Cerebral folate deficiency and CNS inflammatory markers in Alpers disease

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Article history:
Received 2 August 2009
Received in revised form 19 August 2009
Accepted 19 August 2009
Available online 22 August 2009

Keywords:
Mitochondria
Folate–antibodies
Epilepsy
Neopterin, Cytokines
POLG

Abstract
We describe a 3.5-year-old female with Alpers disease with a POLG genotype of p.A467T/p.G848S and with a lethal outcome. Laboratory investigation revealed elevated CSF neopterin, IL-6, IL-8, IFN-γ, reduced CSF 5-methyltetrahydrofolate (5MTHF), and increased serum as well as CSF folate receptor blocking autoantibodies. Treatment with oral Leucovorine (5-formyl-tetrahydrofolate) was initiated at 0.25 mg/kg bid, and later increased to 4 mg/kg bid. Under treatment CSF levels of 5MTHF, seizure frequency and communicative abilities improved. Over a time span of 17 months, CSF levels of IL-6 and IFN-γ decreased, levels of folate receptor blocking autoantibodies continued to raise, whereas CSF IL-8 remained elevated 1500-fold above normal. The child died without apparent stress at the age of 5.5 years.

Alpers disease, a neurodegenerative disease usually presents in the first years of life as a progressive encephalopathy with multifocal myoclonic seizures, developmental regression, cortical blindness and early death. The underlying genetic defect has been attributed to mutations of the catalytic subunit of the mitochondrial DNA polymerase-γ leading to an organ-specific mitochondrial DNA depletion syndrome with reduced activity of respiratory chain enzyme complexes in the brain and the liver. A curative therapy is not available. This case report of Alpers disease provides new insights into the pathophysiology of Alpers disease, where mitochondrial dysfunction in conjunction with inflammatory cytokines and blocking folate receptor autoantibodies may lead to a secondary cerebral folate deficiency syndrome. The treatment of the latter provides relief to the patient without stopping the underlying disease.

Introduction

Alpers disease (OMIM 203700), [1] a spongiform cerebral degenerative disease affects the cerebral cortex, the cerebellum, the basal ganglia and the brain stem. The incidence of Alpers disease is estimated between 1/100,000 and 1/250,000. The affected patients present with a developmental retardation followed by regression, intractable epilepsy, movement disorders, abnormal respiration and at times cortical atrophy and liver dysfunction [2]. Biochemical findings can disclose a dysfunction of the citric acid cycle due to reduced activity of respiratory chain enzymes attributable to mutations of the gene encoding the mitochondrial DNA polymerase enzyme (POLG) [3]. There are so far no biochemicals or inflammatory markers reported and there is no known effective therapy. Valproate as an anticonvulsant drug should be avoided since it is known to induce hepatic failure. Here, we report on novel biochemical findings in a girl with Alpers disease complicated by cerebral folate deficiency and potential treatment options with folinic acid.

Materials and methods

Case report

A 3½-year-old girl presented with acute onset of status epilepticus and somnolence. EEG activity was severely suppressed with focal spike-waves over the temporal regions. The status was initially interrupted by the use of a combination of anticonvulsant drugs but re-emerged in the following days and months and could at times only be suppressed by anesthetic agents.

The girl is the only child of healthy unrelated parents. No progressive neurological diseases are known in the wider family. At the age of 30 months the girl was diagnosed with mental and
motor retardation of about 8–12 months. A video taken by the local pediatrician at age 2½ years showed an ataxia with dysmetria and muscular hypotonia. Her IQ was estimated at a value of 75. At the age of 2 years she had developed simple speech, her weight and height curves never surpassed the 3rd percentile. She had no specific dysmorphic features nor increased susceptibility to infectious diseases. The child died without apparent neurodegeneration at the age of 5.5 years. An autopsy was not obtained.

**Neuroimaging**

Cranial computer tomography (CCT) on day two of the acute disease was compatible with a generalized brain edema, whereas magnetic resonance imaging (MRI) of the CNS at the same time showed no abnormalities of either white or grey matter. A second MRI 3 months after disease onset showed mild cerebral atrophy and an 8 × 11 mm ischemic lesion in the left thalamus.

**Laboratory investigations**

Laboratory or skin examinations excluded the following diseases: sphingolipidoses, gangliosidoses, disorders of glycolysis, ceroid lipofuscinosis type 1 and 2, Laron disease, myoclonic epilepsy with ragged red fibers, and pyridoxine/pyridoxal-5-phosphate-dependent epilepsy. Normal laboratory values were obtained for liver enzymes, coagulation parameters, lactate, lactate–pyruvate ratio, vitamin B12, homocysteine, folic acid (serum), organic acids (urine), amino acids (urine, serum) acylcarnitines (dried blood), neopterin, and biotin (urine). Ammonia was increased intermittently up to three times the normal values (143 μmol/L; normal: 10–48 μmol/L). Cerebral spinal fluid (CSF) analysis was normal for white blood count, amino acids, bacterial or viral infections. Lactate and protein were elevated (lactate: 3.1 mmol/L; normal: 0.8–2.3 mmol/L, protein: 2.9 g/l, normal: 0.1–0.3 g/l).

Neurotransmitter metabolites and folates in CSF were investigated as described previously [4]. CSF and folate receptor (FR) autoantibodies of the blocking type were measured in CSF and plasma [5].

**Results**

**CSF investigations**

Neurotransmitter and folate analysis of CSF showed a markedly decreased 5-methyltetrahydrofolate (5MTHF) concentration, in the presence of increased neopterin, interleukin-6 (IL-6), interleukin-8 (IL-8) and interferon-γ (IFN-γ) (Table 1).

Folate receptor (FR) autoantibodies of the blocking type were present at week 32 and week 52 in serum (0.34 pmol/mL, 0.98 pmol/mL; normal: <0.2 pmol/mL) and at 52 weeks in CSF (1.2 pmol/mL; normal: <0.2 pmol/mL) (Table 1). The two metabolites of dopamine and serotonin homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5HIAA) as well as 3-α-methyl-dopa (3OMD) were at the upper normal range or even increased at all times, independently of the 5MTFH therapy.

Protein content in CSF wars repeatedly above expected values (1.3; 1.9; 2.9, normal: 0.1–0.3 g/L).

**Muscle biopsy**

Respiratory chain enzyme analysis performed on quadriceps muscle showed normal activities of complexes I, II, II + III, III, and IV compared to the mitochondrial marker enzyme citrate synthase (Friedrich Baur Institute, University of Munich, Germany). Mitochondrial DNA (mtDNA) quantification performed on the muscle biopsy material was not done.

**Epilepsy and electroencephalogram**

Repeated status epilepticus required invasive ventilation and at times the use of more than four anticonvulsant drugs simultaneously. Valproic acid as an anticonvulsant agent was not administered since an underlying metabolic disturbances could not be excluded and the EEG pattern with unilateral occipital rhythmic high-amplitude delta with superimposed polyspikes was suggestive of for Alpers disease [6].

**Molecular examination**

Molecular testing revealed a compound heterozygous mutation in the POLG-1 gene on chromosome 15q25. A missense mutation in the polymerase domain in exon 16 leading to p.G848S exchange (c.2542G > A) and a missense mutation in the linker region in exon 7 leading to p.A467T exchange (c.1399G > A) was identified. The first mutation was derived from the mother the latter from the father of the child, both being healthy. Both mutations have previously been described as compound heterozygosity in patients with Alpers disease [3].

**Treatment**

Faced with the deteriorating clinical situation of an intractable epileptic syndrome impressing as *epilepsia partialis continua*, refractory to common antiepileptic drugs, we asked for parental consent to administer pharmacologic doses of folic acid (5-formyltetrahydrofolate, Leucovorine) in order to compensate for the intracerebral folate deficiency. Treatment was started with a daily oral dose of 2 × 0.25 mg/kg body weight and later increased to 2 × 4 mg/kg/body weight.

A remarkable clinical improvement could be observed thereafter. After 2 weeks we could start to reduce the anticonvulsant treatment from four to two drugs. For 17 months under a combined anticonvulsant drug regime with Phenytoin and Vigi-

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<table>
<thead>
<tr>
<th>Weeks since disease onset at 3.5 years</th>
<th>5HIAA (nmol/L)</th>
<th>HVA (nmol/L)</th>
<th>3OMD (nmol/L)</th>
<th>5MTHF (nmol/L)</th>
<th>Neo (nmol/L)</th>
<th>Bio (nmol/L)</th>
<th>IL-6 (pg/mL)</th>
<th>IL-8 (pg/mL)</th>
<th>IFN-γ (pg/mL)</th>
<th>FRAA (pmol/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>CSF</td>
<td>Serum</td>
<td>CSF</td>
<td>Serum</td>
<td>CSF</td>
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<td>Serum</td>
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<td>Serum</td>
</tr>
<tr>
<td>8</td>
<td>406</td>
<td>1167</td>
<td>122</td>
<td>22.9</td>
<td>78.3</td>
<td>20.1</td>
<td>&gt;300</td>
<td>n.d.</td>
<td>0.7</td>
<td>n.d.</td>
</tr>
<tr>
<td>12</td>
<td>259</td>
<td>882</td>
<td>130</td>
<td>73.1</td>
<td>100.5</td>
<td>18.0</td>
<td>23.4</td>
<td>n.d.</td>
<td>1.3</td>
<td>n.d.</td>
</tr>
<tr>
<td>32</td>
<td>529</td>
<td>955</td>
<td>205</td>
<td>58.2</td>
<td>57.6</td>
<td>13.9</td>
<td>5.7</td>
<td>n.d.</td>
<td>0.2</td>
<td>0.98</td>
</tr>
<tr>
<td>52</td>
<td>402</td>
<td>1121</td>
<td>145</td>
<td>20.5</td>
<td>84.5</td>
<td>15.0</td>
<td>7.5</td>
<td>152</td>
<td>0.2</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Normal: 2–4 years</td>
<td>105–299</td>
<td>211–871</td>
<td>&lt;50</td>
<td>63–111</td>
<td>9–30</td>
<td>10–30</td>
<td>&lt;3.1</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>&lt;0.2</td>
</tr>
</tbody>
</table>

* Under folic acid substitution; n.d., not determined; 5HIAA, 5-hydroxyindoleacetic acid; HVA, homovanillic acid; 3OMD, 3-α-methyl-dopa; 5MTHF, 5-methyltetrahydrofolate; Neo, neopterin; Bio, biotin; IL-6, interleukin-6; IL-8, interleukin-8; IFN-γ, interferon-gamma; FRAA, folate receptor autoantibodies.
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at 1500 times the expected value (Table 1).

On the clinical side we witnessed an improvement of communication skills of the child. Whereas before there was no interaction possible between the parents and their daughter, a prompt re-

sponse of the child could be observed when the child was spoken to or touched by the parents. The girl was able to indicate physical discomfort such as a full bladder. Cortical blindness seemed much less apparent, swallowing difficulties were diminished. At the age of 5½ the girl passed away without an apparent deterioration.

During folic acid supplementation follow-up investigations showed a rise in CSF FR-autoantibody titers coinciding with a de-

crease of 5MTHF levels prompting us to increase the folic acid doses. At disease onset IL-6 was increased 3000 times the expected value and almost approached normal values in the course of the disease, whereas IL-8 still remained elevated with an increment at 1500 times the expected value (Table 1).

Discussion

Although the understanding of the molecular defects of Alpers disease has increased over recent years, the exact relationship be-
tween the molecular defect and the phenotype remains very com-
plex. Clayton described in 1982 how the 16569-bp human mitochondrial genome is replicated and repaired by DNA polymerase-γ (POLG) [7]. The first pathogenic mutations of POLG were de-
scribed in 2001 in families with autosomal dominant chronic progressive external ophthalmoplegia [8]. Tissue-specific mtDNA deletions were later identified as causes of deficiencies in mito-
ochondrial oxidative phosphorylation [9].

Different and overlapping disease entities ranging from psychi-
 atric disturbances to primary neurological diseases and endocrine disturbances have been associated with POLG mutation. Mutations in POLG gene account for approximately 90% of the classical pheno-
type of Alpers disease [10]. One in 50 people are thought to be si-
 lent heterozygote carriers of POLG mutations.

Most cases with childhood onset Alpers disease are associated with at least one mutation in the linker region of the POLG gene and one in the polymerase domain. The most common disease mutation in POLG associated with Alpers disease, as in our patient, is the G-to-A mutation at nucleotide 1399 in exon 7 of POLG caus-
ing an alanine to threonine amino acid substitution at codon 467. In the Belgian population the p.A467T mutation has been observed at a frequency of 0.6% without showing clinical signs [6].

In Alpers disease the p.A467T mutation is either homozygous or paired in trans with other mutations in POLG. In the case of com-
 pound heterozygosity there is a polymerase activity of only 4% comparing to the wild-type DNA [11]. It is thus the nature of the mutation in the second POLG allele in compound heterozygous pa-
tients that influences the severity and type of the disease. More prolonged disease courses with less severe symptoms have been described and seemed to be associated with a homozygous muta-
tion [10,12].

The symptomatic epilepsy in Alpers disease is usually refractory to common antiepileptic drug treatment. Epileptic status is often the terminating event. The use of valproic acid as an anticonvul-
sant drug has repeatedly led to a terminal hepatic failure and should be avoided [13].

The natural course of the disease is progressive with death occurring in most cases within 6–12 months after the onset of the disease. A gene dosage effect is thought to be an important
determinant for age of onset, the severity, and course of the disease.

We justified giving 5-formyltetrahydrofolate (calcium folinate) in the form of Leucovorin taking the following into consideration: Folic acid is a water soluble vitamin that is converted into various reduced forms involved in synthesis of purine and pyrimidine bases, methylation of genomic DNA, conversion of homocysteine to methionine, transfer of methyl-groups, and formation of the ac-
tive methyl-group donor S-adenosylmethionine, used in various methylation reactions. Furthermore folates exert a direct influence on brain function through the enhanced biosynthesis of phospho-
 lipids and hence myelination.

The physiological plasma folate form 5MTHF is actively trans-
ferred across the blood–brain barrier by a folate receptor medi-
ated endocytotic process in choroid epithelial cells and reaches 2–4-fold higher concentration in the spinal fluid as compared to serum.

A balanced diet usually provides sufficient quantities of folate to the CNS. However, the presence of folate receptor autoantibodies, oxidative stress and secondary inflammatory responses are able to block folate transfer across the choroid plexus to the CNS. Such primary and secondary forms of cerebral folate deficiency (CFD) with an encephalopathic symptomatology have extensively been described in the infantile-onset CFD syndrome [14], mitochondrial encephalopathies [15], Kearns-Sayre syndrome [16], Rett syn-
drome [17,18], and Aicardi-Goutières syndrome [19]. The use of antifolate drugs in rare inborn errors of metabolism like deficiency of dihydropteridine reductase, methylenetetrahydrofolate reduc-
tase, aromatic aminoacid decarboxylase and 3-phosphoglycerate dehydogenase should be excluded [14].

The low CSF levels of 5MTHF in our patient can be explained by blocked transport across the blood–brain barrier due to the pres-
ence of FR autoantibodies as well as by increased utilization and catabolism of 5MTHF due to oxidative stress [16]. Another expla-
nation may be a defective transport due to ATP depletion in the choroid plexus, as seen in Kearns-Sayre syndrome.

Of interest are the relative high concentrations of neurotrans-
mittor metabolites 5HIAA, HVA, and 3OMD in CSF. Both serotonin and catecholamines metabolism are intimately tied to both mito-
ochondria and inflammation. An inflammatory event with IFN-γ stimulation is known to lead to both increased tryptophan degra-
dation (via indoleamine dioxygenase) and neopterin production (via GTP cyclohydrolase I) [20].

The substitution with high doses of folic acid will increase 5MTHF plasma concentrations even in the presence of blocking FR autoantibodies facilitating thereby an increased uptake of 5MTHF into the CSF compartment via the folate receptor. To treat the cerebral folate deficiency we have chosen an initial oral dose of 2 x 0.25 mg/kg body weight Leucovorine and later adapted the

dose to reach age specific levels of CSF 5MTHF. We refrained from further augmenting the dose, since it is known that high folic acid doses can decrease blood levels of anticonvulsant drugs such as Phenobarbital and thereby potentially interfere with seizure con-
trol [21]. The medication was well tolerated and followed by a marked improvement of the child, though high CSF IL–8 levels re-
 minded of the persisting pro-inflammatory process.

Considering the possible mechanism of the observed effects, we speculate that depletion of folates within the CNS could lead to a partial repair of previously deranged folate-dependant pathways and mitochondrial dysfunction. To our knowledge in disorders of mtDNA deletions the role of folic acid substitution has not been evaluated. At the moment we do not know to what extent the pro-
duction of folate receptor autoantibodies in Alpers disease contribute to its pathophysiology, whether it represents a consistent finding or it is merely an epiphenomenon of the cerebral inflam-
atory response.
The unraveling of insufficient polymerase-γ activity and mitochondrial depletion as the underlying cause of Alpers disease has been followed by many case reports of the natural evolution of this devastating disease for which a treatment to alleviate symptoms is still lacking. Following published experience of the positive effects of increasing cerebral folate in those patients with a decreased CSF 5MTHF concentration we observed for a prolonged period, a remarkable clinical improvement after folinic acid substitution. Our encouraging experience provides an impetus to further clinical and laboratory studies.

Acknowledgments

This work was supported in part by The Swiss National Science Foundation Grant No. 3100A0-1199852/1 (to N.B.) and HD051880 from the National Institutes of Health, USA (to E.V.Q.).

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