Role of Intramuscular Levofolate Administration in the Treatment of Hereditary Folate Malabsorption: Report of Three Cases

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Abstract Hereditary folate malabsorption is a rare autosomal recessive disorder caused by impaired active folate transport across membranes and into the central nervous system due to loss-of-function mutations in proton-coupled folate transporter (PCFT). Newborns with this condition have initially normal folate stores, but as they are unable to absorb dietary folate and use rapidly their stores because of their growth demands, symptoms appear in the early infancy. Significant neurological morbidity usually follows the initial non-specific clinical presentation and delayed initiation of treatment. High dose oral and parenteral folinic acid treatment have been previously reported in literature to improve the clinical outcome without achieving optimal cerebrospinal fluid (CSF) folate levels though. The active isomer of 5-formyltetrahydrofolate, also known as levofolinic acid, is available for administration. We report our experience in achieving normal (age dependent) CSF 5-Methyltetrahydrofolate (5-MTHF) levels following daily intramuscular administration of levofolinic acid in three patients with HFM. Follow-up assessment with repeated lumbar punctures has shown a stabilization of 5-MTHF levels within normal range. Clinical features and brain MRI findings had as well either improvement or stabilization. To

the best of our knowledge, we provide as well for the first time data in regard to the im levofolate treatment dosage.

Introduction

Hereditary folate malabsorption (HFM; OMIM 229050) is a rare autosomal recessive disorder caused by impaired active folate transport across membranes and into the central nervous system due to loss-of-function mutations in proton-coupled folate transporter (PCFT) (Kronn and Goldman 2008; Qiu et al. 2006; Zhao et al. 2009). The severe systemic folate deficiency concomitant with impaired transport of folate across the blood-choroid plexus-brain barrier represents the pathophysiological basis of the disease and results in a marked deficiency of cerebrospinal fluid (CSF) folate (Zhao et al. 2007, 2009; Geller et al. 2002).

Infants with HFM come to medical attention in early infancy when they present with non-specific systemic findings such as failure to thrive, feeding difficulties, diarrhea, megaloblastic anemia, and/or pancytopenia in the context of really low baseline serum folate concentrations (Kronn and Goldman 2008; Zhao et al. 2017). One of the most unique aspects of the disease is an immune deficiency which occurs in a setting of significant hypogammaglobulinemia resulting in infections with unusual organisms (Zhao et al. 2017; Borzutzky et al. 2009). Neurologic symptoms may present concomitantly or develop progressively without adequate treatment (Kronn and Goldman 2008; Zhao et al. 2017). Developmental delay, peripheral neuropathy, movement disorder, behavioral issues, and seizures have been observed in the vast majority of reported

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patients with HFM (Kronn and Goldman 2008; Zhao et al. 2017). Diagnosis is suspected on the basis of the above-mentioned clinical features and hematological findings, which should prompt the clinician to measure CSF 5-methyltetrahydrofolate (5-MTHF) levels which are usually very low or undetectable. Confirmation of the diagnosis is made by detection of biallelic disease causing mutations in the SLC46A1 gene.

Early diagnosis is important for the initiation of treatment. Previous literature reports recommend high doses of oral or intramuscular (im) 5-formyltetrahydrofolate (5-formylTHF), also known as folinic acid, aiming to improve or normalize 5-MTHF CSF levels before irreversible damage occurs (Kronn and Goldman 2008; Zhao et al. 2017). Nevertheless, those levels are difficult to achieve even in patients who have been receiving high doses of parenteral folinic acid (Torres et al. 2015). The active isomer of 5-formylTHF, also known as levofolinic acid, is as well available for administration. Anecdotal observations suggest that the active isomer might be more effective, especially in patients with neurological involvement and refractory seizures (Kronn and Goldman 2008). Herein we report three cases of patients with HFM who received im levofolate treatment. Follow-up assessment with repeated lumbar punctures has shown a normalization of their trough 5-MTHF levels and stabilization of clinical features and brain MRI findings. Trough CSF 5-MTHF levels were measured 24 h post-levofolate dose and prior to the next dose. Based on achieving normal CSF 5-MTHF results, to the best of our knowledge, we provide for the first time data in regard to the im levofolate treatment dosage.

Case Presentation 1

The first patient is the only son of non-consanguineous parents of British origin, born at term following an uneventful pregnancy. This patient's clinical features were previously reported (Shin et al. 2011). He presented to his local hospital at 2 months of age with failure to thrive, pallor, and diarrhea. He was diagnosed with *Pneumocystis jirovecii* pneumonia. Initial work-up showed megaloblastic anemia, neutropenia, undetectable serum folate with normal vitamin B12 levels. IgG levels were normal but IgA and IgM levels were low. Following initial intravenous folinic acid replacement patient was discharged home on oral folic acid supplementation of 1.5 mg/day and immunoglobulin replacement therapy.

Follow-up at 6 months of age demonstrated macrocytic anemia, neutropenia, low serum/red cell folate, and no specific immunodeficiency abnormalities. The dose of oral folic acid was increased. At the age of 13 months the patient showed developmental arrest, movement disorder,

and signs of central motor neuron involvement. The oral folic acid supplementation was again increased. At this point patient had a normal brain MRI and a lumbar puncture was unsuccessful. Even on high doses of oral folic acid patient continued to have the same clinical features and hematologic abnormalities. Based on the above constellation of findings HFM was suspected. Undetectable CSF 5-MTHF levels were in agreement with the clinical diagnosis (Table 1). Genetic analysis confirmed the diagnosis and patient was found to be compound heterozygous, with a mutant allele from his mother (c.204-205delCC) in exon 1 resulting in a frameshift starting at position N68 with early termination of translation, and a mutant allele from his father located in exon 2 (c.1004 C>A) resulting in an A335D loss-of-function point mutation (Shin et al. 2011). At this point patient was started on im folinic acid which led to an improvement of his clinical features; nevertheless, CSF 5-MTHF levels remained suboptimal.

Annual reviews showed delay of gross/fine motor skills, significant visual perceptual difficulty as well as slow witnessed continuous acquisition of skills with support. At the age of 5½ years the patient developed sudden mood swings and drop attacks. He also started having episodes of generalized tonic-clonic seizures, on a weekly basis, and complex partial seizures on a daily basis (up to 30 episodes/day). Epileptic activity was confirmed with an EEG. Patient was started on antiepileptic treatment and im levofolinic acid was introduced. Results from a repeat lumbar puncture 1 year following the initiation of levofolate shown normal levels of 5-MTHF and brain MRI. Currently, at the age of 10 years the patient remains seizure free for 3½ years without any antiepileptic medication and continues to have a normal 5-MTHF CSF level. To date, no local or systemic complications have emerged from the use of im injections.

Case Presentation 2

Patient 2 is the first male child of non-consanguineous parents of Pakistani origin born at term after an uncomplicated pregnancy. He presented at 2 months of age with failure to thrive and irritability. On clinical examination patient presented subtle facial dysmorphism and hypospadias. Initial laboratory work-up showed severe pancytopenia requiring red blood cell and platelet transfusion. Plasma folate level was virtually zero and vitamin B12 was low. IM vitamin B12 and oral folic acid supplementation was started. Good progress was initially documented although plasma folate levels remained suboptimal and total plasma homocysteine was elevated. Vitamin B12 level normalized with im treatment within 2 months, however, patient was lost to follow-up.

Table 1 Biochemical and neuroimaging findings in Patient 1

Age	CSF 5-MTHF	Medication	Imaging
17 months	<10 nmol/L ref. 72–305	Folinic acid, 5 mg/day, im (0.4 mg/kg/day)	Normal intracranial appearances
2 years 6 months	19 nmol/L (trough level) ref. 52–178	Folinic acid, 12 mg/day, im (0.8 mg/kg/day)	Non-specific myelination delay and lack of white matter bulk
3 years	31 nmol/L (trough level) ref. 52–178	Folinic acid, 12 mg/day, im (0.71 mg/kg/day)	Subcortical white matter of bilateral cerebral hemispheres better myelinated, unchanged lack of white matter bulk, no other intracranial changes
5 years	14 nmol/L (trough level) ref. 52–178	Folinic acid, 12 mg/day, im (0.6 mg/kg/day)	No abnormality demonstrated
6 years	17 nmol/L (trough level) ref. 72–172	Sodium levofolate, 15 mg/day, im (0.61 mg/kg/day)	Normal intracranial and intra-spinal appearances
7 years	52 nmol/L (trough level) ref. 72–172	Sodium levofolate, 20 mg/day, im (0.78 mg/kg/day)	Normal intracranial and intra-spinal appearances
10 years	62 nmol/L (trough level) ref. 46–160	Sodium levofolate, 50 mg/day, im (1.13 mg/kg/day)	

Six months later, while in Pakistan, patient underwent brain CT scan, following new onset epilepsy. The scan demonstrated basal ganglia calcification and cerebral atrophy. At the age of 17 months the patient was admitted to PICU because of status epilepticus. Clinical examination revealed truncal hypotonia, movement disorder, and dystonia with poor visual interaction. EEG at that point showed hypsarrhythmia. Because of the above constellation of findings a lumbar puncture for a full CSF work-up was done. The CSF 5-MTHF was undetectable with the rest of findings within normal range. Gene mutation analysis confirmed the diagnosis of hereditary folate malabsorption due to a homozygous c.198C>A, p.Cys66* mutation causing a premature stop codon. This mutation has been previously reported in literature in a patient who was compound heterozygous (Min et al. 2008), but not in a homozygous state. Patient was started initially on oral folinic acid supplementation and 4 months later on im daily injections which led to clinical and biochemical improvement (Table 2 and Fig. 1).

Although on im folinic acid therapy, patient's neurologic progress was turbulent with recurrent PICU admissions for status epilepticus, development of infantile spasms and subsequently myoclonic jerks which required combined antiepileptic drugs. At the age of 2 the patient was started im levofolate treatment which led to the normalization of his 5-MTHF levels. Currently, at the age of 5 years the patient continues to have significant neurological deficit and intractable seizures, auditory and visual impairment, and medication controlled movement disorder.

Case Presentation 3

Patient 3 is the younger brother of Patient 2. Parents have declined antenatal testing. The pregnancy was uneventful and patient was delivered at term in good condition.

The investigations which were carried out on the second day of life showed mild thrombocytopenia, with normal serum folate, red cell folate, and homocysteine. CSF 5-MTHF level was nevertheless really low. Genetic analysis confirmed the presence of the same mutation as in the case of his brother in homozygous state. Levofolate treatment was started at a dose of 5 mg/kg/day as one intramuscular daily dose (Table 3). Trough CSF 5-MTHF levels at 1 month of age showed normalization. Dose per kg decreased with age because of increasing weight and follow-up assessment at the age of 6 months showed low CSF 5-MTHF levels. Dose was increased to 4.5 mg/kg/day. The neurologic progress and the brain MRI findings are normal up to date.

Discussion

HFM is a rare autosomal recessive condition affecting folate metabolism. So far 37 families have been described worldwide, out of which 30 have as well genetic confirmation (Zhao et al. 2007, 2017; Diop-Bove et al. 2013; Mahadeo et al. 2011; Atabay et al. 2010). Our patients were found to have mutations already described in literature as pathogenic (Shin et al. 2011; Min et al. 2008). HFM is a

Table 2 Biochemical and neuroimaging findings in Patient 2

Age	CSF 5-MTHF	Medication	Imaging
17 months	<10 nmol/L ref. 72–305	Folinic acid, 10 mg/day, oral	Ponto-cerebellar hypoplasia in addition to generalized lack of cerebral volume and myelination, bilateral symmetrical mature hemorrhagic infarction of the basal ganglia, symmetrical periventricular, and deep white matter change over the frontal lobes (Fig. 1)
19 months	<10 nmol/L (peak sample) ref. 72–305	Folinic acid, 30 mg/day, oral	
21 months	11 nmol/L (peak sample) ref. 72–305	Folinic acid, 15 mg/day, im (2.5 mg/kg/day)	
2 years	31 nmol/L (trough level) ref. 52–178	Sodium levofolate, 20 mg/day, im (1.3 mg/kg/day)	No progression of prominent white matter changes and ponto-cerebellar volume reduction
2 years 10 months	50 nmol/L (trough level) ref. 52–178	Sodium levofolate, 50 mg/day, im (3.3 mg/kg/day)	
4 years 6 months	106 nmol/L (trough level) ref. 52–178	Sodium levofolate, 40 mg/day, im (1.6 mg/kg/day)

Fig. 1 MRI brain T1 weighted image showing basal ganglia calcifications (a), ponto-cerebellar hypoplasia, (b) T2 weighted image, (c) showing white matter changes over the frontal lobes

multisystemic disease, which emphasizes the major role of folate in different tissues and organs. Patients' clinical features have been recently summarized (Erlacher et al. 2015). Findings include poor feeding, faltering growth, megaloblastic anemia or even pancytopenia, diarrhea, immune dysfunction, and neurological manifestations with progressive deterioration (Kronn and Goldman 2008; Erlacher et al. 2015; Kishimoto et al. 2014). In our small cohort, Patient 1 and 2 had typical features of the disease, which in combination with low 5-MTHF led to the diagnosis of HFM. The abnormal findings on brain MRI

of patient 2 include calcification of the basal ganglia. Intracranial calcifications have been reported in other patients with HFM, typically presenting in the cortex or basal ganglia (Geller et al. 2002; Ahmad et al. 2015; Wang et al. 2015).

There have been no formal studies assessing the different folate formulations, dosing, and routes of administration that provide an evidence based regimen for optimal treatment. Most publications agree that parenteral administration of folinic acid is the only effective treatment for HFM. Even though anemia, immune dysfunction, and

Table 3 Biochemical and neuroimaging findings in Patient 3

Age	CSF 5-MTHF	Medication	Imaging
Day 2	12 nmol/L ref. 72–305	Sodium levofolate, 15 mg/day, im (5 mg/kg/day)	
1 month	140 nmol/L (trough level) ref. 72–305	Sodium levofolate, 15 mg/day, im	Brain MRI – normal
6 months	30 nmol/L (trough level) ref. 72–305	Sodium levofolate, 15 mg/day, im (2 mg/kg/day)	

gastrointestinal symptoms are reversible with the oral or parenteral administration of folinic acid and normalization of blood folate levels is easily achieved, it has been proven that CSF levels remain suboptimal (Zhao et al. 2017; Borzutzky et al. 2009; Torres et al. 2015; Min et al. 2008; Erlacher et al. 2015; Kishimoto et al. 2014; Wang et al. 2015). Thus, the major challenge remains to achieve normal CSF 5-MTHF levels for treatment of neurological disease (Zhao et al. 2017).

This paper presents for the first time evidence of the effective correction of trough CSF 5-MTHF levels using im levofolate in three patients with HFM. Two out of three patients were treated initially with oral and/or parenteral folinic acid, the racemic stable form of folate, which led to correction of the systemic manifestations. Nevertheless, CSF folate levels remained unsatisfactory, below the age dependent values, which prompted us to use levofolate, the pharmacologically active isomer of 5-formylTHF. Daily im administration of the medication led to the normalization of trough 5-MTHF levels in the CSF and those levels remain stable and within normal range so far. Brain MRI findings had been stabilized. Clinical features of patients remained stable as well, while Patient 1 had a significant improvement in regard to his seizure activity. Initial dose in each patient was decided based on trough CSF 5-MTHF levels, and previous dose of folinic acid in combination with current knowledge that the biologic impact of the active isomer is twice that of the racemic compound at the same dose (Kronn and Goldman 2008). Hence, by using the active isomer, we were able to deliver double the amount of folate that would be delivered in the case the same dose of the racemic isoform was used. As a general remark we would say that the dose of im levofolate which is required for the normalization of trough 5-MTHF CSF levels seems to be higher in neonates and infants and decreases with increasing age. This could demonstrate a higher demand of folate at the beginning of life and is in accordance as well with the fact that CSF folate levels are much higher in infancy and decrease with the advancing age (Zhao et al. 2017). Nevertheless, this theory has to be

confirmed and appropriate dosing can only be decided when data from more patients with HFM on im levofolate treatment are available. In addition it should be emphasized that individual CSF 5-MTHF levels may be influenced by multiple factors such as growth spurts, infections, and medication. These factors may explain to some degree the variation observed in our patients. Therefore the treatment regime should always be interpreted with regard to the overall clinical outcome and not just the CSF 5-MTHF levels. Finally, our results are based on assessment of trough CSF 5-MTHF levels. This represents a limitation of the study as 5-MTHF levels could have a great variation during the day which would not be depicted with just a single measurement.

Finally, this paper describes the youngest patient diagnosed with HFM and treated on the second day of life. This patient had only mild thrombocytopenia without any additional systemic or neurological findings.

Our patients did not experience any local or systemic complications as a consequence of the im injections. However, lifelong daily im administration is challenging and development of alternative and effective route of administration would be welcomed by the families.

Take Home Message

Intramuscular levofolate administration represents an alternative treatment option in HFM which seems to result in normal CSF 5-MTHF levels and improvement of clinical features.

Details of the Contribution of Authors

EM has written part of the paper.

PG has supervised and provided corrections for the paper.

SP has done the laboratory analysis and provided comments for the paper.

SJH has done the laboratory analysis and provided comments for the paper.

SB has supervised and written part of the paper.

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Compliance with Ethics Guidelines

Conflict of Interest

Emanuela Manea, Paul Gissen, Simon Pope, Simon J. Heales, and Spyros Batzios declare that they have no conflict of interest.

Informed Consent

No experimental procedures were done in the human subjects which are reported in this paper so no ethical approval was required. All tests were done as part of the normal review of the patients. Parents gave informed consent for the publication of the paper.

Animal Rights

No animal is involved in this study.

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

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