Severe Neonatal Holocarboxylase Synthetase Deficiency in West African Siblings

Mauricio De Castro · Dina J. Zand ·
Uta Lichter-Konecki · Brian Kirmse

Received: 14 July 2014 / Revised: 15 September 2014 / Accepted: 18 September 2014 / Published online: 18 February 2015
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Abstract In multiple carboxylase deficiency (MCD), the biotin-dependent carboxylases have decreased activity due to either biotinidase deficiency or holocarboxylase synthetase (HS) deficiency. We report the case of two siblings from Ghana, the first of which presented shortly after birth with profound lactic acidosis and a urine organic acid profile consistent with MCD. In the first sibling, treatment with pulverized biotin tablets (20 mg) was begun immediately, but the patient died at 10 days of age from cardiac arrest secondary to refractory metabolic acidosis. Autopsy revealed a biotin bezoar. Sequencing of *HCLS* showed homozygosity for a novel missense variant (p.G241W). The second sibling had a similar presentation at birth: severe metabolic acidosis and respiratory distress. A urine organic acid profile was consistent with HS deficiency; he was treated with biotin powder (20 mg), and after 24 h, the lactate decreased significantly; by day 5 of life, the patient was tolerating 40 mg of biotin, feeding by mouth and off all other medications and support. This is the first report of the p.G241W mutation. To our knowledge, this is also the first mutation described in West African patients with HS deficiency and the cases demonstrate that it is biotin responsive. Additionally, our experience suggests that the

powdered form of biotin supplementation may be more digestible than tablets for the treatment of severe neonatal HS deficiency.

Introduction

Multiple carboxylase deficiency (MCD) (OMIM#253260, #253270) is a disorder of organic acid metabolism caused by a defect in the biotin pathway. Signs and symptoms include severe, refractory metabolic/lactic acidosis, seizures, hypotonia, respiratory distress, ataxia, impaired consciousness, skin rash, and alopecia (Wolf 2000). Biochemical findings include hyperammonemia, elevated lactic acid, and a specific pattern in urine organic acids including elevations of 3-OH-propionic acid, lactate, and 3-methylcrotonylglycine (Suormala et al. 1998). Clinically, MCD can be divided into an infantile/juvenile form, caused by the inability to recycle endogenous biotin due to biotinidase deficiency (*BTD*) (EC 3.5.1.12), and a neonatal form, caused by holocarboxylase synthetase deficiency (*HLCS*) (EC 6.3.4.11). This distinction is not always clear-cut as patients with HS deficiency can present later in childhood (Sakamoto et al. 2000).

Biotin is covalently bound through its carboxyl group to an inactive apocarboxylase forming in the process the active holoenzyme (Moss 1971); this process is catalyzed by *HLCS*. Additionally, biotin has been found to regulate gene expression (Rodriguez-Melendez and Zemleni 2003), biotinylate histones (Narang et al. 2004), and play a role in systemic processes such as development and immunity (Baez-Saldaña et al. 1998). In the USA, biotin is available in tablet and capsule form. No intravenous form of the drug is available.

We report the case of two West African siblings that presented with severe neonatal HS deficiency and had

Communicated by: Bridget Wilcken

Competing interests: None declared

M. De Castro (✉)
Medical Genetics Branch, Department of Health and Human Services,
National Human Genome Research Institute, National Institutes of
Health, 35 Convent Drive, MSC 3717, Room 1B207,
Bethesda, MD 20892-3717, USA
e-mail: mauricio.decastro-pretelt@nih.gov

D.J. Zand · U. Lichter-Konecki · B. Kirmse
Genetics and Metabolism, Children's National Health System,
Washington, DC, USA

divergent outcomes. They were found to have a novel missense mutation in the *HLCS* gene.

Subjects, Methods, and Results

Case 1

The first patient is a 39-week-old male born to a 36-year-old mother and a 34-year-old father; both are originally from Ghana. The patient was 3,850 g at birth and had a crown-heel length of 53 cm.

Thick, meconium stained fluid was noted during delivery; deep suctioning was not performed, as the baby appeared vigorously active immediately. Apgars at 1 and 5 min were 8 and 9, respectively. The patient was transferred from the delivery room to the newborn nursery for routine care. In the nursery, bi-basilar crackles and increased respiratory effort were noted, a chest X-ray at the time showed patchy infiltrates. On DOL 2, the patient was promptly transferred to the Intensive Care Unit where an arterial blood gas showed a pH of 7.0 and a base deficit of -26 ; in light of the profound acidosis, metabolic testing was performed (acylcarnitine profile, free/total carnitine, urine organic acids, and plasma amino acids).

The patient continued to deteriorate requiring ventilatory support, total parenteral nutrition, and antibiotics for suspected sepsis. The acylcarnitine profile and urine organic acids showed elevations in C-3 and C5-OH and propionate and 3-MCC, respectively; the presumptive diagnosis of MCD was made. The patient was started on biotin supplementation. The form of biotin available in the hospital at the time was tablets, which were crushed at the bedside using a pill crusher and administered via NG tube (20 mg QID). Over the next 3 days, the patient's acidosis failed to improve and seizures ensued. He was placed on several antiepileptics without improvement; a bicarbonate infusion and vasopressors were started as well. Over the next 24 h, the patient developed profound encephalopathy (EEG showed burst suppression pattern with 4–6 s bursts of medium to high-voltage sharp activity interspersed with 10 or more second interval of background suppression) and liver and kidney failure. On DOL 9, he developed bradycardia, followed by pulseless electrical activity; CPR was performed for 10 min, but the patient died.

Autopsy revealed several changes consistent with MCD including changes in skeletal muscle resembling ragged red fibers (observed in metabolic disorders), cystic changes in the brain, and fatty infiltration of the liver. Other gross pathologic findings included multiple areas of liquefactive necrosis in the brain indicating previous infarctions, an enlarged heart weighing 24.6 g (vs. expected 19 g) and a thrombus in a medium-sized pulmonary artery with an area

of infarction. In the stomach, a 69 g bezoar of yellow material was found. The mass was soft and gritty, with no specific odor; there were similar findings in the lung, which showed areas in which the airways were filled with a nonstaining material similar to the material in the stomach. Microscopic analysis of the mass revealed a faintly basophilic material with the appearance of folded string; the same substance was found lining the gastric and intestinal mucosa and to be the main component of the large mass found in the stomach. The identification of the bezoar was made by a pediatric forensic pathologist at Children's National Medical Center; based on the macroscopic (the color was similar to the tablets) and histological appearance, the pathologist felt certain the bezoar was made of biotin. The material was not only found in the stomach but also in the airways, suggesting aspiration of the material as well. The child had been on NPO; otherwise, the only enteral medication he received was the biotin.

A homozygous mutation in *HLCS* was found in exon 5: c.721G>T (p.G241W) by Sanger sequencing from the Emory Genetics Laboratory (Atlanta, GA).

Case 2

The second patient is a 39-week-old male born via vaginal delivery 8 years after the birth of his deceased brother. The parents had 2 healthy, non-affected children in the intervening years. The patient weighed 2,640 g and had Apgars of 4 and 8 at 1 and 5 min, respectively.

The delivery was complicated by presence of meconium and a nuchal cord, the patient was grunting and tachypneic in the nursery, and he was transferred to the NICU on DOL 1 shortly after birth. An arterial stick showed significant metabolic acidosis (pH = 7.26 and base deficit of -20); basic laboratory work showed hyperammonemia, elevated CK, and hyperlactatemia. Due to his brother's diagnosis 8 years before, a diagnosis of *HLCS* deficiency was suspected. The patient was started on a bicarbonate infusion and biotin supplementation at 5 mg PO QID (20 mg daily). Over the next 24 h (DOL 2), the patient's clinical course worsened to include seizures and worsening acidosis. He required CPAP and dopamine infusion.

Preliminary results of the acylcarnitine profile showed severe elevations of C3 and C5-OH; the urine organic acid panel revealed large peaks of propionyl and methylcrotonyl metabolites; all findings consistent with MCD. Since this particular mutation had not been described in the literature, we had presumed this to be a biotin-unresponsive case (Mayende et al. 2012). Because of his brother's history of bezoar, we treated with powdered biotin, 20 mg divided QID. Six hours later, the lactate had decreased from the maximum linearity of the instrument (>20 mg/dL) to 17 mg/dL. Over the next 24 h (DOL 3), the patient was

weaned off hemodynamic support and the lactate level dropped below 10 mg/dL, he was taken off CPAP, and oral feedings were begun. Given his response to biotin, we decided to increase his dose from 20 to 40 mg/day. On DOL 4, the patient was completely off all support; lactate was 1.5 mg/dL and was vigorously bottle-feeding. Brain MRI showed changes consistent with MCD: bilateral caudothalamic and intraventricular germinal matrix hemorrhages, bilateral frontal and anterior temporal horn subependymal cystic lesions and generalized signal abnormalities throughout the white matter. The patient was discharged from the hospital 10 days after birth and is currently 9 months old. On follow-up, he has continued to do well on 40 mg of biotin and 150 mg/kg/day of levocarnitine supplementation; he is closely followed by Medical Genetics and Neurology. His mutation was confirmed via clinical Sanger sequencing, homozygous for c.721G>T, p.G241W.

Discussion

To our knowledge, this is the first time that the p.G241W mutation has been reported and the first mutation described in West African patients. The mutation appears to be biotin responsive.

The exact incidence of HS deficiency is not known and varies between populations. It has been estimated to be less than 1:100,000 in Japan (Narisawa et al. 1982) and as high as 1:10,000 in the Faroe Islands. Studies describing mutations in African populations are limited (Yang et al. 2001). Although the age of presentation can be variable, more than half of cases of HS deficiency manifest in the newborn period with the rest of the cases being reported in children months after birth and in rare cases even up to age 8 (Sakamoto et al. 2000). Most cases respond to oral biotin therapy; in some patients, however, there seems to be progression of the disease even with doses of biotin as high as 200 mg/day (Baumgartner and Suormala 1997; Wilson et al. 2005). These particular cases, dubbed “biotin unresponsive,” are limited to a handful of mutations known to affect the N-terminal domain of the protein (Mayende et al. 2012).

Some mutations are limited to specific ethnic groups. IVS10+5G>A, a common western European mutation, has been determined via polymorphic microsatellite marker identification to come from the Faroe Islands (Yang et al. 2001). The mutations p.L237P and c.782delG are founder mutations in the Japanese (Sakamoto et al. 1998). Other mutations such as p.R508W and p.V550M have been seen across different ethnic groups and belonging to different haplotypes hinting to a recurrent mutation mechanism for these particular changes (Yang et al. 2000).

In these two cases, the mutation p.G241W replaces a highly conserved (through Baker's yeast) glycine with a tryptophan in position 241; the Grantham score (a quantitative measure of the chemical dissimilarity between two aminoacids) between them is high at 184 (0–215), representing a large physicochemical difference. Of the multiple available transcripts for the *HLCS* gene, we used NM_000411.6 for our analysis, used as the reference transcript in HGMD. The p.G241W mutation localizes to exon 5 which is the largest and one of the most commonly affected exons; other well-characterized exon 5 mutations include p.L237P, c.782delG, and c.655dupA. It is interesting to note that p.L237P and c.782delG are the most common mutations in the Japanese population and as such have been well characterized; these mutations are known to abolish enzyme activity (Yang et al. 2001). The p.L237P mutation is described in the literature as a non-Km mutant as it does not affect the putative biotin binding domain, and recent research showed that mutations at this site (and more generally between amino acids 159–314) affect a structured domain present in the N-terminal portion of the protein abolishing *HLCS* activity by affecting the rate of dissociation from its substrate (Mayende et al. 2012). The clinical presentation in our patients was similar to that observed in Japanese patients with the p.L237P mutation, that of severe, neonatal form responsive to biotin.

Finally, we do not have a satisfactory explanation as to why the first patient had a fatal outcome or whether the bezoar played a role. There are no reports in the literature of a biotin bezoar in general, let alone in MCD. Pharmacobezoars are unusual entities and have been described for a number of drugs; their formation is usually associated with enteric-coated drugs or presence of binding agents (Stack and Eapen 1995). The available literature on the subject states that the cause for the majority of cases of pharmacobezoars is GI obstruction and/or decreased motility. Many factors are known to affect the rate of absorption of a drug: dissolution rate, particle size, solvates, ionization state, and drug pK_a . Tablets are more likely to form bezoars owing to their more difficult disintegration and absorption, while finely granulated powder has improved dissolution and absorption (Shargel and Andrew 1999). In the first sibling, we suspect some degree of gastroparesis owing to the critical severity of his illness in addition to the intrinsic characteristics of the tablet (presence of binding) contributed to the formation of the bezoar.

It is also possible that the first sibling's more refractory clinical course was due to a relative delay in the initiation of biotin. Biotin was started as soon as the presumptive diagnosis was made in both cases but was on DOL 2 for the first sibling and on DOL 1 for the second sibling. This 24 h delay could have theoretically played a role in the first

siblings having been farther along the course of multisystem critical illness and thus less responsive to therapy.

In conclusion, we present the case of two brothers from West Africa with HS deficiency caused by a novel missense mutation that is responsive to biotin clinically. Whenever available, we suggest using the powdered (capsule) form of biotin for the treatment of neonatal HS deficiency.

Synopsis

The *HLCS* mutation G241W is biotin responsive, and powdered biotin may be more bioavailable than crushed biotin tablets for the treatment of neonatal HS deficiency.

Compliance with Ethics Guidelines

Conflict of Interest Statements

Mauricio De Castro, Dina J. Zand, Uta Lichter-Konecki, and Brian Kirmse declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by the any of the authors.

Details of the Contributions of Individual Authors

Mauricio De Castro was involved in the management of the second patient in the report and wrote the main manuscript.

Dina J. Zand was involved in the management of the first patient in the report and contributed to the manuscript.

Uta Lichter-Konecki was involved in the management of the first patient in the report and contributed to the manuscript.

Brian Kirmse was involved in the management of the second patient in the report and contributed to the manuscript.

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