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Cone ERG Responses in Patients with Smith-Lemli-Opitz Syndrome (SLOS)

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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 SLOS patients 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 rods as has been demonstrated in the rat model of SLOS.

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 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

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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, 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 one year [8,9]. The patients' ERG results were compared to previously reported data from 13 control subjects aged 8 to 40 years [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 minutes 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 Figure 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:

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

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 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 Figure 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 Figure 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 SLOS patients 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 disc membrane of the rod outer segment. The amplitude parameter, R_{ROD} , depends on the number 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 SLOS patients 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 SLOS patients 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 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 SLOS patients 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.

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References

1. Irons M, Elias ER, Salen G, Tint GS, Batta AK. Defective cholesterol biosynthesis in Smith-Lemli-Opitz syndrome. *Lancet* 1993;341(8857):1414. [PubMed: 7684480]
2. Moebius FF, Fitzky BU, Lee JN, Paik Y-K, Glossmann H. Molecular cloning and expression of the human $\Delta 7$ sterol reductase. *Proceedings of the National Academy of Sciences* 1998;95(4):1899–1902.
3. Tint GS, Irons M, Elias ER, et al. Defective cholesterol biosynthesis associated with the Smith-Lemli-Opitz syndrome. *N Engl J Med* 1994;330(2):107–13. [PubMed: 8259166]
4. Tint GS, Salen G, Batta AK, et al. Correlation of severity and outcome with plasma sterol levels in variants of the Smith-Lemli-Opitz syndrome. *J Pediatr* 1995;127(1):82–7. [PubMed: 7608816]
5. Porter FD. RSH/Smith-Lemli-Opitz syndrome: a multiple congenital anomaly/mental retardation syndrome due to an inborn error of cholesterol biosynthesis. *Mol Genet Metab* 2000;71(1-2):163–74. [PubMed: 11001807]
6. Elias ER, Hansen R, Irons M, Quinn N, Fulton AB. Rod photoreceptor responses in children with Smith-Lemli-Opitz syndrome. *Archives of Ophthalmology* 2003;121:1738–1743. [PubMed: 14662594]
7. Fliesler SJ, Peachey NS, Richards MJ, Nagel BA, Vaughan DK. Retinal degeneration in a rodent model of Smith-Lemli-Opitz syndrome: electrophysiologic, biochemical, and morphologic features. *Arch Ophthalmol* 2004;122(8):1190–200. [PubMed: 15302661]
8. Fulton AB, Hansen RM. The development of scotopic sensitivity. *Investigative Ophthalmology and Visual Science* 2000;41:1588–1596. [PubMed: 10798680]
9. Hansen RM, Fulton AB. Development of the cone ERG in infants. *Investigative Ophthalmology and Visual Science* 2005;46(9):3458–3462. [PubMed: 16123452]
10. Wongpichedchai S, Hansen RM, Koka B, Gudas VM, Fulton AB. Effects of halothane on children's electroretinograms. *Ophthalmology* 1992;99:1309–1312. [PubMed: 1513585]
11. Hood DC, Birch DG. Phototransduction in human cones measured using the a-wave of the ERG. *Vision Research* 1995;35(20):2801–10. [PubMed: 8533321]
12. Hood DC, Birch DG. Human cone receptor activity: the leading edge of the a-wave and models of receptor activity. *Vis Neurosci* 1993;10:857–871. [PubMed: 8217936]
13. Pugh EN Jr, Lamb TD. Amplification and kinetics of the activation steps in phototransduction. *Biochimica et Biophysica Acta* 1993;1141:111–149. [PubMed: 8382952]
14. Lamb TD, Pugh EN Jr. A quantitative account of the activation steps involved in phototransduction in amphibian photoreceptors. *Journal of Physiology* 1992;449:719–758. [PubMed: 1326052]
15. Bradshaw K. Contribution of post-receptor cells to the a-wave of the human photopic electroretinogram. *Vision Research* 2007;47(22):2878–88. [PubMed: 17850841]
16. Robson JG, Frishman LJ. Photoreceptor and bipolar cell contributions to the cat electroretinogram: A kinetic model for the early part of the flash response. *J Opt Soc Am* 1996;13:613–622.
17. Bush RA, Sieving PA. A proximal retinal component in the primate photopic ERG a-wave. *Investigative Ophthalmology and Visual Science* 1994;35:635–645. [PubMed: 8113014]
18. Lo Cascio GP. A study of vision in cerebral palsy. *American Journal of Optometry and Physiological Optics* 1977;54:332–337. [PubMed: 900255]
19. Freidburg C, Allen CP, Mason PJ, Lamb TD. Contributions of cone photoreceptors and post-receptor mechanisms to the human photopic electroretinogram. *Journal of Physiology* 2004;556(3):819–834. [PubMed: 14990682]
20. Cideciyan AV, Jacobson SG. An alternative phototransduction model for human rod and cone ERG a-waves: normal parameters and variation with age. *Vision Research* 1996;36:2609–2621. [PubMed: 8917821]

21. Robson JG, Saszik SM, Ahmed J, Frishman LJ. Rod and cone contributions to the a-wave of the electroretinogram of the macaque. *J Physiol* 2003;547(Pt 2):509–30. [PubMed: 12562933]
22. Cideciyan AV, Jacobson SG. An alternative phototransduction model for human rod and cone ERG a-waves: normal parameters and variation with age. *Vision Res* 1996;36:2609–2621. [PubMed: 8917821]
23. Hood DC, Birch DG. Rod phototransduction in retinitis pigmentosa: estimation and interpretation of parameters derived from the rod a-wave. *Invest Ophthalmol Vis Sci* 1994;35(7):2948–61. [PubMed: 8206712]
24. Moskowitz A, Hansen RM, Akula JD, Eklund SE, Fulton AB. Rod and rod-driven function in achromatopsia and blue cone monochromatism. *Invest Ophthalmol Vis Sci* 2009;50(2):950–8. [PubMed: 18824728]
25. Boesze-Battaglia K, Damek-Poprawa M, Mitchell DC, et al. Alteration of retinal rod outer segment membrane fluidity in a rat model of Smith-Lemli-Opitz syndrome. *J Lipid Res* 2008;49(7):1488–99. [PubMed: 18344409]
26. Fliesler SJ, Vaughan DK, Jenewein EC, Richards MJ, Nagel BA, Peachey NS. Partial rescue of retinal function and sterol steady-state in a rat model of Smith-Lemli-Opitz syndrome. *Pediatr Res* 2007;61(3):273–8. [PubMed: 17314682]
27. Tserentsoodol N, Szein J, Campos M, et al. Uptake of cholesterol by the retina occurs primarily via a low density lipoprotein receptor-mediated process. *Mol Vis* 2006;12:1306–18. [PubMed: 17110914]
28. Fliesler, SJ. Retinal degeneration in a rat model of Smith Lemli Opitz Syndrome: Thinking beyond cholesterol deficiency. In: Anderson, R.; Lavail, MM.; Hollyfield, J., editors. *Recent advances in retinal degeneration research*. Springer; New York: 2009. In press
29. Perkins GA, Ellisman MH, Fox DA. Three-dimensional analysis of mouse rod and cone mitochondrial cristae architecture: bioenergetic and functional implications. *Molecular Vision* 2003;9:60–73. [PubMed: 12632036]
30. Punzo C, Kornacker K, Cepko CL. Stimulation of the insulin/mTOR pathway delays cone death in a mouse model of retinitis pigmentosa. *Nat Neurosci* 2009;12(1):44–52. [PubMed: 19060896]
31. Richards MJ, Nagel BA, Fliesler SJ. Lipid hydroperoxide formation in the retina: correlation with retinal degeneration and light damage in a rat model of Smith-Lemli-Opitz syndrome. *Exp Eye Res* 2006;82(3):538–41. [PubMed: 16199034]
32. Vaughan DK, Peachey NS, Richards MJ, Buchan B, Fliesler SJ. Light-induced exacerbation of retinal degeneration in a rat model of Smith-Lemli-Opitz syndrome. *Exp Eye Res* 2006;82(3):496–504. [PubMed: 16360150]

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

Fig. 2. Sample records. (A) Cone mediated ERG records from 9-year-old patient 2. (B) Fit of Eq. 1 (dashed lines) to the a-wave. The calculated values of S_{CONE} and R_{CONE} are shown.

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.

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).

Table 1

Characteristics of patients with Smith-Lemli-Opitz Syndrome

Pt. #	Age yr	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	0.20	undetectable	1.00	no mutation detected

^aNormal 7-DHC value: 0.036 ± 0.020 mg/dL^bNormal 8-DHC value: 0.061 ± 0.033 mg/dL^cRatio = Cholesterol/Total Sterol

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)		