evaluated using rank order correlation. Two-factor analysis of variance (ANOVA) was used to evaluate the amplitude and implicit time of the cone-driven b-wave in patients and controls. The significance level of all tests was $P \leq 0.01$.

Results

Sample ERG responses and model fits (Eq. 1) to the a-waves are shown in Fig. 2. Although the average values of S_{CONE} and R_{CONE} in patients with SLOS are below the mean values in controls (Table 2), the differences were not statistically significant. There is

considerable overlap between the SLOS and control data (Fig. 3). Neither of the cone parameters was correlated with the concentrations of cholesterol, 7-DHC, 8-DHC, or the ratio of cholesterol to total sterol levels (Table 1).

In Fig. 4, the patients' cone parameters, S_{CONE} and R_{CONE} , are compared to their previously reported rod response parameters [6]. For each patient, rod and cone photoresponse parameters (S_{CONE} and S_{ROD} , R_{CONE} and R_{ROD}) were expressed as a percent of the normal mean (100%). On average, in patients with SLOS, S_{CONE} was 89% of the normal value while S_{ROD} was only 64% of normal. Saturated amplitudes, R_{CONE} (77%) and R_{ROD} (84%), were mildly attenuated and did not differ significantly from normal. The amplitude and implicit time of the cone-driven b-waves (Fig. 5) and response to 30 Hz flicker (Table 2) in patients with SLOS did not differ significantly from those in controls.

Discussion

There is no significant dysfunction of the cone photoreceptors or cone-driven post-receptor retina in these patients with Smith-Lemli-Opitz syndrome. In contrast to the rod photoresponse, the sensitivity parameter of the cone photoresponse, S_{CONE} , did not differ significantly between patients with SLOS and control subjects. The corresponding parameter for the rod photoresponse, S_{ROD} , obtained from the same patient cohort in the same ERG test session was significantly attenuated (Fig. 4).

The sensitivity parameter, S_{ROD} , depends on the movement of transduction cascade proteins within the disk membrane of the rod outer segment. The amplitude parameter, R_{ROD} , depends on the number

Table 2 Comparison of ERG cone response parameters in patients with Smith-Lemli-Opitz syndrome (SLOS) and control subjects (mean \pm SEM)

ERG parameter	Patients ($n = 12$)	Controls ($n = 13$)	<i>t</i> value	<i>P</i>
S_{CONE} , (phot td) ⁻¹ s ⁻³	66.6 (6.2)	79.1 (4.9)	-1.59	0.124
R_{CONE} , μV	35.9 (3.1)	45.2 (2.8)	-2.23	0.035
30 Hz flicker				
Amplitude, μV	92 (10.5)	123 (9)		
Implicit time, ms	33 (0.2)	33 (1)		

Fig. 3 Photoresponse parameters in 13 patients with SLOS and 13 control subjects. **a** S_{CONE} is the sensitivity parameter. **b** R_{CONE} is the amplitude of the saturated cone response. The horizontal lines indicate the mean for each group

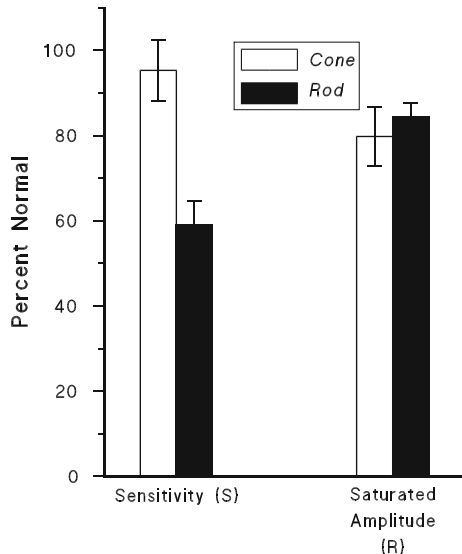
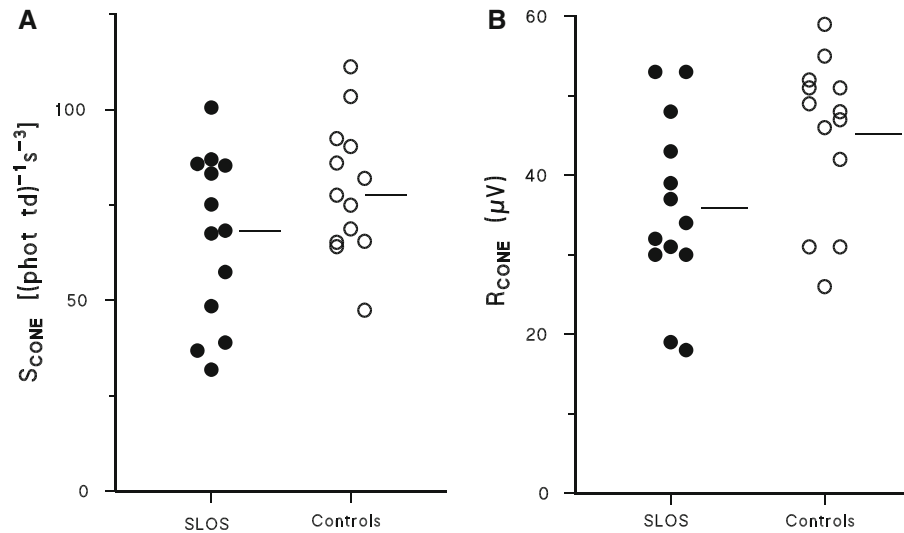


Fig. 4 Sensitivity parameters, S_{CONE} and S_{ROD} , and saturated amplitude parameters, R_{CONE} and R_{ROD} , as a percent of the mean (100%) in control subjects

of channels in the rod outer segment membrane available for closure by light and, thus, on length of the rod outer segment. R_{ROD} is also influenced by the number of rods [22–24]. Because R_{ROD} was normal, we reasoned [6] that low S_{ROD} was the consequence of impaired mobility of the transduction cascade proteins in the SLOS outer segment rather than short outer segments and reduced quantum capture by rhodopsin, the first protein in the transduction cascade. In the rat model of SLOS, loss of docosahexaenoic acid (DHA)

reduces fluidity of rod outer segment membranes and regenerability of rhodopsin [25].

It is important to note that all of the patients with SLOS examined in this study (Table 1) had been supplemented from an early age with dietary cholesterol, which raised blood cholesterol and lowered sterol precursors as much as possible. Cholesterol has been shown to cross the blood-retinal barrier in both the SLOS rat model [26] and normal rats [27]. This is in striking contrast to the blood-brain barrier, which is essentially impervious to blood-borne cholesterol [28]. In the SLOS rat model, dietary cholesterol supplementation results in increased levels of cholesterol and decreased levels of 7-DHC in the retina, as well as partial rescue of cone function [26]. In SLOS rats given cholesterol supplementation starting from weaning (postnatal day 28) and proceeding for up to 2 months, the amplitude and implicit time of the cone b-wave were near normal, whereas unsupplemented SLOS rats had significant deficits in cone b-wave amplitude and timing [26]. On the other hand, the rod responses in supplemented and unsupplemented rats did not differ significantly [26]. In view of the apparent consistency between the results obtained in human patients with SLOS and the rat SLOS model, it seems unlikely that structural [29] or nutritional [30] characteristics render cones immune to the effects of SLOS. Rather, we suspect that the cholesterol supplementation has protected the cones.

Why should cholesterol supplementation protect cones more effectively than rods? Perhaps, as in the

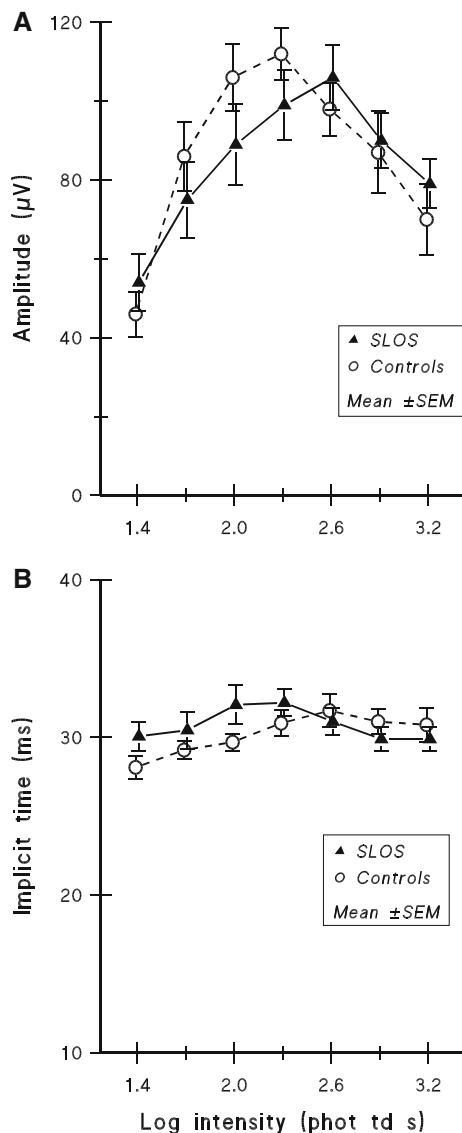


Fig. 5 Cone-driven b-wave amplitude (**a**) and implicit time (**b**) as a function of stimulus intensity. Mean and standard error of the mean for patients with SLOS and controls are shown. ANOVA showed no significant differences in either amplitude ($F = 0.164$; $df 1,6$; ns) or implicit time ($F = 1.58$; $df 1,6$; ns)

rat model of SLOS, the rods are more vulnerable to secondary damage by oxidation of lipids and proteins [31] that escalates with exposure to light [32]. This has led to the proposal for treatment of SLOS with antioxidants in addition to cholesterol [28].

The ERG studies lead us to the following suggestions for management of children with SLOS. Early diagnosis followed by prompt supplementation with cholesterol is advisable; among the possible benefits

is the preservation of photopic function. Extrapolating from the studies of the rat model, protection of the SLOS retina from excessive light by the use of dark glasses and hats with brims is also recommended. Although DHA levels in whole retina and rod outer segment membranes were markedly reduced in the SLOS rat model compared to age-matched controls [25], there was no evidence of systemic DHA or generalized omega-3 fatty acid deficiency in the SLOS rat model. Hence, it is not clear that DHA dietary supplementation will provide any significant beneficial effect for patients with SLOS with regard to preservation of retinal structure or function. We await the results of a pre-clinical trial of combined dietary antioxidants plus cholesterol supplementation in the rat SLOS model (S. J. Fliesler, personal communication) and a clinical trial in human patients (E. R. Elias, personal communication) in hopes that antioxidants will add benefit to cholesterol supplementation that is already the standard of care for patients with SLOS. Electroretinography is the outcome measure of choice if a clinical antioxidant trial is undertaken.

Acknowledgments Supported in part by R01EY010597 from the National Eye Institute, National Institutes of Health to ABF and M01-RR02172 from the National Center for Research Resources, National Institutes of Health to the Children's Hospital Boston General Clinical Research Center. The authors thank Dr. Steven Fliesler for helpful discussions during the course of this study and for providing pertinent information ahead of publication.

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