

Steroid Metabolic Consequences of 7-Dehydrosterol Reductase Deficiency (SLO)

Fred Chasalow^{1,2*} and Sandra Blethen³

¹Managing Partner, IOMA LLC, USA

²Visiting Professor, Department of Laboratory Medicine, VAMC, San Francisco, CA, USA

³Consultant, USA

*Corresponding Author: Fred Chasalow, Visiting Professor, Department of Laboratory Medicine, VAMC, San Francisco, CA, USA.

Received: April 27, 2020; Published: May 18, 2020

DOI: 10.31080/ecpe.2020.09.00720

Abstract

Background: Individuals affected with Smith-Lemli-Opitz (SLO) syndrome have 7-dehydro-sterol reductase deficiency. This enzyme catalyzes the last step in cholesterol biosynthesis. Earlier, we showed that infants with SLO failed to produce polar steroids during the immediate neonatal period. In 2018, we reported the isolation and characterization of the four major polar steroids, which are phosphocholine esters. One of the four steroids has 21 carbon atoms. The other three have 23 carbon atoms. The phosphocholine steroids are spiral steroids. Their structure is similar to spironolactone and they may also function as potassium sparing diuretics. Now that we know the identity of each steroid, we have reexamined serum from patients with SLO.

Methods: For this study, the phosphocholine steroid concentration in 12 serum samples: six from affected individuals and six from obligate heterozygotes were determined by mass spectroscopy. We anticipated that the serum from affected individuals would have high levels of the precursors and low levels of the active compound and its metabolites.

Results: The actual observations did not fit the expected pattern. All 12 samples had low levels of the four phosphocholine steroids that we had previously detected in cord serum obtained from normal infants. There was no difference between males and females. The serum samples from the obligate heterozygotes had a novel, extra steroid phosphoester which we designated as C369.

Conclusion: We propose that, after the neonatal period, the diet is rich in potassium and there is no continuing need for a potassium retaining hormone, similar to spironolactone. However, in times of biochemical stress, there might be a basis for replacement hormone therapy. In view of the severe consequences to the affected individuals during pregnancy and the neonatal period, we propose that C369 may provide a compensating selective advantage to account for the persistence of these mutations in the world genome.

Keywords: SLO; DLM; Ionotropin; Potassium Sparing Diuretics; 7-Dehydrosterol Reductase

Abbreviations

SLO: Smith-Lemli-Opitz Syndrome; 7DCHR: 7-Dehydrosterol reductase - the underlying enzyme deficiency in SLO; CRM: Cross-Reacting Material; CRM-S: CRM specific for antibodies based on sulfate haptens; DLM: CRM Specific for Digoxin Antigens; DHEA-S: Dehydroepiandrosterone Sulfate Ester; PC: Phosphocholine; PE: Phosphoethanolamine; Δ: Double Bond Equivalents - the sum of alkenes, rings, and carbonyls in a molecule; Cxxx: PC ester of a steroid with a xxx Da fragment; Exxx: PE ester of a steroid with a xxx Da Fragment. The steroid would have a formula with a mass of xxx + 17. The H⁺ ion of the PC ester would have a mass ion of xxx + 183 Da. In serum extracts, Na⁺ cation of PC ester has a mass ion of xxx + 183 + 22 Da. In serum extracts, Na⁺ cation fragments with loss of trimethylamine - 59 Da

Background

In 1964, Smith, Lemli, and Opitz recognized a malformation syndrome (SLO) with distinctive facial features, microcephaly, intellectual disability and learning or behavioral problems [1]. In 1984, we investigated a child with similar malformations, our index case [2]. At 3 days of age, the child's serum had high levels of a steroid that cross reacted with 17-hydroxyprogesterone antibodies and very high levels of steroids that cross-reacted with some, but not all, antibodies to DHEA-sulfate, indicating compounds identified were not DHEA-sulfate but had some structural similarity. The high 17-hydroxyprogesterone levels would be expected for a child with 21-hydroxylase deficiency, but the child was hypokalemic and had no symptoms of hyponatremia. To determine if the cross-reacting material detected with steroid sulfate antibodies (CRM-S) was DHEA-sulfate, we extracted the serum in a hydrophilic solvent and chromatographed it in a similar solvent. There were two peaks of CRM-S, but neither co-eluted with authentic DHEA-sulfate. We obtained a serum sample from an infant of similar age and repeated the extraction and chromatography. We expected to find one peak of CRM-S but there were four peaks and none of the four was DHEA-sulfate. However, we couldn't collect sufficient serum from patients with SLO to permit identification of the CRM-S.

In 1989, we collected cord serum samples, from unaffected infants, extracted and flash chromatographed the extract. Each fraction was evaluated for CRM material with antibodies specific for digoxin (digoxin-like material - DLM), DHEA-sulfate and androstene sulfate [3]. There were three peaks of DLM but there were four peaks of CRM-S. A sample of adult male serum was treated in the same way. There was only one major peak and it was both a DLM and a CRM-S. Thus, one of the DLMS in cord serum could also be present in adult male serum, but a new method would be needed to identify the compounds unique to cord serum.

In 1994, Irons recognized SLO is a cholesterol biosynthetic deficiency, in particular, 7-dehydrosterol reductase deficiency (7DHCR) [4]. This enzyme reduces 7-dehydrosterols to sterols, including cholesterol. Shackelford investigated the neutral sterols [5]. He noted the presence of high concentrations of $\Delta^7(8)$ and $\Delta^8(9)$ unsaturated adrenal steroid metabolites or precursors. There were also high serum levels of pregnantriols and he proposed these might be diagnostic for SLO.

In 2018, we reported isolation of DLM from mammals, specifically humans, pigs and cattle [6]. The DLM, which we named Ionotropin, is the PC ester of a steroid with 23 carbon atoms. There were no prior reports of a mammalian steroid with either feature. The structure was confirmed both by ^{31}P -NMR and by MS fragmentation analysis. Ionotropin shares structural features with specific potassium sparing diuretics, such as spironolactone. Based on the similarity, we proposed that Ionotropin also functions as a potassium sparing hormone. This suggests that the SLO patients would be deficient in Ionotropin and that this might account for their hypokalemia. Now that we know the identity of each steroid, we have reexamined serum from older patients with SLO.

This paper describes an investigation of the PC sterols in serum from patients with 7DHCR deficiency (SLO) and obligate heterozygotes.

Methods

Sample preparation

Miltefosine (Sodium salt of PC hexadecanol ester) (Cayman Chemical, Ann Arbor, MI) was used as a recovery marker. For analysis, 10 μl of a 0.2 mg/ml solution (2 μg) of miltefosine was added to 0.2 ml of serum (10 $\mu\text{g}/\text{ml}$). The diluted serum was extracted with 0.8 ml of acetonitrile, centrifuged and filtered with Whatman Syringe filters of 0.2 μm pore size [7]. (If the peak intensity of miltefosine and the unknown were equal, then the serum concentration of the unknown might be about 10 mg/L or 20 μM . In contrast, morning cortisol levels are 100 - 200 $\mu\text{g}/\text{L}$ and normal DHEA-sulfate levels in young adults are 3 - 5 mg/L).

Mass spectroscopy

The extract was analyzed by direct injection into the electrospray source of an LTQ-XL quadrupole ion trap mass spectrometer (MS) (Thermo Scientific, San Jose, CA). Flow rate was 10 $\mu\text{l}/\text{min}$. The capillary temperature was 275 C. Spray voltage was 4.4 volts. Ten mass

spectra scans were collected and averaged for quantification. Each sample was also analyzed when 60 volts was applied at the source to fragment the molecules. Representative peaks were selected for MS/MS analysis and, in some cases, MS^N analysis to identify parent/fragment ion relationships. For selection of the parent ions for collision-induced dissociation, the isolation width was set to 2. All spectra were generated in the positive ion mode. To restrict cross contamination, the capillaries were washed between samples with 4:1 acetonitrile/water until the ion counts dropped to below 1×10^3 , i.e. less than 1% of the intensity observed in the samples being investigated [7].

Characteristic fragments were formed by collision-induced dissociation: [i] 183 Da derives from the PC fragment, [ii] 22 Da derives from Na⁺ replacing H⁺, [iii] 59 Da derives from loss of trimethylamine fragment in the PC, [iv] phosphoester peaks differing by 42 Da are attributed to PE rather than PC steroids and [v] 17 Da must be added to the Cxxx designation to evaluate the mass of the steroid when the PC fragments. These values illustrate how the phosphosteroids can be identified from the fragments.

Serum samples

The investigation of SLO patients is a joint effort of Dr. Fred Chasalow and Dr. Forbes Porter. PC steroids were measured on samples already collected and that had been stored by Dr. Porter for future investigation. In addition to patients diagnosed with SLO, we also evaluated samples from obligate heterozygotes. There were 12 samples: 6 affected (3 males, 3 females) and 6 obligate heterozygotes (3 males, 3 females).

Results

Table 1 shows the quantitative results on all 12 samples. Figure 1 and 2 display typical mass spectra obtained from obligate carriers for SLO syndrome; Figure 3 and 4 display mass spectra obtained from patients with SLO syndrome. There was little difference in the concentration of the spiral lactones with 23 carbon atoms (mass ions at $m/z = 542, 544$ and 546 Da) between the carriers and the affected individuals. However, we were not able to obtain samples during the first two weeks postpartum when potassium recovery was important and there were high levels of phosphoesters in normal infants [8].

Designation										
	m/z		518	542	546	341	369	381	475	313
+/+		F	0.53	0.11	0.21	0.08	0.08	0.16	1.08	0.04
+/+		M	0.50	0.17	0.25	0.17	0.09	0.26	0.89	0.07
+/+		F	0.93	0.19	0.37	0.03	0.12	0.03	0.60	0.04
+/+		M	0.58	0.13	0.19	0.25	0.13	0.27	0.41	0.12
+/+		M	0.56	0.25	0.25	0.25	0.17	0.17	0.77	0.08
+/+		F	0.65	0.14	0.12	0.07	0.10	0.37	0.59	0.03
+/	H	F	0.70	0.30	0.22	0.11	0.45	0.05	0.99	0.07
+/	H	F	0.58	0.16	0.13	0.65	0.67	0.62	1.13	0.29
+/	H	M	0.83	0.37	0.22	0.73	0.76	0.29	0.70	0.29
+/	H	M	0.81	0.21	0.21	0.81	0.89	0.45	0.72	0.39
+/	H	F	0.84	0.50	0.27	0.07	0.98	0.02	0.53	0.02
+/	H	M	1.32	0.25	0.23	0.16	1.45	0.01	0.70	0.20

Table 1: Relative intensity of specific mass ions compared to the intensity of miltefosine.

This table compares the intensity of the specific ions to the ion intensity of miltefosine at $m/z = 430$ Da. Values in **red** should be noted. The ion at $m/z = 341$ is the steroid fragment from the PE ester of Itonotropin. Note there are high levels of C369 in the carriers but not in the affected individuals. The individuals with SLO would not have a functional $\Delta 7$ -sterol reductase enzyme and, thus, could not synthesize C369. The function of C369 remains to be determined.

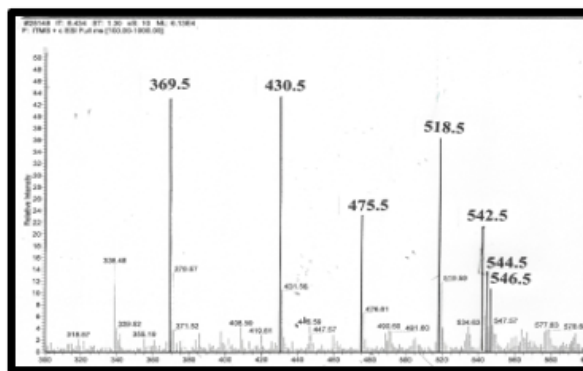


Figure 1: Mass spectrum obtained on serum from female obligate heterozygote.

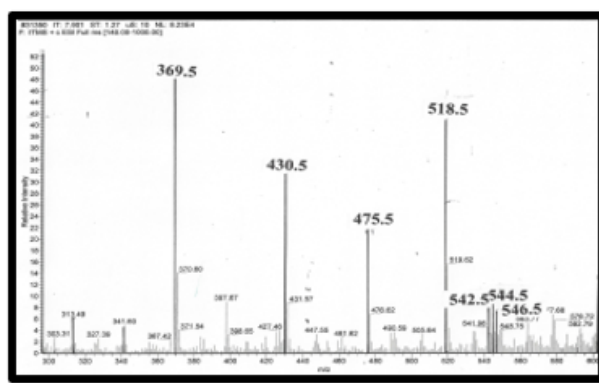


Figure 2: Mass spectrum obtained on serum from a male obligate heterozygote.

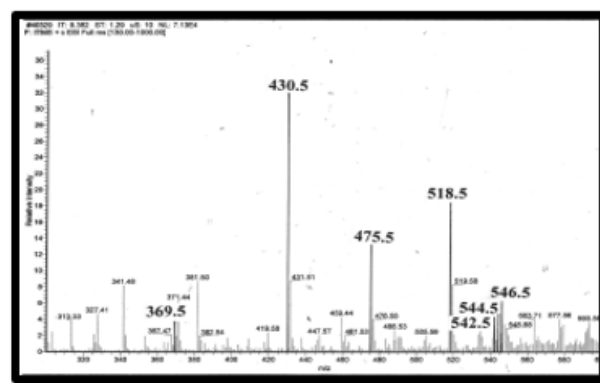


Figure 3: Mass spectrum obtained on serum from female with SLO syndrome.

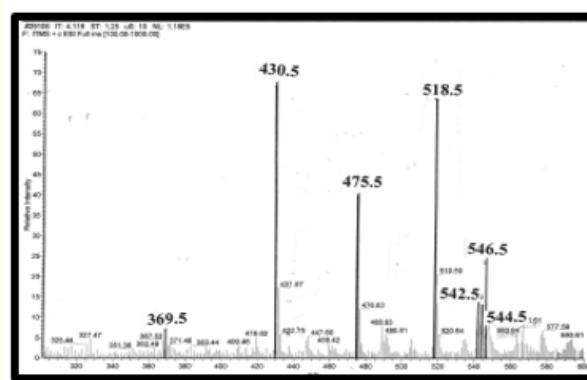


Figure 4: Mass spectrum obtained on serum from male with SLO syndrome.

In addition to the spiral lactones with 23 carbon atoms, there were two other mass ions in some of the samples: [1] an ion at $m/z = 475$ Da was present in all of the samples and [2] an ion at $m/z = 369$ Da was absent from patients with SLO but present in the samples from SLO carriers. The following sections describe what we know about each of the mass ions we observed.

Identification of mass ions

430 Da: This is the mass ion associated with the Na^+ salt of miltefosine. This compound was added as a marker to monitor recovery. Note the absence of either the [a] mass ion at 446 Da, the K^+ salt and of [b] the ion at 408 Da, the H^+ cation. This confirms that most of the ions present are Na^+ cations. This observation simplifies understanding of the origin of the individual ions actually observed.

518 Da: (C313) This is the Na^+ salt of the PC ester of 3β - 17α -dihydroxy pregna-5,7-dien-20-one (C313). Shackleton identified this steroid component as one of the major 7-dehydrosterols in the neutral fraction [5]. The composition of the steroid is $\text{C}_{21}\text{H}_{30}\text{O}_3$.

542 Da, 544 Da and 546 Da: (C337, C339, C341, respectively) These three ions are PC-spiral lactones with 23 carbon atoms in the Na^+ cation form (Figure 5) [6]. All three contribute to the total DLM observed. The m/z ion at 542 Da (C337) compound is the 5, 7-dialkene; the m/z ion at 544 Da (C339) compound is the 5-alkene; the m/z ion at 546 Da compound (C341) has both alkenes reduced. The 546 Da compound has been named Ionotropin. Ionotropin shares the ring B stereochemistry with digoxin. 7DCHR catalyzes the reduction of C337 to C339. Patients with the 7DCHR mutation should lead to accumulation of the 542 Da compound, but only if either of the metabolites - C339 or C341- were needed.

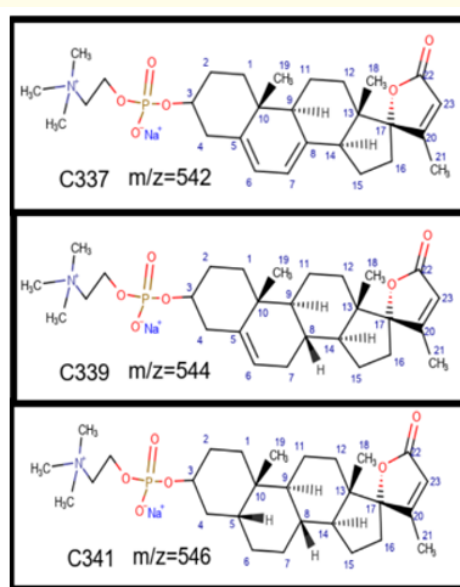


Figure 5: The structure of the spiral lactones with 23 carbon atoms.

475 Da: (C329) As these were serum extracts, the phosphoester ions were Na⁺ cations, rather than either H⁺ ions or K⁺ ions. Molecules containing only carbon, hydrogen and oxygen must have an 'even' mass. However, the observed ion mass is an odd number (475 Da), indicating it must be a fragment. MS-MS analysis confirms that the 475 Da ion was a fragment of an ion with m/z=534 Da (See figure 6). The corresponding H⁺ ion would have a mass of 512 Da (m/z = 512-183 = 329 Da) and would be designated C329. The intact steroid molecule would have a mass of 346 Da (m/z: 329+17 = 346 Da).

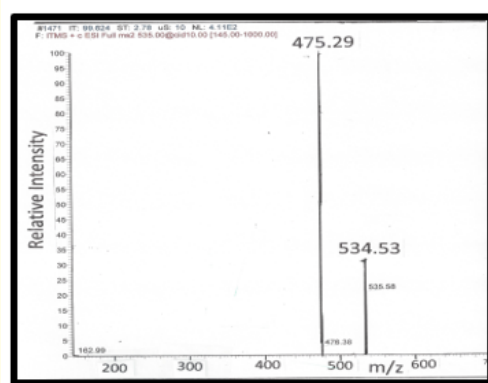


Figure 6: MS-MS analysis of 534 ion showing the 475 ion as the major fragment. The steroid is C329.

369 Da: (E369) This is the first report of a steroid fragment with a mass of 369 Da. The PC would have m/z = 552 Da and the corresponding PE ester would have m/z = 510 Da. PCs and PEs fragment by different paths. The Na⁺ ion of a PC-steroid fragments by loss of 59 Da - loss of trimethylamine. The Na⁺ ion of PE- fragments by loss of 141 Da which is the entire phosphoester leaving only the steroid fragment. Thus, the m/z = 369 Da ion indicates that it is derived from the PE-steroid with an m/z = 510 Da. The intact steroid would have m/z = 386 Da.

Discussion

Structure and biosynthesis of the ion at 475 Da (C329)

Table 2A shows a trial and error analysis of possible compositions that could generate a steroid molecule at m/z = 346 Da (C329). The composition on Line 2 (C₂₁H₃₀O₄) is the best fit. Line 4 fails based on Delta because 7 Delta are needed and Line 4 requires 6 Delta. Line 5 fails because 3 oxygens are needed to be a substrate for lactone formation. Figure 7 shows a possible structure corresponding to line 2. The simplest biosynthetic pathway for C329 is hydroxylation of C313. The substrate for the hydroxylase could be either the PC or the PE and the substrate for the conversion of the PE to the PC could be either E313 or E329. N-methylation is required to convert Exxx to Cxxx. However, as we identified both PE and PC forms for each intermediate, either one of the steps could be the step at which N-methylation occurs.

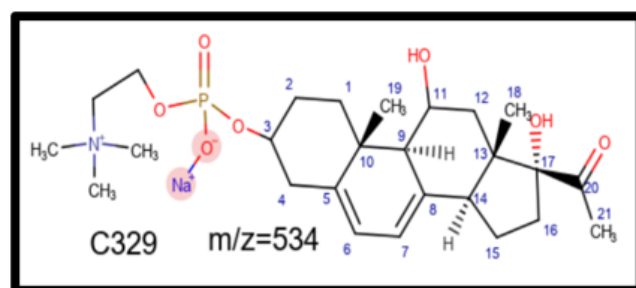


Figure 7: The proposed structure for C329.

C329 was detected by MS at m/z = 475 Da. This ion is generated by loss of trimethylamine from the PC ester. The chemical formula is C₂₁O₄H₃₀ with delta of 7. A similar fragment is generated by C313.

Structure and biosynthesis of the ion at 369 Da (E369)

The PC ester of C369 would have a parent ion at $m/z=552$ Da ($m/z: 369+183$); the PE-ester (E369) would have a parent ion at $m/z = 510$ Da ($m/z = 369+141$). To date, the only other sample we found with an ion at $m/z = 369$ Da was a bovine ovarian extract. Ovarian extracts had two major ions - 554 Da (C371) and 510 Da (E369). On MS-MS fragmentation, both ions fragmented with loss of 18 Da, as would be expected if the first fragmentation step was loss of a hydroxy group (See figure 8). No other PC steroids examined fragmented with loss of 18 Da. Table 2B indicates the composition on Line 15 could only have one alkene [9]. The detection of both C371 and E369 in the extracts suggests that E369 is converted *in situ* to C371 in two steps - N-methylation and $\Delta 7$ - $\Delta 8$ reduction. We have no evidence as to the order of the two steps. The precursor (E329) has both a $\Delta 5$ -6 and a $\Delta 7$ -8 alkene in the B-ring, but the $\Delta 5$ reductase doesn't reduce $\Delta 5$ -6 alkenes when the $\Delta 7$ -8 alkene is present. Thus, the alkene in E369 must be in the $\Delta 5$ -6 position and the $\Delta 7$ -8 must have been the one that was reduced. This would account for its absence in the SLO patients who would not be able to reduce the $\Delta 7$ -8 alkene.

Line	Carbons	Oxygens	C+O	H-Req	H-Max	Delta
1	21	3	300	46	44	-1
2	21	4	316	30	44	7
3	21	5	340	14	44	15
4	22	3	312	34	46	6
5	23	2	312	38	48	7
6	23	3	324	22	48	13

Table 2A: Possible formulas for C329, a molecule of mass 346 Da with a fragment of 329 Da.

Line	Carbons	Oxygens	C+O	H-Req	H-Max	Delta
11	21	6	348	38	44	4
12	23	4	340	48	48	0
13	23	5	356	32	48	8
14	24	3	336	50	50	0
15	24	4	352	34	50	81
16	24	5	368	18	50	16
17	25	3	348	38	52	7
18	25	4	364	22	52	15

Table 2B: Possible formulas for C369, a steroid molecule of mass 386 Da with a fragment at 369 Da.

These two tables are trial and error analysis of possible chemical formulas containing only carbon, oxygen and hydrogen atoms to generate a mass of 346 Da (Table 2A) or 386 Da (Table 2B). Each line shows a possible combination of carbon and oxygen atoms. The C+O column shows how many Da would be provided by the specific combination of carbon and oxygen atoms on that line. H-Req shows the required number of hydrogen atoms necessary to complete the molecule. Delta is the sum of rings, double bonds and other features that reduce the required number of hydrogens. Because a steroid has 4 rings, delta can't be less than 4. Delta can't be more than 12 without an aromatic A ring. The lines in RED show the only combinations that can make steroid-like molecules. See figure 7 and 8 for proposed structures. The Line 15 molecule already has 4 oxygens at carbons 3, 11, 17 and 22. There is no obvious site for the fifth oxygen to generate Line 13. The 21-hydroxylase enzyme requires its substrate to have a delta-4, 3-ketone structure and could not use a phosphoester as a substrate.

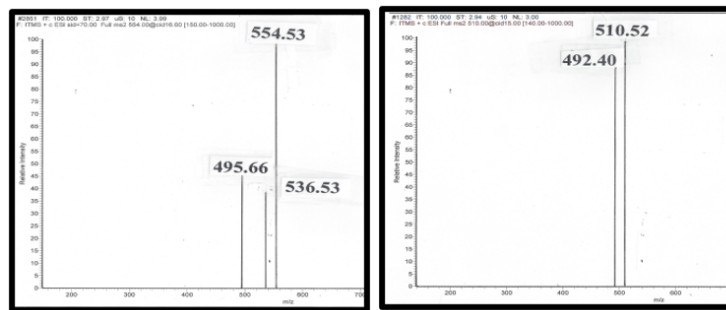


Figure 8: MS-MS analysis of C371 and E369.

Left panel: MS-MS analysis of $m/z = 554$ Da ion showing fragments of 18 and 59 Da.

Right Panel: MS-MS analysis of $m/z = 510$ Da showing fragment of 18 Da.

The absence of the 59 Da fragment indicates it is not a PC ester but a PE ester.

There are four common hydroxylases – 11-hydroxylase, 17-hydroxylase, 18-hydroxylase and 21-hydroxylase. To form a spiral steroid, we need the 17-hydroxyl to form the lactone ring. The 21-hydroxylase enzyme is specific for Δ^4 -3-ketones and the 3-phosphoester could not be a substrate. If there were an 18-hydroxy group, it could not fragment with loss of water as was observed with E369. Thus, the most likely location for the extra hydroxyl group is the 11-hydroxy group. Figure 9 shows one possible structure that would be consistent with the 369 Da fragment.

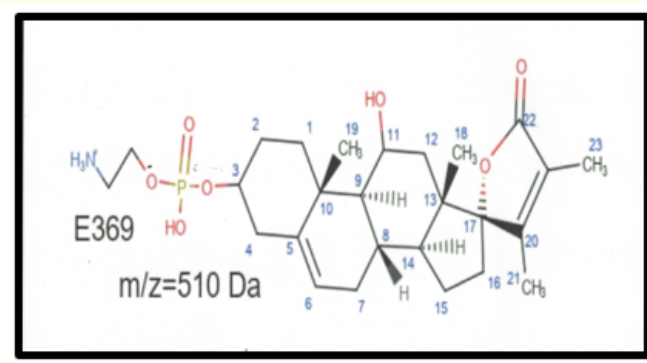


Figure 9: Proposed structure for E369.

Function of spiral lactones

The proposed structure of Ionotropin (C341) includes a spiral lactone E-ring [3]. Spironolactone also has a spiral lactone E-ring and regulates NaK-ATPase. On the basis of this similarity, we have proposed that the spiral lactones, such as Ionotropin or C369, function as potassium sparing hormones. This process could be important for ovulation.

Relation to SLO syndrome

Affected patients: During pregnancy, fetal nutrition is provided via the placenta. Plasma is 5 mM potassium. Hence, it is not surprising that an infant with hypokalemia is small because potassium is required for cell growth. Similarly, gonads, cardiac and renal function all

require potassium. These tissues are all under-developed in affected infants. However, after the immediate post-partum period, diet is replete in potassium but patients still grow slowly.

In the serum from the patient samples we had, all 6 patients had low levels of Ionotropin (C341) but the levels were not significantly different from the levels in the carriers. We attribute the low levels of Ionotropin and its precursors in the patients to the high levels of potassium in their diet, obviating the need for enhanced renal recovery of potassium. Patients with Bartter syndrome have hypokalemia, alkalosis, and normal to low blood pressure [13]. Patients with that syndrome benefit from treatment with potassium sparing diuretics, such as triamterene. As SLO patients have a somewhat similar presentation, but caused by a different inborn error, replacement therapy with a potassium sparing diuretic might be indicated. Perhaps, hypokalemia should be assessed and treated when an SLO patient is sick, in analogy to patients with 21-hydroxylase deficiency who require extra sodium and glucocorticoids when they are sick.

Obligate heterozygotes for SLO syndrome

All six of the obligate heterozygotes had high levels of E369. This seems to be characteristic for the syndrome. In view of the adverse consequences of SLO, there must be a selective advantage for carriers to preserve the genes in the general population. One possibility, suggested by its presence in bovine ovarian extracts, is that it may have an important role to play in gonadal function. Note that, whether or not, our proposed structure is correct, first, there is an ion of $m/z = 369$ Da in the serum of the obligate heterozygotes and in ovarian extracts and, second, its function is completely unknown.

This is the second paper from my laboratory reporting on the quantitation of PC steroids in human serum samples. In the first study, there were 40 samples from pregnant women, half of whom had pre-eclampsia [7]. Three of the 40 had high levels of E369. Overall, the incidence of E369 is similar to the incidence of carriers of 7DCHR deficiency [14].

Conclusion

The observation that there was no accumulation of spiral steroid precursors in patients more than a few weeks of age confirms our initial observation [2]. The defects in renal, gonadal and cardiac development point to a role for the spiral steroids during development. However, whether or not there is a continuing need for the spiral steroids in normal individuals remains to be determined. As there are at least 5 spiral steroids with lactone rings, measurement of total DLM will probably not be diagnostic. There may consequences to a spiral steroid deficiency that might be alleviated by replacement hormone therapy.

Ethics Approval and Consent to Participate

Samples were collected by Dr. Forbes Porter of the NICHD. and stored for future investigation. Coded samples were received in the laboratory without personal identification.

Funding

This work was funded by a grant from the Smith-Lemli-Opitz Foundation with partial support by the Research Service of the United States Department of Veterans Affairs, VA Medical Center, San Francisco, CA.

Authors' Contributions

FC initiated the current investigation, generated the laboratory data, and wrote the major portion of the manuscript. SB participated in the discovery process and in the writing of the manuscript.

Acknowledgements

We acknowledge Dr. Forbes Porter and Dr. Christopher Wassif of the NICHD for providing serum samples from patients and obligate heterozygotes. This work was partially supported by the Research Service of the United States Department of Veterans Affairs, by Dr. Gary Jarvis, VA Medical Center, San Francisco, CA and by Dr Constance John, VA Medical Center, San Francisco, CA. This work was encouraged by Dr. Larry Kunstadt of Warwick Advisors. Marvin Applets were used for drawing, displaying and characterizing chemical structures and reactions, Product Version 20.1 ChemAxon (<https://www.Chemaxon.com>).

Bibliography

1. Smith DW., *et al.* "A newly recognized syndrome of multiple congenital anomalies". *The Journal of Pediatrics* 64 (1964): 210-217.
2. Chasalow F., *et al.* "Possible abnormalities of steroid secretion in children with Smith-Lemli-Opitz syndrome and their parents". *Steroids* 46 (1985): 827-843.
3. Chasalow F and Blethen S. "Characterization of Digoxin-like Material in Human Cord Serum. Steroid Formation, Degradation, and Action in Peripheral Tissues". *The Annals of the New York Academy of Sciences* 595 (1990): 212-220.
4. Irons M., *et al.* "Defective cholesterol synthesis in the SLith-Lemli-Opitz syndrome". *Lancet* 341 (1993): 141.
5. Shackleton C., *et al.* "Identification of 7(8) and 8(9) unsaturated adrenal steroid metabolites produced by patients with 7-dehydrosterol-delta-7-reductase deficiency (Smith-Lemli-Opitz Syndrome)". *The Journal of Steroid Biochemistry and Molecular Biology* 82 (2002): 225-232.
6. Chasalow F and Pierce-Cohen L. "Iontropin is the mammalian digoxin-like material (DLM). It is a phosphocholine ester of a steroid with 23 carbon atoms". *Steroids* (2018).
7. Chasalow F., *et al.* "Spiral steroids as potential markers for pre-eclampsia: a pilot study". *Steroids* 151 (2019): 108466.
8. Chasalow F. "A new concept for the regulation of electrolytes in pregnancy: The role of Iontropin, the endogenous potassium sparing hormone". *EC Pediatrics* 7.6 (2018): 527-532.
9. Chasalow F. "Spiral Phosphocholine Steroids and DLM in Chicken Eggs (*Gallus gallus domesticus*)". *EC Paediatrics* 8.10 (2019): 01-12.
10. Haram K., *et al.* "The HELLP syndrome: clinical issues and management. A Review". *BMC Pregnancy Childbirth* 9 (2009): 8.
11. Chasalow FI. "A New Concept: Iontropin might be a Factor in Mobilization for [a] the Fight or Flight Response and [b] Child Birth". *EC Pediatrics* 7.9 (2018): 909-918.
12. Hamlyn J., *et al.* "11- Hydroxylation in the biosynthesis of endogenous ouabain: multiple implications". *Annals of the New York Academy of Sciences* 986 (2003): 685-693.
13. Colussi G. Bartter syndrome. Orphanet encyclopedia (2005).
14. Nowaczyk M., *et al.* "Frequency and ethnic distribution of the common DHCR7 mutation in Smith-Lemli-Opitz syndrome". *American Journal of Medical Genetics* 102 (2001): 383-386.

Volume 9 Issue 6 June 2020

© All rights reserved by Fred Chasalow and Sandra Blethen.