

-
4. Blau, N., Thöny, B., Cotton, R.G., Hyland, K. Disorders of tetrahydrobiopterin and related biogenic amines. Chapter 78 in: Scriver, C.R. et al: *The metabolic and molecular bases of inherited disease*. Vol II, 8th ed, McGraw-Hill Companies, 2001.
 5. Fahn, S. The spectrum of levodopa-induced dyskinesias. *Ann. Neurol.* 47, Suppl 1: S2-S11, 2000.
 6. Nutt, J.G. Clinical pharmacology of levodopa-induced dyskinesia. *Ann. Neurol.* 47, Suppl 1: S160-S166, 2000.
 7. Tanaka, Y., Matsuo, N., Tsuzaki, S., Araki, K., Tsuchiya, Y., Niederwieser, A. On-Off phenomenon in a child with Tetrahydrobiopterin deficiency due to 6-pyruvoyl tetrahydropterin synthase deficiency (BH₄ deficiency). *Eur. J. Pediatr.* 148 (5), 450-452, 1989.
 8. Birnbacher, R.; Scheibenreiter, S.; Blau, N.; Bieglmayer, C.; Risch, H.; Waldhauser, F. Hyperprolactinemia, a tool in treatment control of tetrahydrobiopterin deficiency: endocrine studies in an affected girl. *Pediatr. Res.* 43 (5), 472-477, 1998.

ADDRESS FOR CORRESPONDENCE

Stefan Vlaho MD
Department of Pediatric Neurology
Johann Wolfgang Goethe-University
Theodor-Stern-Kai 7
60596 Frankfurt/Main, Germany
Tel.: 0049-(0)69-6301-6482
Fax: 0049-(0)69-6301-5765
e-mail: stefan.vlaho@kgu.de

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, which was further confirmed by measurement of enzyme activity 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, dihydrobiopterin 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.

Metabolite	Patient L.R.	Controls
HVA	76	>148
5-HIAA	<10	> 68
MHPG	<10	> 28
3-OMD	30	< 50
DOPA	17	< 25
5-HTP	2	< 10
Neopterin	26	< 20
Biopterin	55	< 30

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.

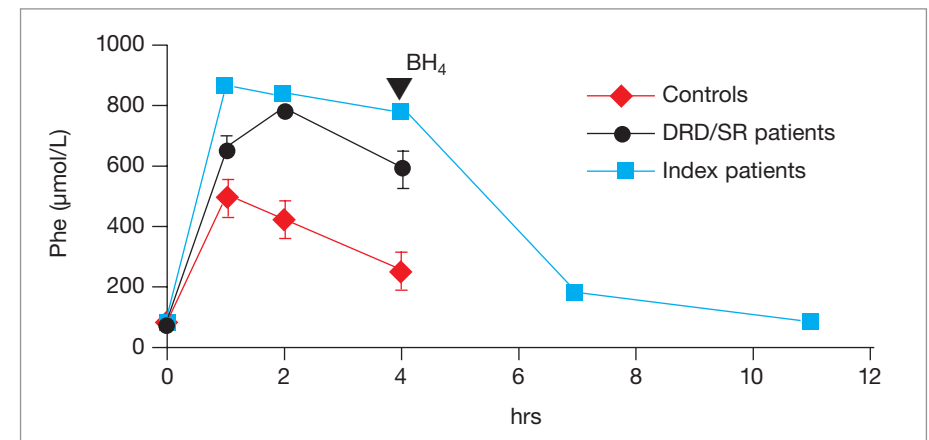


Fig. 1: Loading test with phenylalanine (100 mg/kg body weight) resp. BH₄ (20 mg/kg body weight)

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 μ U/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 consanguinity 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 μ M, 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.

Acknowledgements

This work is supported in part by the Swiss National Science Foundation grant no. 31-66953.01 (to NBS).

REFERENCES

1. Bonafé L, Thöny B, Penzien JM, Czarnecki B, Blau N. Mutations in the sepiapterin reductase gene cause a novel tetrahydrobiopterin-dependent monoamine neurotransmitter deficiency without hyperphenylalaninemia. *Am J Hum Genet* 69: 269-277, 2001.
2. Elzaouk L, Osmani H, Romstad A, Friedman J, Maccolin M, Thöny B, Blau N. Sepiapterin reductase deficiency: Molecular analysis in a new case presenting with neurotransmitter deficiency without hyperphenylalaninemia. In: Milstien S, Kapatos G, Levine RA, Shane B, editors. Chemistry and Biology of Pteridines and Folates. Norwell: Kluwer Academic Publishers, 2002: 277-284.
3. Blau N, Bonafé L and Thöny B. Tetrahydrobiopterin deficiencies without hyperphenylalaninemia: diagnosis and genetics of dopa-responsive dystonia and sepiapterin reductase deficiency. *Mol Genet Metab* 74:172-85:2001, doi: 10.1006/mgme.2001.3213.
4. Bonafé L, Thöny B, Leimbacher W, Kierat L, Blau N. Diagnosis of DOPA-responsive dystonia and other tetrahydrobiopterin disorders by the study of biopterin metabolism in fibroblasts. *Clin Chem* 47: 477-485, 2001.
5. De Mattos-Dutra A, De Freitas MS, Schroder N, Zilles AC, Wajner M and Pessoa-Pureur R. Methylmalonic acid reduces the in vitro phosphorylation of cytoskeletal proteins in the cerebral cortex of rats. *Brain Res* 763: 221-31, 1997.

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.: +31-205665904
Fax: +31-206962596
E-mail: n.g.abeling@amc.uva.nl