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RESEARCH REPORT

Treatment with Mefolinate (5-Methyltetrahydrofolate), but Not Folic Acid or Folinic Acid, Leads to Measurable 5-Methyltetrahydrofolate in Cerebrospinal Fluid in Methylenetetrahydrofolate Reductase Deficiency

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Abstract *S*-adenosyl methionine, which is formed from methionine, is an essential methyl donor within the central nervous system. Methionine is formed by the enzyme methionine synthase for which 5-methyltetrahydrofolate (5-MTHF) and homocysteine are substrates. Patients with severe methylenetetrahydrofolate reductase (MTHFR) deficiency cannot make 5-MTHF and have extremely low levels in the CSF. As a consequence, methylation reactions in the CNS are compromised, and this is likely to play an important role in the neurological abnormalities that occur in MTHFR deficiency. Although treatment with oral betaine can remethylate homocysteine to methionine in the liver, betaine crosses the blood-brain barrier poorly, and CSF levels of methionine remain low. We report three patients with severe MTHFR deficiency (enzyme activity $\leq$1% of controls) who had undetectable levels of CSF 5-MTHF at diagnosis and while on treatment with either folic acid or calcium folinate. Only treatment with oral 5-MTHF given as calcium mefolinate at doses of 15–60 mg/kg/day resulted in an increase in CSF 5-MTHF.

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Background

Severe methylenetetrahydrofolate reductase (MTHFR) deficiency is a rare autosomal recessive condition leading to a wide spectrum of neurological symptoms, mainly encephalopathy, hypotonia, microcephaly, seizures, developmental delay and episodes of apnoea (Burda et al. 2015; Huemer et al. 2015). Hydrocephalus is an additional rare but recognised complication. The condition generally presents in infancy and is associated with a high morbidity and mortality.

MTHFR is an enzyme required for the formation of 5-methyltetrahydrofolate (5-MTHF), a form of folate able to cross the blood-brain barrier and which is necessary as a substrate for the remethylation of homocysteine to methionine by methionine synthase (Fig. 1). A deficiency of this enzyme results in a low methionine and hence a reduction in *S*-adenosylmethionine, with elevated homocysteine levels (Watkins et al. 2012). Although the pathology in MTHFR deficiency is not fully understood, *S*-adenosylmethionine, an important methyl donor, is required for the formation and maintenance of myelin in the brain; a defect in methylation is likely to contribute to the neurological sequelae seen in the condition (Jadavji et al. 2012). Additionally, raised levels of homocysteine may result in an increased risk of thrombosis.

If treatment is started early, it can improve the outcome for children with MTHFR deficiency, although the outlook is still often rather poor. The treatment consists of betaine, hydroxocobalamin and folate (in various forms) which work in combination to reduce homocysteine, increase methionine, and increase CSF folate levels (Diekman et al. 2014). Betaine lowers homocysteine and increases methionine levels by acting as a methyl donor for the remethy-

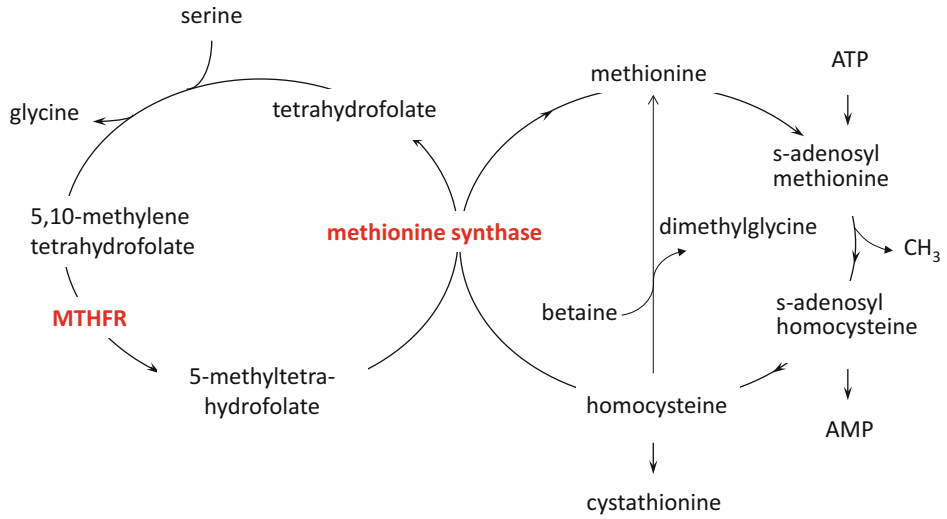

Fig. 1 Folate metabolism and related enzymes. *MTHFR* methylenetetrahydrofolate reductase

lation of homocysteine to methionine, although the homocysteine levels remain elevated above normal. Methylcobalamin is derived from hydroxycobalamin and, along with 5-MTHF, is a cofactor in methionine production. Folate is given in an attempt to increase 5-MTHF levels.

This report will focus on the various forms in which folate can be given in practice and their effects in MTHFR deficiency. In a mouse model of severe MTHFR deficiency, 5-MTHF given to mothers during pregnancy and lactation led to reduced mortality in the pups, but this effect was not seen with folic acid treatment (Li et al. 2008). A previous single case (reported in abstract form only) showed better clinical effects when treated with 5-MTHF compared with folinic acid but no change in the CSF 5-MTHF levels, which remained undetectable (El-Gharbawy et al. 2011). In this report we will present three unrelated patients with severe MTHFR deficiency whose CSF 5-MTHF remained undetectable when treated with folate or folinic acid but improved when this was changed to relatively high dosages of 5-MTHF, commercially available as calcium mefolinate.

Patient Cases

Patient 1

Patient 1 is a female born by normal vaginal delivery at term with a birth weight of 3.04 kg. She is the second child of healthy, non-consanguineous parents. She presented to her local hospital at 5 weeks of age with lethargy, reduced feeding and low temperatures. Treatment for sepsis with intravenous fluids and antibiotics was started but despite this she deteriorated further. A CT head showed areas of low attenuation but no acute changes. Blood tests revealed a raised homocysteine of 176 μmol/L (normal <18 μmol/L) and low methionine of <2 μmol/L (normal range 18–62 μmol/L). Urine organic acid analysis showed no increase in methylmalonic acid and CSF 5-MTHF was undetectable. A skin biopsy was undertaken which confirmed the diagnosis of MTHFR deficiency, with clear deficiency of fibroblast MTHFR activity of around 1 % of the mean control activity with no increase in the presence of added cofactor, flavin adenine dinucleotide. Molecular genetic investigations showed her to be homozygous for c.1781G>A in *MTHFR*.

Following diagnosis patient 1 was commenced on betaine (200 mg/kg/day), hydroxycobalamin (10 mg/day po) and folic acid (5 mg/day). She made a satisfactory clinical recovery from her acute presentation, and the plasma homocysteine and methionine improved being 110 μmol/L and 31 μmol/L, respectively, after 2 weeks. However, at 2 months of age, the CSF 5-MTHF was undetectable and folic acid was changed to calcium folinate. At 11 months of age, the CSF 5-MTHF was still undetectable; calcium folinate was then changed to mefolinate 15 mg/day. At 15 months of age on this treatment, CSF 5-MTHF had increased to 17 nmol/L. However, at 42 months of age, CSF 5-MTHF was again undetectable, but it transpired that due to a pharmacy error at 18 months of age, calcium folinate had been given instead of mefolinate for this period of time. Mefolinate was then restarted at 30 mg twice daily; repeat CSF 5-MTHF levels at 55 months of age had increased to 26 nmol/L (Table 1). At 5 years of age, she has normal speech and is fully mobile, attends a normal school and has only mild learning difficulties.

Table 1 CSF 5-MTHF concentrations and medication in our three patients

Patient	Age	Medication	Dose (mg/day)	CSF 5-MTHF (nmol/L)[a] (reference range 52–178 nmol/L)
1	2 months	Folic acid	5	0
	10 months	Calcium folinate	10	0
	1 year 3 months	Mefolinate	15	17
	3 years 10 months	Calcium folinate	60	0
	4 years 8 months	Mefolinate	60	26
2	3 months	Folic acid	5	0
	1 year 7 months	Mefolinate	15	18
	5 years 11 months	Mefolinate	45	33
3	5 years 8 months	Calcium folinate	15	0
	6 years 3 month	Mefolinate	15	18

[a] All CSF samples were collected at least 4–6 h after the last oral dose of medication. CSF 5-MTHF measurements were made by HPLC in the laboratory of Prof Simon Heales, London

Patient 2

Patient 2 is a male born by normal vaginal delivery at term. He is the first child of distantly consanguineous parents. He first presented at 20 days of age with a bronchiolitic illness and at this point was noted to be below the 0.4th centile for both weight and head circumference. At 8 weeks of age, he was readmitted with poor feeding and mild respiratory distress associated with an abnormal, jerky breathing pattern. He was also found to have some developmental delay – he had nystagmus and was unable to fix and follow and also had central hypotonia. Urine tests during admission revealed homocystinuria. Blood tests showed a raised homocysteine level of 204 μmol/L and low methionine level of 14 μmol/L. There was no increase in methylmalonic acid on urine organic acid analysis.

A diagnosis of 5-MTHFR deficiency was suspected and treatment with betaine (100 mg/kg/day), hydroxycobalamin (10 mg/day po) and folic acid (5 mg/day) was started. The plasma homocysteine and methionine levels responded quickly to treatment, with levels of 109 μmol/L and 64 μmol/L, respectively, within 3 days. However, the patient clinically deteriorated with intermittent sunsetting of the eyes, a bulging fontanelle and increasing head circumference. Cranial ultrasound showed dilatation of the lateral ventricles, and CT scan showed dilatation of all four ventricles with periventricular oedema. An MRI scan revealed further abnormalities with marked hydrocephalus, atrophy of forebrain, pons and medulla and severe hypoplasia of the cerebellum with opening of the inferior aspect of the fourth ventricle into the foramen magnum, indicating a Dandy-Walker malformation.

At 11 weeks of age, he underwent surgery for insertion of a ventriculoperitoneal shunt. CSF analysis at this time showed no sign of infection, but CSF 5-MTHF was undetectable. The diagnosis of 5-MTHFR deficiency was subsequently confirmed by skin biopsy with severely reduced MTHFR activity (0.7% of the mean control value) in extracts of cultured skin fibroblasts. Molecular genetic investigations showed the patient to be homozygous for c.1530G>A in exon 9 of *MTHFR*. This variant is not predicted to result in an amino acid change in the MTHFR protein, but because it changes the last nucleotide of exon 9, there was suspicion that it may affect splicing; this was confirmed by cDNA sequencing from cultured skin fibroblasts (Burda et al. 2015)

The patient continued on betaine treatment and the vitamin B12 was changed to oral administration (previously intramuscular). The folate treatment was changed to mefolinate 15 mg/day and a repeat CSF 5-MTHF level at 23 months of age was 18 nmol/L. The mefolinate has since been increased to 45 mg/day and the CSF 5-MTHF level has risen to 33 nmol/L.

Following insertion of the VP shunt, his breathing pattern, responsiveness and feeding improved. There was also an improvement on imaging, with reduction in size of the lateral and third ventricles and resolution of periventricular oedema. More clinical improvement was seen 1 month following surgery as he was able to fix and follow with no strabismus or nystagmus. Although there was some progression of development, his head circumference remained below the 0.4th centile and developmental milestones continue to be delayed. He sat without support at 18 months of age, crawled at 2 years and walked at 3.5 years. He acquired his first words at approaching 5 years of age. He has cortical visual impairment and has now developed an intermittent divergent squint. He also requires feeding via a gastrostomy.

Patient 3

Patient 3 is a female born by normal vaginal delivery at term with a birth weight of 2.8 kg. She is the third child of consanguineous (first cousin) parents. Her older siblings and parents are all healthy. There were concerns regarding microcephaly and development from an early age, as she could only sit unsupported at 1 year of age, stood without support at 18 months and walked with support at 5 years. By 5 years of age, she had not developed any expressive language. There were also reports of possible seizures at 8 months of age so she was commenced on sodium valproate.

At 5.5 years of age, she presented with a 6-week history of lethargy and poor feeding and required intubation due to deterioration with hypothermia and respiratory depression. Initial investigations were performed, and an MRI brain showed diffuse generalised atrophy of cerebral hemispheres, cerebellum, and brainstem, with reduced volume of both grey and white matter. Blood tests revealed a raised total homocysteine of 256 μmol/L and a methionine of 0 μmol/L. Urine organic acid analysis was normal and CSF 5-MTHF was undetectable. Subsequent studies on cultured skin fibroblasts confirmed showed MTHFR activity to be approximately 1 % of controls.

She was commenced on treatment with betaine (200 mg/kg/day), hydroxycobalamin (10 mg/day po) and folic acid (5 mg/day). Her level of consciousness improved over the next few weeks, but she required a tracheostomy and was ventilator dependent for 4 months. CSF 5-MTHF remained undetectable on folic acid and after being changed to calcium folinate 15 mg/day. She was then switched to mefolinate 15 mg/day, and a repeat CSF 5-MTHF was measurable with a level of 18 nmol/L, 7 months after presentation. The mefolinate has since been gradually increased to 45 mg/day.

On review 9 months after presentation, she was able to vocalise, use her hands and mobilise on her knees. Her muscle tone and reflexes were mildly increased in her arms and markedly increased in her legs. Her vision and hearing were normal. The sodium valproate was stopped at this time and there have been no concerns regarding seizures. On review at 7 years, she was able to take a few steps with the assistance of a walking frame. She was also able to say her name and demonstrated some understanding of language.

Discussion

The main treatment used to treat severe MTHFR deficiency is betaine, with the aim to increase CSF methionine levels and reduce blood homocysteine levels. However, despite betaine working to increase the plasma methionine levels, it crosses the blood-brain barrier poorly (Kempson et al. 2014). It is suggested that an increase in blood methionine and *S*-adenosylmethionine, as a result of betaine treatment, may provide a sufficient supply of methionine and *S*-adenosylmethionine to the CNS for methylation reactions and that providing this is the case the developing brain may be tolerant to low 5-MTHF (Strauss et al. 2007). Indeed, some patients with severe MTHFR deficiency diagnosed and treated early with betaine may do reasonably well (Diekman et al. 2014). Unfortunately long-term outcome in most other patients is poor, as demonstrated in our report by patient 2, who developed hydrocephalus, and by patient 3 who was diagnosed late. Diekman (Diekman et al. 2014) reviewed the published literature on the outcome of patients with MTHFR deficiency treated with betaine. They found that of the 36 patient included in their study, only those five patients treated early with betaine had satisfactory outcome both in terms of growth and cognitive development. However, the number of early treated patients reported by Diekman is small, and the period of their follow-up was only 1–3 years. Clayton et al. (1986) concluded that defective methyl folate metabolism is the key to neurological damage in this condition and that treatment should be directed towards maintaining methionine turnover as well as a supply of folate to the CNS. If folate levels within the brain remain very low, there may still be a risk of demyelination/subacute degeneration occurring later despite treatment with betaine. Consequently, it would seem appropriate to provide treatment with a form of folic acid that will both increase brain 5-MTHF in addition to using betaine to increase blood levels of methionine and *S*-adenosylmethionine that may cross the blood-brain barrier.

As demonstrated in our case reports, folic acid can be given in various forms and these have varying effects on CSF 5-MTHF levels. Commercial synthetic folic acid is slowly converted to tetrahydrofolate by dihydrofolate reductase and may inhibit the uptake of 5-MTHF into the brain and cause further clinical deterioration (Clayton et al. 1986). Calcium folinate is rapidly converted to tetrahydrofolate, but it can only cross the blood-brain barrier following further metabolism to 5-MTHF, a reaction that requires MTHFR activity (Levitt et al. 1971).

Schiff et al. reported six children with MTHFR deficiency (Schiff et al. 2011) One patient who previously had mild cognitive delay presented at the age of 11 years with an abrupt neurological deterioration. In this child CSF 5-MTHF increased to 15.5 and 10.4 nmol/L with oral 5-MTHF at 45 mg/day but subsequently on folinic acid at 20 mg/day still managed to maintain a level of 19 nmol/L. Since this patient had relatively late-onset disease, it is likely that he had some residual MTHFR activity that was able to covert some THF to 5-MTHF, hence the limited response to folinic acid treatment.

Although we were only able to achieve CSF 5-MTHF levels between 18 and 33 nmol/L with oral 5-MTHF in our patients, this may be sufficient. A patient diagnosed at 4 years with congenital folate malabsorption, a disorder in which there is folate deficiency and impaired transport of folate into the CNS, had at the time of diagnosis undetectable levels of CSF 5-MTHF (Torres et al. 2015). With twice daily intramuscular folinic acid, CSF levels increased to between 18 and 46 nmol/L. At 7 years of age, she had reached all developmental milestones and had a normal MR brain scan. The authors concluded that these levels of CSF 5-MTHF may be enough to eradicate CNS disease.

In conclusion, folic acid and calcium folinate both appear to be ineffective in increasing CSF levels of 5-MTHF in severe early-onset MTHFR deficiency. Treatment should include oral 5-MTHF as calcium mefolinate, rather than other forms of folic acid, and relatively high doses are required.

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Take-Home Message

In severe methylenetetrahydrofolate reductase deficiency, measurable 5-methyltetrahydrofolate in cerebrospinal fluid is only achieved with mefolinate (5-methyltetrahydrofolate) supplements and not with either folic acid or folinic acid**.**

Compliance with Ethics Guidelines

Conflict of Interest

Dr. Linzi Knowles, Dr. Andrew Morris and Prof. John Walter declare that they have no conflict of interest.

Informed Consent

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000 (5). Informed consent was obtained from all patients for being included in the study.

This manuscript details the results of investigations and treatment that were undertaken as part of routine clinical care and not as part of a research study. Consent for treatment and routine investigations was obtained from the parents of all patients reported in this article.

Details of the Contributions of Individual Authors

All authors contributed equally to the planning, conduct and reporting of the work described in the article.

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