

Sepiapterin reductase deficiency in a 2-year-old girl with incomplete response to treatment during short-term follow-up

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Summary Sepiapterin reductase (SR) catalyses the last step in the tetrahydrobiopterin biosynthesis pathway; it converts 6-pyruvoyl-tetrahydropterin (6-PTP) to BH₄ in an NADPH-dependent reaction. SR deficiency is a very rare autosomal recessive disorder with normal phenylalanine (Phe) concentration in blood

and diagnostic abnormalities are detected in CSF. We present a 16-month-old girl with SR deficiency. From the newborn period she presented with an adaptation regulatory disorder. At the age of 3 months, abnormal eye movements with dystonic signs and at 4.5 months psychomotor retardation were noticed. Since that time axial hypotonia with limb spasticity (or rather delayed reflex development), gastro-oesophageal reflux and fatigue at the end of the day has been observed. Brain MRI was normal; EEG was without epileptiform discharges. Analysis of biogenic amine metabolites in CSF at the age of 16 months showed very low HVA and 5-HIAA concentrations. Analysis of CSF pterins revealed strongly elevated dihydrobiopterin (BH₂), slightly elevated neopterin and elevated sepiapterin levels. Plasma and CSF amino acids concentrations were normal. A phenylalanine loading test showed increased Phe after 1 h, 2 h and 4 h and very high Phe/Tyr ratios. SR deficiency was confirmed in fibroblasts and a novel homozygous g.1330C>G (p.N127K) *SPR* mutation was identified. On L-dopa and then additionally 5-hydroxytryptophan, the girl showed slow but remarkable progress in motor and intellectual ability. Now, at the age of 3 years, she is able to sit; expressive speech is delayed (to 1 1/2 years), passive speech is well developed. Her visual-motor skills, eye-hand coordination and social development correspond to the age of 2 1/2 years.

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References to electronic databases: Phenylalanine hydroxylase: EC 1.14.16.1. Tyrosine hydroxylase: EC 1.14.16.2. Tryptophan hydroxylase: EC 1.14.16.4. Nitric-oxide synthase: EC 1.14.13.39.

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Abbreviations

3-OMD 3-*O*-methyldopa
5-HIAA 5-hydroxyindoleacetic acid
AR aldose reductase

BH ₂	dihydrobiopterin
BH ₄	tetrahydrobiopterin
CR	carbonyl reductase
DHFR	dihydrofolate reductase
DHPR	dihydropteridine reductase
GTPCH	GTP cyclohydrolase
HVA	homovanillic acid
L-dopa	levodopa
NOS	nitric-oxide synthase
PTPS	6-pyruvoyltetrahydropterin synthase
SR	sepiapterin reductase
TH	tyrosine hydroxylase
TPH	tryptophan hydroxylase

Introduction

Sepiapterin reductase (SR) deficiency is an autosomal recessive disorder with normal phenylalanine (Phe)

concentration in blood and cerebrospinal fluid (CSF) and cannot be detected through neonatal screening programmes for PKU. SR is one of the enzymes involved in BH₄ biosynthesis and regeneration (Blau et al 2001). SR catalyses the last step in tetrahydrobiopterin (BH₄) biosynthesis, converting 6-pyruvoyltetrahydropterin to tetrahydrobiopterin (BH₄) in an NADPH-dependent reaction (Fig. 1) (Hyland 1999; Pearl et al 2004).

In the alternative pathway, 6-pyruvoyltetrahydropterin is reduced to 6-lactoyltetrahydropterin (1'-oxoTHP) by aldose reductase (AR) and carbonyl reductase (CR). Carbonyl reductase converts 6-pyruvoyltetrahydropterin to both 1'-oxoTHP and 2'-oxoTHP (Fig. 1) (Hyland 1999).

In the salvage pathway CR and SR convert sepiapterin to dihydrobiopterin (BH₂), which is reduced further to BH₄ by dihydrofolate reductase (DHFR). In human brain BH₂ cannot be reduced further to

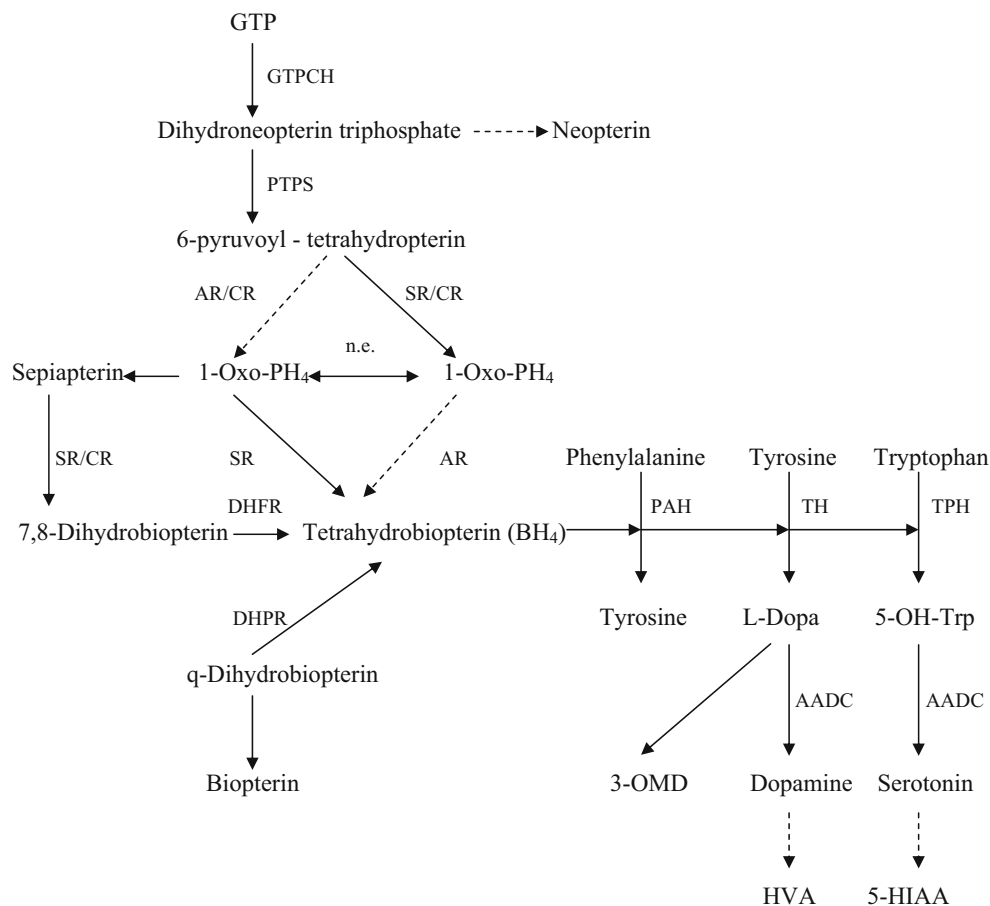


Fig. 1 Metabolic pathway of biogenic amines: phenylalanine hydroxylase (PAH), tyrosine hydroxylase (TH), tryptophan hydroxylase (TPH), homovanillic acid (HVA), 5-hydroxyindoloacetic acid (5-HIAA), 3-ortho-methyldopa (3-OMD), aromatic amino acid decarboxylase (AADC), and biosynthesis of tetrahydro-

biopterin: guanosine triphosphate cyclohydrolase (GTPCH), 6-pyruvoyl-triphosphate (6-PTPS), aldose reductase (AR), carbonyl reductase (CR), sepiapterin reductase (SR), dihydrofolate reductase (DHFR), dihydropteridine reductase (DHPR)

BH₄ because of low DHFR activity (Fig. 1) (Blau et al 2001).

BH₄ plays an essential role as the cofactor for phenylalanine hydroxylase (EC 1.14.16.1), tyrosine hydroxylase (TH; EC 1.14.16.2), tryptophan hydroxylase (TPH; EC 1.14.16.4) and nitric-oxide synthase (NOS; EC 1.14.13.39). Two enzymes—TH and TPH—are rate-limiting in the biosynthesis of dopamine and serotonin (Fig. 1). These monoamine (dopamine and serotonin) neurotransmitters play an important role in motor movements, sleep, thermoregulation, learning, memory and emotional behaviour (Hyland 1999; Surtees 1999).

SR deficiency causes increase of dihydrobiopterin (BH₂) and sepiapterin, which are competitive inhibitors of tyrosine and tryptophan hydroxylases. BH₂ and sepiapterin displaces pre-bound BH₄ from NOS, resulting in elevated superoxide anion and peroxy nitrite, a very neurotoxic anion (Blau et al 2001).

Materials and methods

Patient

This girl was the first child born to young, healthy Polish parents who have not declared consanguinity but who came from the same urban agglomeration at Zag³êbie (Silesia). There was no history of genetic disorders in the families. Pregnancy and delivery at 40 weeks of gestation were normal. Her birth weight was 3000 g, head circumference 35 cm, Apgar score 9. The neonatal period was normal. At about 3 months of age she developed episodes of prolonged upward rolling or horizontal eye movements and also tongue movements with episodic crying, without loss of consciousness, occurring many times a day. Since the 4th month of life psychomotor retardation with truncal instability and dystonic movements and postures have been observed. The symptoms exhibited diurnal variations, becoming worse at the end of the day. Excessive sleepiness with daytime napping was noted, but she had problems with initiating and maintaining sleep. She was always better after sleep.

On physical examination at the ages of 7 and 10 months her head circumference was normal; she was floppy, but her limbs were often stiff. Very brisk deep tendon reflexes, foot clonus and Moro reflex were observed. Postural reflexes and the righting reactions were absent. Ocular movements were initially misinterpreted as epileptic, but

video-electroencephalography (v-EEG) during sleep revealed no epileptiform discharges. During wakefulness, horizontal eye rotations to the right accompanied by beta activity over the right frontal region in v-EEG were observed. Brain MRI was normal. Her neurological status has worsened continuously.

Expanded metabolic screening performed at the age of 12 months was negative and further neurotransmitter study was suggested. The cerebrospinal fluid sample was taken according the standard protocol at the age of 16 months and stored at -20°C until further investigation.

At the age of 2 years the girl had poor head control, was unable to sit, and presented with hypotonia and dystonic movements. Inconsolable crying with upward rolling of eyes was frequent.

Biochemical analyses

The neurotransmitter metabolites homovanillic acid (HVA), 5-hydroxyindoleacetic acid (5-HIAA), 3-*O*-methyl dopa (3-OMD) and levodopa (L-dopa) were measured in CSF by high-performance liquid chromatography (HPLC) with electrochemical detection (Blau et al 2001). Dihydrobiopterin, biopterin and sepiapterin were separated using reversed-phase HPLC with fluorimetric detection (Howells et al 1986).

A phenylalanine loading test was performed by oral administration of Phe at a dose of 100 mg/kg per day. Blood samples were obtained before and 1 h, 2 h, 4 h and 6 h after Phe intake. Phe concentrations were measured by HPLC with UV-Vis detection, using a reversed-phase column, 3.9 mm × 300 mm, Waters, PicoTag system.

Sepiapterin reductase activity was measured in fibroblasts as described earlier (Bonafe et al 2001).

Molecular analyses

Molecular study of the *SPR* gene was performed according earlier described approach (Bonafe et al 2001). Genomic DNA of the proband and both parents was isolated from dry blood spot.

Results

Analysis of CSF completed at the age of 25 months revealed significantly decreased concentrations of

Table 1 Biochemical data before and during treatment

Metabolite	Before therapy	After 2 months of therapy (L-dopa/benserazide, 12 mg/kg per day)	After 5 months of therapy (L-dopa/benserazide, 20 mg/kg per day and 5-hydroxytryptophan, 10 mg/kg per day)	Control range
HVA (nmol/L) (CSF)	79.7	119	154	200–800
5-HIAA (nmol/L) (CSF)	12.3	<12.5	<5	100–400
3-OMD (nmol/L) (CSF)	nd	46	332	<50
L-Dopa (nmol/L) (CSF)	nd	nd	27	<25
Biopterin (nmol/L) (CSF)	24	nd	nd	10–30
BH ₂ (nmol/L) (CSF)	69	nd	nd	0.4–14
Sepiapterin (nmol/L) (CSF)	14.7	nd	nd	<0.5
Prolactin (ng/ml) (plasma)	15.3	nd	nd	2.7–19.7

nd, non determined

HVA and 5-HIAA, and normal 3-OMD. Dihydrobiopterin and sepiapterin concentrations in CSF were highly elevated, while biopterin concentration was normal (Table 1). Plasma and CSF amino acids levels were normal. The phenylalanine loading test showed a significant increase of phenylalanine after 1 h (525.3 μ mol/L; control 103.6 μ mol/L). Phenylalanine/tyrosine ratio after 1 h was 11.06 (control 1.46) (Lopez-Laso et al 2006; Sauders-Pullman et al 2004).

Plasma prolactin concentration was normal (15.3 ng/ml; control range 2.7–19.7) (Table 1).

In fibroblasts, SR activity was severely decreased (14 mU/mg protein; controls 99–185).

Molecular analysis of the *SPR* gene in the patient revealed a novel mutation g.1330C>G (p.N127K) in homozygous state. Data on its expression and activity are not available. Both parents were shown to carry the p.N127K mutation on one allele.

Treatment

The patient has been treated since the age of 26 months with levodopa/benserazide. The doses of L-dopa have been slowly increased from 12 up to 20 mg/kg per day. After 5 months, 5-hydroxytryptophan was added with doses slowly increased from 10 up to 16 mg/kg per day. An increase of dopamine metabolites and HVA concentrations was noted. However, the 5-HIAA concentration remained very low (Table 1) (Pearl et al 2004).

After 2 months of therapy, the patient was able to sit and evident progress in motor and intellectual ability was observed. She demonstrated a transient worsening of the neurological status (mainly during

infection), especially in the intensity of oculogyric crises and dystonic posturing of the limbs. Excessive sleepiness subsided.

The results of neuropsychological assessment in the 3-year-old patient after 11 months of treatment with L-dopa and 5-hydroxytryptophan were as follows:

- Gross motor skills delayed (age range under 12 months).
- Visual-motor skills, eye–hand coordination delayed (age range 28 months).
- Good development of passive speech (age range 34 months).
- Expressive speech delayed (age range 18 months).
- Social development (age range 30 months).
- Emotional irritability and instability.
- On physical examination: hypotonia with brisk deep tendon reflexes and right foot clonus, preserved body-righting and equilibrium reactions, with isolated and small-amplitude choreic movements.

Discussion

Tetrahydrobiopterin deficiencies form a group of rare inherited neurological diseases that lead to monoamine neurotransmitter deficiency with or without hyperphenylalaninaemia (HPA). Sepiapterin reductase (SR) deficiency is especially rare. So far 23 patients have been identified worldwide (excluding 7 with Malta's founder mutation) (source www.biopku.org/dbsearches/BIODEF_Start.asp). Most of the SR-deficient probands carry novel ('private') homozygous mutations.

Diagnosis of SR deficiency, like other dopa-responsive dystonias presenting without HPA, is difficult because the disorder cannot be detected through neonatal screening programmes for PKU. The condition can be recognized only by analysis of pterins and neurotransmitter metabolites in CSF. The diagnosis is usually delayed. Our patient is one of the youngest identified up to now.

Analysis of CSF showed very low concentration of HVA and 5-HIAA, normal biopterin and elevated dihydrobiopterin and sepiapterin. The normal concentration of biopterin and elevated BH₂ are explained by the existence of the *salvage pathway* in the BH₄ biosynthesis with the additional two enzymes carbonyl reductase and SR (Blau et al 2001; Hyland 1999).

All 23 reported patients with SR deficiency have been tabulated in the BIODEF database (http://www.bh4.org/BH4_databases_biodef.asp). All patients presented mainly with dopa-responsive dystonia with diurnal variation (Abeling et al 2006; Bonafe et al 2001; Echenne et al 2006; Friedman et al 2006; Neville et al 2005; Verbeek et al 2008).

Except for one, all the patients with SR deficiency started with symptoms in the first year of life. In the majority of cases the gross motor and cognitive skills were delayed. The truncal hypotonia, dystonic movements, stiffness of limbs, eyes rolling up and diurnal fluctuations were frequent. Under levodopa treatment the muscle tone normalized and oculogyric crises disappeared. Our patient had clinical features rather similar to those of previously described patients, but for the first time we extended the case study by neuropsychological assessment after 11 months of treatment. It is noteworthy that response to treatment may be incomplete, as in the described patient. She suffered from hypersomnolence and problems with initiating and maintaining sleep. These problems disappeared after treatment. Hypersomnolence has previously been reported in only two patients (Friedman et al 2006). The horizontal eye movements also subsided. The upward rolling of eyes disappeared when L-dopa was given at a dose of 12 mg/kg per day.

The current clinical abnormality is remarkable gross motor retardation; at the age of 3 years the patient cannot walk independently. Social, emotional and passive speech development are well preserved.

Our patient on treatment, similarly to earlier described patients, has demonstrated transitory worsening mainly during infections: weakness, intensification of oculogyric crises, and dystonic posturing of the limbs (Abeling et al 2006; Echenne et al 2006; Pearl 2006; Pearl et al 2004).

Conclusion

The diagnosis and treatment of SR-deficient patients is still a challenge for the majority of paediatricians because of the low number of cases, limited access to diagnostic methods and lack of personal experience. The two-step approach for such very rare diseases, with centralized metabolic CSF neurotransmitter screening at the national level and the verification step at the European level, seems very reasonable and useful in practice. Such an approach should help to achieve satisfactory early detection and treatment of all newborn affected infants with SR deficiency.

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