CSF 5-Methyltetrahydrofolate Serial Monitoring to Guide Treatment of Congenital Folate Malabsorption Due to Proton-Coupled Folate Transporter (PCFT) Deficiency

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Abstract Hereditary folate malabsorption is characterized by folate deficiency with impaired folate transport into the central nervous system (CNS). This disease is characterized by megaloblastic anemia of early appearance, combined immunodeficiency, seizures, and cognitive impairment. The anemia and immunologic disease are responsive but neurological signs are refractory to folic-acid treatment. We report a 7-year-old girl who has congenital folate deficiency and SLC46A1 gene mutation who is unable to transport folate from her gut to the circulatory system and consequently from the blood to the cerebrospinal fluid (CSF). As a result she developed undetectable 5-methyltetrahydrofolate levels in her plasma and CSF and became immunocompromised and quite ill. Intramuscular treatment with 5-formyltetrahydrofolate (folinic acid) was therapeutic at her presentation and has been successful preventing other signs and symptoms of hereditary folate malabsorption even at relatively low CSF levels. Although difficult, early detection and diagnosis of cerebral folate deficiency are important because folinic acid at a pharmacologic dose may normalize outcome in PCFT gene defects, as well as bypass autoantibody-blocked folate receptors and enter the

cerebrospinal fluid by way of the reduced folate carrier. This route elevates the 5-methyltetrahydrofolate level within the central nervous system and can prevent the neuropsychiatric disorder. CSF levels of 5-methyltetrahydrofolate between 18 and 46 nmol/L may be sufficient to eradicate CNS disease.

Introduction

Hereditary folate malabsorption is a rare inborn error of metabolism, first reported by Lubby et al. in 1961 (Lubby et al. 1961). This autosomal recessive disease is due to mutations in the SLC46A1 gene, which encodes the proton-coupled folate transporter (PCFT) (Qiu et al. 2006). It presents in early infancy with megaloblastic anemia, poor growth, diarrhea, infections, stomatitis, hypotonia, and progressive neurological deterioration including severe developmental delay and seizures that could be refractory to treatment (Diop-Bove et al. 1993–2014; Fernandes et al. 2012; Scriver et al. 1995; Zhao et al. 2012). To date 31 patients have been reported in the literature (Atabay et al. 2010; Borzutzky et al. 2009; Corbeel et al. 1985; Diop-Bove et al. 2013; Geller et al. 2002; Hansen and Blau 2005; Jebnoun et al. 2001; Kishimoto et al. 2014; Lanzkowsky et al. 1969; Lasry et al. 2008; Malatack et al. 1999; Ponz et al. 1898; Rosenblatt and Fenton 2001; Santiago-Borrero et al. 1973; Shin et al. 2011; Sofer et al. 2007; Steinschneider et al. 1990; Su 1976; Urbach et al. 1987; Wang et al. 2014; Zhao et al. 2007). It is imperative that the patients be treated very early and effectively with 5-formyltetrahydrofolate (folinic acid) in order to prevent irreversible neurological disease (Surtees 2001; Urbach et al. 1987; Wang et al. 2014; Whitehead 2006). The use of intramuscular folinic acid since infancy was reported to allow normal development and

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health in a 27-year-old woman with severe disease (Min et al. 2008). The purpose of this report is to demonstrate the levels of cerebrospinal fluid (CSF) 5-methyltetrahydrofolate (5-CH₃-THF) that are associated with normal neurological outcome in a child with hereditary folate malabsorption. Our goal was to use the concentration of 5-methyltetrahydrofolate in spinal fluid as a biomarker to guide treatment.

Human cells cannot synthesize folate *de novo*, but require folate for many metabolic functions, including the conversion of serine to glycine; the catabolism of histidine; the synthesis of thymidylate, methionine, and purine; and the DNA chromatin methylation that regulates gene expression (Cains et al. 2009). Folate is a B vitamin, essential for brain metabolism. It participates in the *de novo* synthesis of purines and thymidine and together with cobalamin represents an important cofactor for homocysteine remethylation and *S*-adenosylmethionine production in the brain. As with other vitamin deficiency states, folate deficiency may be the consequence of acquired or inherited disorders. Several inborn errors of metabolism can lead to defective folate transport or impaired metabolism, resulting in systemic folate deficiency and, obligatorily, 5-methyltetrahydrofolate depletion in the nervous system detectable by assay of CSF levels (Rosenblatt and Fenton 2001).

We report a child with severe systemic folate deficiency due to proton-coupled folate transporter deficiency who has done remarkably well with intramuscular administration of folinic acid and careful monitoring of CSF 5-methyltetrahydrofolate levels. Her diagnosis, hematological, and immunological rehabilitation was the subject of a previous report (Borzutzky et al. 2009).

Case Report

A 7-year-old female, born to non-consanguineous parents of Puerto Rican descent, developed frequent emesis and failure to thrive at 3 months of age. Her weight dropped from the 25th percentile to the 5th percentile. She was able to smile responsively, follow past the midline, vocalize sounds, and lift her head. At 4 months of age, she developed cough, coryza, low-grade fever, irritability, and increased emesis without diarrhea, for which she was hospitalized at another institution. Chest X-ray revealed diffuse interstitial pneumonia. Laboratory studies showed severe normocytic anemia (hemoglobin 6.7 g/dL, MCV 76 μm^3) with 0.4% reticulocyte count, mild neutropenia (1.4×10^9 cells/L) with hypersegmentation, eosinophilia (1.1×10^9 cells/L), and a normal absolute lymphocyte count (3.4×10^9 cells/L). Serum folate levels were undetectable. She was treated with antibiotics, a packed red blood cell transfusion and a single replacement dose of intravenous folinic acid (2 mg/kg) prior to transfer to Boston Children's Hospital.

Upon admission, bronchoalveolar lavage revealed *Pneumocystis jiroveci*. In addition, active cytomegalovirus (CMV) infection was documented by p65 antigenemia, PCR positivity in the bone marrow, and positive staining for CMV in endoscopic biopsies of esophagus and duodenum. Testing for HIV by ELISA was negative. Treatment was started with trimethoprim-sulfamethoxazole and ganciclovir. High-frequency ventilation was initially required due to respiratory distress, but gradual improvement allowed extubation after 12 days. The clinical course was complicated by *Klebsiella pneumoniae* bacteremia, requiring treatment with ceftriaxone.

Bone marrow aspirate performed 10 days after the initial dose of folinic acid was normal except for decreased iron staining and rare neutrophil hypersegmentation, without evidence of megaloblastic hematopoiesis. At that time, blood folate levels were still low. Continued treatment with intravenous folate resulted in normalization of plasma levels. However, 5-methyltetrahydrofolate in the CSF was undetectable. The patient was then switched to parenteral folinic acid to facilitate CSF penetration. Progressive clinical improvement, with normalization of hematological and immunological parameters was observed. The CSF 5-methyltetrahydrofolate improved to 33 nmol/L on 5 mg IM folinic acid daily. Dosing has been increased since then to 50 mg daily of IM folinic acid at 7 years of age with the goal of normalizing CSF 5-methyltetrahydrofolate. During this period, her neurological exam was only significant for hypotonia. Her brain MRI was normal at presentation, 7 months and 2 years of age. At the age of 7 years, she has had no more serious infections since infancy. She has reached all developmental milestones appropriate for her age and is doing well in school.

Her mother had a cousin diagnosed at a young age with anemia that was treated with vitamins and got better. We were unable to obtain more details. However, he has not required further treatments and is healthy.

Results

The goal of our treatment plan was to maintain CSF 5-methyltetrahydrofolate within the normal range. To that end, we adjusted the dose of folinic acid based on the measurements of 5-methyltetrahydrofolate in CSF obtained approximately every 6 months during the first few years of life considered critical for future development.

The CSF 5-methyltetrahydrofolate values are presented in Table 1. The doses shown were those the patient was taking at the time of the lumbar puncture before any adjustments were made. The samples were obtained in the fasting state early in the morning. Some samples were

Table 1 CSF 5-methyltetrahydrofolate (5-CH₃-THF) levels and IM folinic acid therapy

Age (months)	6	8	13	15	18	26	34	44	53	65	84
Sample collection time	1200	0752	0728	0800	0945	0918	1100	0810	0950	0918	1030
CSF 5-CH ₃ -THF nmol/L ^a	18	33	23	28	29	19	42	33	27	39	46
Serum folate nmol/L ^b	178	179	>261	152	113	>261	>261	>261	>261	>261	>261
Dose (mg/kg/day)	0.5	1	1.5	1.5	1.5	2	2.5	2.5	3	3	3
Total daily dose in mg	2	5	7.5	10	12.5	15	20	20	30 (15bid)	40 (20bid)	50 (30 am 20 pm)

^a CSF 5-CH₃-THF normal range: 40–187 nmol/L

^b Serum folate normal range: 4–45 nmol/L

obtained between 1 and 4 h after the injection but the mother could not recall this information with certainty. Since it was difficult to achieve therapeutic values and the half-life of folinic acid is about 6 h, we recommended to split the dose but her compliance has not been very good with the second dose. The first 4 years IM injections were given in the legs, but since then, she has been injected in her arms too. She had no local or systemic side effects. The samples were collected and sent to the Medical Neurogenetics laboratory in Atlanta, GA, for analyses, according to the recommendations of Keith Hyland. Her current dose is 50 mg/day IM of folinic acid taken 30 mg IM in AM and 20 mg IM in PM (3 mg/kg/day). As seen in the table, we were not able to achieve parameters considered normal consistently of cerebrospinal fluid 5-methyltetrahydrofolate. Despite the fact that levels were between 18 and 46 nmol/L (mean 35 nmol/L), our patient's cognitive development and motor function are within normal limits. The CSF levels of this magnitude were associated with serum levels between 113 and >261 nmol/L.

Her weight, length, and head circumference are near the third percentile, but this most likely is familial because the measurements of weight and length are proportional and her mother has the same phenotype and similar percentiles. She currently does not have failure to thrive which has been reported in other cases of hereditary folate deficiency.

She is currently in first grade with neither motor nor cognitive problems. Her neurological, developmental, and neuropsychological evaluations showed no significant abnormalities. To evaluate her cognitive abilities, she was administered the DAS-II, Early Years, Upper Level Battery at 6.6 years of age. The DAS-II measures intelligence in terms of verbal, nonverbal, and spatial reasoning abilities. Verbal abilities were in the average range (standard score of 100, 50%). Nonverbal skills were in low average range (standard score 83, 13%). Her ability to use categorical reasoning to perceive increasingly complex similarities among stimuli was in the average range. In contrast she scored in the low average range on a task of abstract visual

reasoning and pattern recognition. Visual motor skills were average (standard score of 93, 32%). Selected subtests of the Wechsler Individual Achievement Test (WIAT-III) were administered. She performed in the average range (standard score of 93, 23%) on early reading. Math problem-solving skills were low average (standard score of 89, 23%). She was also administered the Beery-Buktenica Developmental Test of Visual Motor Integration, Sixth Edition (VMI-6); Developmental Neuropsychological Assessment, Second Edition (NEPSY-2); Behavior Rating Inventory of Executive Function (BRIEF); Behavior Assessment System for Children, Second Edition, Parent Rating Scale (BASC-2-PRN); Children's Depression Inventory, Second Edition (CDI-2); and Roberts Apperception Test, Second Edition (Roberts-2) and did not show abnormalities.

Her father completed 1 year of college and is currently a singer, and her mother obtained a bachelor's degree and currently stays at home. They are both from Puerto Rico but neither of them nor their ancestry are from the Villalba region where the carrier prevalence of the SLC46A1 mutation is 6.3% compared to 0.2% of the rest of the island (Mahadeo et al. 2010).

Discussion

Levels of 5-methyltetrahydrofolate in CSF between 18 and 46 nmol/L may be sufficient to eradicate CNS disease. Folate is an essential cofactor for one-carbon methyl transfer reactions and crucial for a variety of biological processes, such as synthesis and repair of DNA (Blount et al. 1997; Kronenberg et al. 2008; Linhart et al. 2008), regulation of gene expression (Ghoshal et al. 2006; Pogribny et al. 2008), and synthesis of amino acids and neurotransmitters (Fournier et al. 2002; Shinohara et al. 2006). Since the 1940s, folate-deficient states and inborn errors of folate metabolism including defective transport have increasingly been recognized to play a role in the development of a macrocytic anemia, atherosclerosis,

thrombosis, neuropsychiatric disorders, and neural tube defects (Surtees et al. 1993). Clinical conditions associated with abnormal folate status range from malformations such as spina bifida to isolated disorders of cerebral function such as the cerebral folate deficiency (CFD) syndrome (Djukic 2007). Folate is known to occur in higher concentrations in CSF than plasma. As it is not synthesized within CNS, folate must enter the CSF against a concentration gradient from the blood (Chen and Wagner 1975; Suleiman et al. 1981). In various species, a folate binder was found in plasma membrane of choroid plexuses, suggesting a role for this protein in transport of folate to CSF and CNS (Chen and Wagner 1975; Suleiman et al. 1981). Dietary folate is taken up in the gut, metabolized to 5-methyltetrahydrofolate, and then distributed by the bloodstream. Cellular uptake of 5-methyltetrahydrofolate is mediated by the PCFT, the reduced folate carrier (RFC), and by two GPI-anchored receptors, folate receptor alpha (FR α) and beta (FR β) (Matherly and Goldman 2003). Unlike the reduced folate carrier, PCFT has a similar affinity for reduced folate and folic acid, at pH 5.5, ambient in the upper small intestine. A loss-of-function mutation affecting the PCFT gene coding for this transmembrane protein was identified as the cause of hereditary folate malabsorption (Qiu et al. 2006; Zhao et al. 2007). However, PCFT is also expressed in many other tissues, including the brain, where it might export folate from acidified endosomes after FR α -mediated endocytosis (Zhao et al. 2009). After folate binds to the receptors, it is internalized by the epithelial cells through receptor-mediated endocytosis and, from there, passes into the brain interstitium and the cerebrospinal fluid (Henderson 1990; Holm et al. 1991; Wu and Pardridge 1999).

The PCFT plays a key role in intestinal folate absorption and folate transport into the central nervous system (Blount et al. 1997). Loss-of-function mutations in the PCFT gene lead to the rare autosomal recessive disorder, hereditary folate malabsorption, characterized by markedly reduced folate levels in blood and CSF (Blount et al. 1997; Diop-Bove et al. 1993–2014; Geller et al. 2002; Green and Miller 1999; Mahadeo et al. 2010; Qiu et al. 2006; Whitehead 2006; Zhao et al. 2007). Folic acid transport deficiency, another rare congenital metabolic defect, is presumably a recessive disorder, given that it is more prevalent in children of consanguineous matings and may simply be hereditary folate malabsorption (Lanzkowsky et al. 1969).

The initial manifestations of the condition may be agitation and insomnia, followed by deceleration of head growth, psychomotor retardation, hypotonia, and later spasticity, ataxia, dyskinesia, gait disturbances, speech, and epilepsy (Hansen and Blau 2005). The clinical manifestations are multisystemic, stressing the central role of folate in many organ systems. Manifestations in the CNS include microcephaly, seizures, and

progressive neurological deterioration along with ataxia and autism. Cranial computed tomography may show calcifications (Geller et al. 2002). Manifestations in the central nervous system are explained by the involvement of folate in the synthesis of methionine and *S*-adenosylmethionine, which are essential for normal development of the central nervous system (Surtees et al. 1993). During early stages of the disease, when folate body stores are depleted but not yet exhausted, the signs are often minimal, and the only abnormality may be neutrophil hypersegmentation (Nath and Lindebaum 1979; Zittoun and Zittoun 1999). Megaloblastic anemia, and both reduced red blood cell count and cerebrospinal fluid folate, and hyperhomocysteinemia are the hallmarks of severe folate depletion. In hereditary folate malabsorption, CSF 5-methyltetrahydrofolate is very low or not detectable. Even when blood 5-methyltetrahydrofolate levels in patients with hereditary folate malabsorption are restored to, or above, the normal levels by folate supplementation, the CSF/blood 5-methyltetrahydrofolate ratio remains low (0.07–0.14) (Geller et al. 2002). Min et al. has suggested CSF levels might not represent intraneural concentrations (Min et al. 2008). The variation might represent different times since last administration. The absence of control studies because of the rarity of the disease precludes definition of optimal therapy (Min et al. 2008).

Treatment with pharmacologic doses of folinic acid, most of which is enzymatically converted in vivo to the physiologically active 5-methyltetrahydrofolate (Levitt et al. 1971; Wright et al. 2003), enters the CSF by way of the reduced folate carrier on the choroid epithelial cells. Treatment of this condition with folinic acid, with a dose of 0.5–1.0 mg/kg body weight/day of a racemic mixture of the DL-form or half of this dose for the levo-form of folinic acid, has resulted in significant improvement of clinical symptoms and increased concentrations of 5-methyltetrahydrofolate in the CSF. Folinic acid is a more stable form of a metabolically active reduced folate compound, in contrast to folic acid which is an oxidized and metabolically inactive folate form. Also, the latter substance is more likely to have adverse effects such as inducing epileptic seizures (Ramaekers and Blau 2004). The starting dose ranges from 0.5 to 1.0 mg/kg/day and can be followed by monthly increments of 0.5 mg/kg/day according to the recommendations by the International Cerebral Deficiency Group (Ramaekers and Blau 2004). We have used doses up to 3 mg/kg/day and prefer to give every 12 h. An interval of 8 h would be more technically correct but is not practical with children. An alternative explanation for our patient excellent outcome might be that the half-life of the folinic acid in the brain or CSF is longer than in the blood. Because the majority of patients respond to treatment with folinic acid, physicians should remain vigilant to the possibility of deficiencies of folate and consider it before drawing any conclusions about the cryptogenic etiologies of

unexplained cases. The deleterious outcomes of untreated patients underscore the importance of making an early diagnosis (Green and Miller 1999).

Conclusion

Folinic acid supplementation is effective and should be initiated as early as possible. It may offer a life-changing therapy in patients with confirmed congenital hereditary folate deficiency. Folinic acid is therapeutic for the hematological and immunological complications, and CSF 5-methyltetrahydrofolate levels between 18 and 46 nmol/L (mean of 35 nmol/L) might be sufficient to prevent the neurological complications that, if untreated, are irreversible. Folinic acid was well tolerated in our patient without side effects. However, treatment of this condition in patients without CSF investigations is not recommended. The remaining questions about how often a lumbar puncture should be performed and what is the appropriate intramuscular dose of folinic acid necessary to prevent an irreversible neurological disorder remains to be determined.

Compliance with Ethics Guidelines

Conflict of Interest:

Alcy Torres, Stephanie A. Newton, Brian Crompton, Arturo Borzutzky, Ellis J. Neufeld, Luigi Notarangelo, and Gerard T. Berry declare that they have no conflict of interest.

Informed Consent

Informed consent was not obtained from the family as lumbar punctures for CSF testing were considered to be a part of routine care for patients with this potentially very serious encephalopathic illness.

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