Molecular Genetics and Metabolism 115 (2015) 157–160

Contents lists available at ScienceDirect

Molecular Genetics and Metabolism

journal homepage: www.elsevier.com/locate/ymgme

Urine sepiapterin excretion as a new diagnostic marker for sepiapterin reductase deficiency

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ARTICLE INFO

Article history:
Received 22 May 2015
Received in revised form 22 June 2015
Accepted 22 June 2015
Available online 25 June 2015

Keywords:
Sepiapterin reductase deficiency
Sepiapterin
Tetrahydrobiopterin biosynthesis

ABSTRACT

Sepiapterin reductase deficiency (SRD) causes depletion of biogenic amines in the brain, early onset motor disorder, and intellectual disability. The diagnostic marker for this rare disease is increased sepiapterin and biopterin in CSF. Through a new analytic methodology we demonstrated accumulation of sepiapterin in urine of four SRD patients several times greater than that found in healthy controls and carriers, regardless of age or treatment. Our findings suggest a new interpretation of current theories of peripheral pterin metabolism and provide a new non-invasive diagnostic tool for children with early onset cryptogenetic developmental delay and/or movement disorder.

1. Introduction

Sepiapterin reductase (EC 1.1.1.153) deficiency (SRD, OMIM#182125) is a rare inherited disorder of the synthesis of the cofactor tetrahydrobiopterin (BH_4) due to mutations in the *SPR* gene. SRD results in reduced activity of aromatic amino acid hydroxylases in the brain with secondary depletion of serotonin and dopamine [1,2]. Unlike other defects of BH_4 metabolism, SRD is not associated with hyperphenylalaninemia, and therefore cannot be detected by newborn screening. Prompt clinical diagnosis, necessary to prevent mental disability and restore normal motor development relies on assessment of biogenic amines and pterins (including sepiapterin) in CSF, or on *SPR* gene sequencing. CSF sampling is invasive and, as such, may potentially bias ascertainment towards the most severely affected subjects or delay diagnosis until a more severe brain derangement becomes evident. With the present study we showed that sepiapterin (Sp) is increased in the urine of patients with SRD and may be reliably detected by the analytic method we developed.

2. Methods

2.1. Patients

Table 1 summarizes clinical features, neurotransmitter precursor treatment (L-DOPA/carbidopa), and genotype of four SRD patients enrolled in the study. All were previously published [2–4]. Cases 3 and 4 were fraternal twins. In three (cases 2–4) urine was collected during treatment. Several urine samples were collected and examined in patient 1 before and during treatment.

Urine from the parents of 3 cases (cases 1, 3 and 4) were also examined. Forty-three normal subjects (mean age 5 years; range 2 months–19 years) were enrolled as controls.

2.2. Materials and methods

Sp was obtained from Schircks Laboratories (Jona, Switzerland). All reagents were purchased from VWR International. Agilent Technologies 1200 HPLC instrument including a microvacuum degaser, a binary pump, a 99-positon cooled auto sampler, heated column, a spectrofluorescence detector and a HPLC Chemstation was used. The excitation and emission wavelengths were 425 and 530 nm respectively. The

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http://dx.doi.org/10.1016/j.ymgme.2015.06.009
1096-7192/

Table 1
Genotype, clinical features, and Sp levels in urine of SR deficient patients.

Pt no	Genotype	Age (y/m)	Sex	Age at onset	Symptoms at presentation	Age at diagnosis y/m	Age at urine examination y/m	Sepiapterin concentration in urine[a]	Therapy	Ref
1	c.448>G c.751>T	2/4	F	3 months	Generalized rest tremor, oculogyric crises, oral dyskinesias, progressive developmental delay	5 m	5 m 7 m 10 m 11 m 17 m 19 m	974 996 886 807 593 509	No treatment L-DOPA/carbidopa; 5-hydroxytryptophan Idem Idem Idem Idem	[3]
2	c.448A>G p.Gln206Alafs55[a]	8/0	M	First few months of life	Developmental delay with generalized hypotonia, dystonic postures of upper limbs	2.5 y	8 y	813	L-DOPA/carbidopa; 5-hydroxytryptophan	[2]
3	c.448A>G c.751A>T	18/6	F	First few months of life	Irritability, gastro esophageal reflux and poor sleep noted first few days of life. Generalized hypotonia and global developmental delay	14 y	18 y 4 m	669	L-DOPA/carbidopa; 5-hydroxytryptophan	[2,4]
4	c.448A>G c.751A>T	18/6	M	First few months of life	Irritability, gastro esophageal reflux and poor sleep noted first few days of life. Generalized hypotonia and global developmental delay	14 y	18 y 4 m	298	L-DOPA/carbidopa; 5-hydroxytryptophan	[2,4]

y/m: years, months.
[a] (μmol/mol creatinine); reference value: 0.0 to 101.7 (mean ± SD: 30.3 ± 19).

column was Spherisorb S5 ODS1250 × 4.6 mm I.D., 5 μm particle size, with an Agilent Technologies ODS Hypersil ODS guard column 20 × 4 mm I.D. The temperature of column compartment was set at 38 °C. A standard solution of Xanthopterin, the most prominent yellow

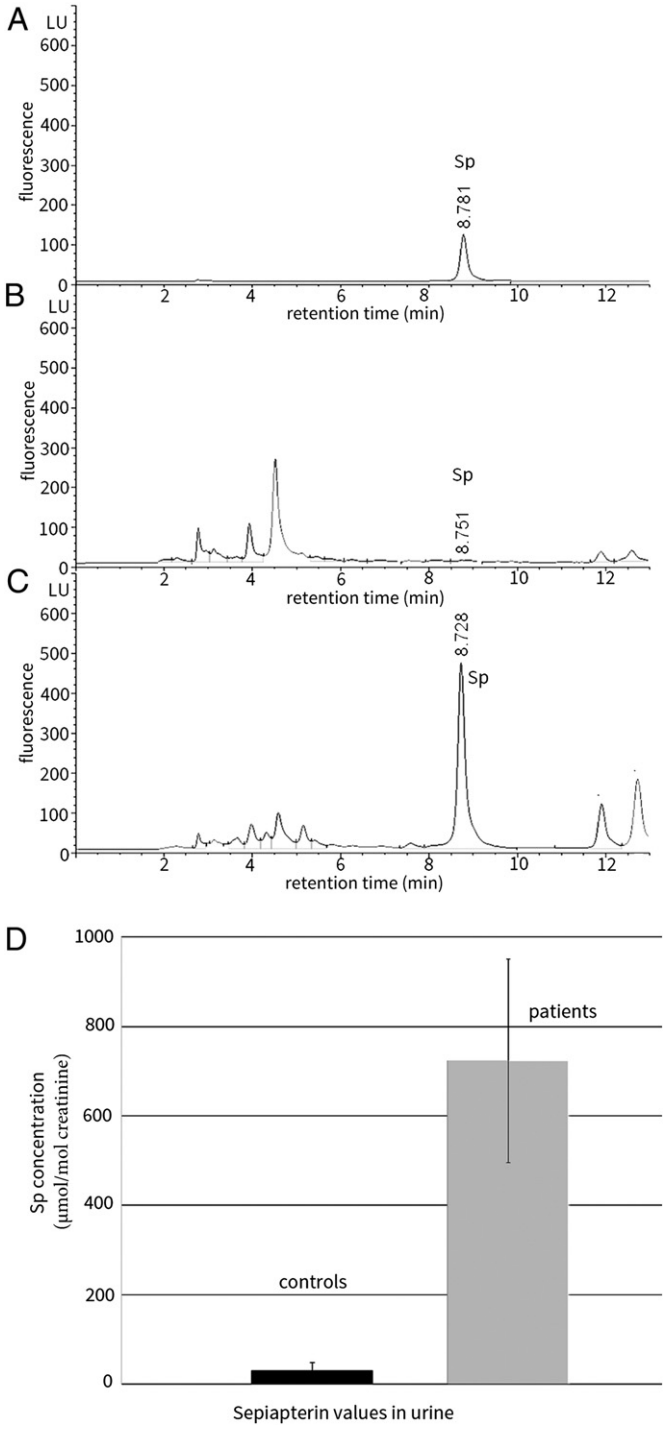

Fig. 1. HPLC analysis of Sp in standard solution and urine. Chromatograms of a standard solution containing Sp 500 nmol/l (A), urine obtained from a normal subject (Sp 3.5 μmol/mol creatinine) (B) and urine from a patient (case 2) affected by SRD (Sp 813 μmol/mol creatinine) (C). The increase of Sp in patient's urine is clearly evident among several signals of unknown compounds. In panel D Sp values (mean ± SD: 727 ± 230 μmol/mol creatinine) detected in SRD patients are compared with those of controls (mean ± SD: 30.3 ± 19 μmol/mol creatinine). Mean patients' age at collection was 5.6 years (range: 5 months–18 years) and mean controls' age at collection was 5.4 years (range: 2 months–19 years).