Cerebral methylmalonic aciduria does not exist: a patient with sepiapterin reductase deficiency

NGGM Abeling1, M Duran1, HD Bakker1, AG van Cruchten1, AEM Stroomer1, N Blau2, B. Thöny2 and BT Poll-The1

1 Academic Medical Center, University of Amsterdam, Laboratory Genetic Metabolic Diseases, Depts of Pediatrics/Emma Children’s Hospital and Clinical Chemistry, Amsterdam, The Netherlands
2 Laboratory of Clinical Chemistry and Biochemistry, University Children’s Hospital Zürich, Switzerland

Abstract

Sepiapterin reductase (SR) deficiency was diagnosed in a 14 year-old girl, who was already known for 12 years with an aspecific form of mild methylmalonic aciduria (MMA-uria) and a progressive neurologic clinical picture.

The SR deficiency was revealed after re-investigation, which was performed because we questioned whether the MMA-uria could explain the neurologic picture and particularly because a movement disorder with dystonia had become more and more prominent in recent years.

CSF analysis indicated a severe overall biogenic amine neurotransmitter deficiency. A loading test with phenylalanine showed a high increase of plasma phenylalanine, followed by a sharp decrease after administration of BH4, indicating a defect of BH4 biosynthesis. An abnormal pterin profile and the demonstration of elevated sepiapterin in the CSF was highly suggestive of SR deficiency in fibroblasts and establishment of a new homoallelic mutation in the SR gene on chromosome 12.

Treatment with low dose L-DOPA and 5-hydroxytryptophan led to a rapid and spectacular clinical improvement. Although we formerly had found an elevated level of methylmalonic acid (MMA) in the CSF and tried to explain the neurologic picture this way, we now are convinced that ‘cerebral MMA-uria’ does not exist.

Introduction

Sepiapterin reductase (SR) deficiency is the most recent inherited defect in tetrahydrobiopterin (BH4) biosynthesis (1,2). In contrast to most other defects in BH4 metabolism, SR deficiency does not present with hyperphenylalaninemia and therefore cannot be detected by the neonatal phenylketonuria screening. The major symptoms of SR deficiency were mental retardation, dystonia with diurnal fluctuations, axial hypotonia and spasticity. All patients reported so far reacted favourably to treatment with oral supplements of L-DOPA and 5-hydroxytryptophan. We describe a 14 year old Dutch girl, born to consanguineous parents, with mild B12-unresponsive methylmalonic aciduria already diagnosed at 2 years of age. SR deficiency was diagnosed at the age of 14.
Materials and methods

The patient, L.R., a girl, had been admitted at 2 years of age because of psychomotor retardation and hypotonia. Metabolic screening of urine and plasma revealed only a mild MMA-uria. Investigations in fibroblasts had shown decreased uptake of (14C) propionate, but normal MMA-CoA mutase and normal cobalamin metabolism. In CSF a small but significant amount of MMA was detected. Despite treatment with a protein-restricted diet, which normalised the MMA-uria, progressive neurologic features, including axial hypotonia, spastic paresis, cerebellar dysfunction and myoclonic movements had occurred. The girl became wheelchair-bound at the age of 6 years. In the last few years a mild dystonic component with diurnal fluctuation became more and more apparent and now at the age of 14, triggered the investigation of neurotransmitters in CSF.

Oral loading was performed with 100 mg/kg of L-phenylalanine and samples drawn at baseline and 1, 2 and 4 h after administration (3). At 4 h BH4 (20 mg/kg) was administered and additional blood samples drawn 3 and 7 h following BH4.

Biochemical analyses

The various metabolite analyses for the diagnosis were performed using established RP-HPLC methods with electrochemical (biogenic amine metabolites) or fluorometric (pterins) detection, or tandem-mass spectrometry (for phenylalanine).

Biogenic amine metabolites and pterins were measured in CSF and urine. Pterins, i.e. BH4 and neopterin, were separated after iodine oxidation and detected at 350/450 nm (excitation/emission). This implies the measurement of the sum of tetrahydrobiopterin, dihydro-biopterin and biopterin. Sepiapterin and other yellow fluorescing pterins were detected using a Jasco fluorimetric detector at 425/530 nm (excitation / emission).

Fibroblast studies

Cell culturing, neopterin and biopterin production in fibroblasts after stimulation with cytokines for 24 h, and SR activity measurement in non-stimulated fibroblasts were performed as described elsewhere (4).

DNA mutation analyses

Mutation analyses of the SR gene were performed in DNA isolated from blood samples of the index patient L.R. and of both parents.

Results and discussion

Diagnosis

CSF analysis (table 1) revealed very low levels of the biogenic amine neurotransmitter metabolites, and normal values of the neurotransmitter precursors L-DOPA, 5-hydroxytryptophan and of 3-O-methyl-DOPA. Oxidized neopterin and biopterin were mildly elevated in CSF, while urine pterins were normal.

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Patient L.R.</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>HVA</td>
<td>76</td>
<td>&gt;148</td>
</tr>
<tr>
<td>5-HIAA</td>
<td>&lt;10</td>
<td>&gt; 68</td>
</tr>
<tr>
<td>MHPG</td>
<td>&lt;10</td>
<td>&gt; 28</td>
</tr>
<tr>
<td>3-OMD</td>
<td>30</td>
<td>&lt; 50</td>
</tr>
<tr>
<td>DOPA</td>
<td>17</td>
<td>&lt; 25</td>
</tr>
<tr>
<td>5-HTP</td>
<td>2</td>
<td>&lt; 10</td>
</tr>
<tr>
<td>Neopterin</td>
<td>26</td>
<td>&lt; 20</td>
</tr>
<tr>
<td>Biopterin</td>
<td>55</td>
<td>&lt; 30</td>
</tr>
</tbody>
</table>

Table 1: Neurotransmitter metabolites and pterins in CSF (in nmol/l). Abbreviations: HVA, homovanillic acid; 5HIAA, 5-hydroxyindoleacetic acid; MHPG, 3-methoxy-4-hydroxyphenylethylene glycol; 3-OMD, 3-O-methyl-DOPA; DOPA, 3,4-dihydroxyphenylalanine.

Hyperphenylalaninemia (HPA) had never been observed, but a Phe loading test caused plasma Phe to rise steeply and stay elevated at > 800 µM for 4 hours, followed by a rapid BH4-induced normalisation (Fig.1). Further investigation of pterins revealed a clear elevation of sepiapterin in the CSF (11 nmol/L, ref n.d.). Sepiapterin could also be detected in the urine (2.3 µmol/l; ref n.d.). These biochemical findings were fully consistent with SR deficiency. In cytokine-stimulated fibroblasts biopterin was severely decreased (17.5 pmol/mg; ref. 158-303), while neopterin was increased (199 pmol/mg; ref. 18-98), which was in accordance with findings in the earlier described SR-deficient patients (3).
SR activity in fibroblasts was severely decreased (18.0 µU/mg protein; ref. 99-185), confirming the SR deficiency. Finally the establishment of a new, homoallelic mutation P163L on exon 2 of the SR-gene in the patient, and heterozygosity in both parents completed the diagnosis.

Treatment and follow-up

Treatment was started with low dose (1 mg/kg/day) L-DOPA and 5-hydroxytryptophan, and induced a rapid and spectacular clinical improvement with respect to strength, energy and mood. A slow but sustained further improvement was observed in the following months and after one year of treatment she is now able to walk short distances with support, perform complex functions like playing computer games, and is bright-tempered.

Discussion

Our patient apparently was affected with two metabolic defects, which seem to be unrelated. The consanguninity of the parents may have been a contributing factor.

The diagnosis of SR deficiency at the age of 14 years had been delayed by an earlier diagnosis of an atypical form of methylmalonic aciduria on the age of 2 years.

However, at the time of the initial ‘diagnosis’ neurotransmitter defects were not yet very well known. Moreover, SR deficiency is not detectable in urine or plasma, which are the usual materials investigated for a metabolic screening. The finding of elevated, though low in an absolute sense, MMA in CSF, was the only metabolic abnormality to possibly explain the purely neurologic clinical presentation, which is quite unusual in classic MMA-uria.

MMA levels measured in CSF of our patient were around 20 µM, which is far below the levels added to rat brain slices (2.5 mM) in experiments suggesting neurotoxic effects of MMA as such in classical MMA-uria patients (5).

Hyperphenylalaninemia does not occur in SR deficiency. Our approach using the novel combined Phe / BH4 loading test has clearly shown the limited peripheral Phe-oxidising capacity when the patient is stressed metabolically.

In SR-deficiency the BH4 depletion as well as the accumulation of dihydrobiopterin and sepiapterin are thought to exert various pathogenic effects in the cerebral compartment, and so far seem to lead to a rather comparable neurologic picture in SR-deficient patients (3).

After the establishment of SR-deficiency we now can conclude, that this disorder is much more likely to explain the neurologic picture than the MMA-uria, and does not justify the hypothesis of a condition to be called cerebral MMA-uria. Treatment with L-DOPA and 5-hydroxytryptophan was (partially) successful. It remains to be investigated, whether additional supply of BH4 to the patient would be of any benefit.

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REFERENCES


ADDRESS FOR CORRESPONDENCE

N.G.G.M. Abeling
Academic Medical Center, University of Amsterdam
Laboratory Genetic Metabolic Diseases, F0-224
Meibergdreef 9
1105 AZ Amsterdam
Tel.: +31-2056665904
Fax: +31-206962596
E-mail: n.g.abeling@amc.uva.nl