First patient in Serbia with biochemically and genetically diagnosed pyridoxine-dependent epilepsy

Prvi bolesnik u Srbiji sa biohemiji i genetički dijagnostikovanom piridoksin zavisnom epilepsijom

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Abstract

Introduction. Pyridoxine-dependent epilepsy (PDE) is a rare autosomal recessive inborn error of metabolism present with early-onset seizures resistant to common anticonvulsants. PDE has been shown to be caused by a defect of a \( \alpha \)-aminoadipic semialdehyde dehydrogenase (also known as ALDH7A1 or antiquitin) in the cerebral lysine degradation pathway. Its deficiency results in accumulation of \( \alpha \)-aminoadipic semialdehyde (\( \alpha \)-AASA), piperidine-6-carboxylate and piperolic acid, which serve as diagnostic markers in urine, plasma and cerebrospinal fluid of the disease. \( \alpha \)-Aminoadipic semialdehyde dehydrogenase is encoded by the ALDH7A1 or antiquitin gene. Diagnosis is confirmed by molecular-genetical analysis.

Case report. We present a first patient in Serbia who was diagnosed clinically, biochemically and genetically. We suspected PDE due to drug-resistant seizures in the seventh day of life when we attempted with pyridoxine. Since that time the patient has taken pyridoxine and the seizures have not recurred. Our patient had markedly elevated \( \alpha \)-AASA in urine while on treatment with individual dosages of pyridoxine. Molecular-genetical analysis identified mutations of the ALDH7A1 (antiquitin) gene. Diagnosis is confirmed by molecular-genetical analysis and pyridoxine withdrawal is no longer needed to establish the diagnosis of „definite” PDE.

Key words: epilepsy; infant, newborn; vitamin B6; genetic diseases, inborn; diagnosis, differential; drug therapy; Serbia.

Introduction

Pyridoxine-dependent epilepsy (PDE) is an autosomal recessive disease which occurs in 1 in 100,000 to 700,000 individuals. At least 100 cases have been reported worldwide.

1. Typically, patients present with neonatal seizures, but atypical manifestations up to 3 years of age, as well as transient response to common anticonvulsants or poor initial response to pyridoxine have been reported. 2–4. Until recently, a definite diagnosis of PDE had been established worldwide.

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upon a successful therapeutic trial with pyridoxine and further proof of pyridoxine after a controlled withdrawal with recurrence of seizures. Recently, PDE has been shown to be caused by a defect of α-aminoadipic semialdehyde dehydrogenase (also known as ALDH7A1 or antiquitin, ATQ), in the cerebral lysine degradation pathway and to catalyze the conversion of α-aminoadipic semialdehyde (α-AASA) to α-aminoadipic acid. α-AASA is in chemical equilibrium with piperideine-6-carboxylic acid (P6C). P6C has been shown to inactivate pyridoxal phosphate (PLP), the active vitamer of pyridoxine, by a Knoevenagel condensation reaction, leading to a severe secondary PLP deficiency. As PLP is a cofactor of various enzymes in the central nervous system, seizures in PDE are more probably due to a perturbation in metabolism of cerebral amino acids and neurotransmitters.

Biochemically, ALDH7A1 or ATQ deficiency is characterized by the accumulation of α-AASA and P6C and by the accumulation of piperideine-6-carboxylic acid (PA), which is formed proximally to the primary enzyme defect. Screening for ATQ deficiency is possible via determination of urinary or plasma α-AASA and P6C, and of plasma PA. The diagnosis is confirmed by molecular analysis.

Molecular analysis has revealed mutations of the ALDH7A1 or ATQ gene in all individuals with PDE and increased PA and/or α-AASA in plasma or urine. Pyridoxine therapy is life-long, but despite treatment many patients with PDE have a disorder of psychomotor development. Gathering evidence on the usefulness of lysine restricted diet is in progress. We report the first patient in Serbia with biochemical and genetically diagnosed classic form of PDE.

Case report

A 6-day old boy was referred to our Neonatal Unit from a maternity hospital due to recurrent episodes of seizures with shrieking, starting within the first days of life. The patient was born to a 23-year-old mother by spontaneous vaginal delivery after an uneventful pregnancy. A full term male neonate had 3,900 g, a length of 57 cm and head circumference 37 cm (97th percentile). At birth, his Apgar scores were 9 and 9, at 1 and 5 min, respectively. He was the first child of healthy parents without known consanguinity. In addition, there was no family history of epilepsy or neurological disorders.

The baby was incapable of tolerating oral feedings. The baby seizures were complex: flexion of the limbs, twisting and jerking of the body and limbs, jerks of facial muscles with blinking, and all accompanied by occasional screams. When applied anticonvulsants (phenobarbital and/or midazolam), the response was short-lived and seizures were repeated. The first electroencephalography (EEG) performed after a seizure that lasted 6 minutes showed a discharge of high voltage bizarre spike-wave complexes and multiple spikes. His lactate level was elevated (up to 12.7 mmol/L, normal < 2 mmol/L) during seizures and normal in the periods without seizures. His ammonia level was 28 μmol/L (normal 42–144 μmol/L), while urine ketones were negative. All other biochemical analyses (glycemia, calcium, magnesium, sodium, potassium) and ultrasound examinations did not indicate the cause of drug-resistant seizures. After about 24 hours, an attempt was made to stop drug-resistant seizures with intravenous iv pyridoxine at a dose of 100 mg. Apnea did not occur during the administration of pyridoxine. EEG monitoring was not performed simultaneously due to technical reasons. He became seizure-free after the first dose. We continued with the administration of iv pyridoxine, 15 mg/kg/day over the next 7 days, and then moved on to oral administration.

Measurement of urinary α-AASA by electrospray ionisation tandem mass spectrometry under pyridoxine treatment was performed at the Biochemistry Department, Institute of Child Health in London. Urinary α-AASA level was markedly elevated (206.46 mmol/mol creatinine; normal at age < 6 months: < 2.0 mmol/mol creatinine). To make gene analysis and establish the cause of seizures we used the patient’s DNA. The result of genetic analysis was performed at the Regional Molecular Genetic Laboratory at Great Ormond Street Hospital for Children in London. Sequence analysis for mutations associated with PDE identified that the patient was a heterozygous for the c.328 C > T mutation in exon 4 of the ALDH7A1 gene resulting in the substitution of arginine with stop codon at amino acid position 110 (p.Arg110*) and a heterozygous single transversion of G > T at a highly conserved donor splice site in intron 17 (c.1566-1 G > T). Both mutations have been reported previously in patients with PDE.

After 3 weeks, the baby was discharged from the hospital in a good clinical condition with oral pyridoxine (50 mg/day). Five months after starting pyridoxine, the patient’s EEG was normal in drowsy and sleep states. His neurological examination showed normal head growth, truncal hypotonia, and mildly decreased peripheral tone in the extremities. At 12 months of age the patient’s neuropsychological testing was done. According to Brunet Lézin-scale development, the overall index was 83 units, i.e. at the level of a 10-month-old infant.

Discussion

Clinical course of events in our patient suggests a classic form of PDE. Seizures occurred in the first days of life. After admission at our Neonatal Unit anticonvulsants were administered, but the attacks were repeated. After 24 hours, pyridoxine was applied, and the seizures ceased completely. During the 12 months of life on pyridoxine treatment, neither seizures have been reported nor was EEG recorded. To make sure that late and masked response is not missed, treatment with oral/enteral pyridoxine should be continued until ALDH7A1 or ATQ deficiency is excluded by negative biochemical and/or genetic testing.

For diagnostic workup of neonates and infants with therapy-resistant seizures, many previous studies recommended serum or/and urine samples for the determination of α-AASA and PA regardless of a therapeutic trial with pyridoxine. While α-AASA is a pathognomonic marker of defects in the ALDH7A1 or ATQ gene, as illustrated by our pa-

Jesić MM, et al. Vojnosanit Pregl 2017; 74(6): 594–597.
tient, PA has the disadvantage of also being elevated secondarily in liver disease and peroxisomal defects like Zellweger syndrome.\(^8,9\) In contrast, \(\alpha\)-AASA is the primary substrate of the affected enzyme, ALDH7A1 or ATQ, and remains more markedly elevated than PA in plasma and urine in all patients while on pyridoxine. Levels of urinary \(\alpha\)-AASA in normal individuals decline during the first year of life: < 6 months: < 2.0 mmol/mol creatinine; 6–12 months: < 1.0 mmol/mol creatinine, > 1 year: < 0.5 mmol/mol creatinine. Pathological values for \(\alpha\)-AASA are several folds above the upper limit of the appropriate reference range and seem to depend on the nature of the mutation, and on the child’s age, treatment, and possibly on nutritional lysine intake. In our patient urinary \(\alpha\)-AASA level was markedly elevated. It is important to note that PA levels in urine normalize under treatment, and patients with mild missense mutations may have near normal PA levels in plasma while on pyridoxine.\(^3,4\)

Mutation analysis of the ALDH7A or ATQ gene is recommended to confirm the diagnosis. \(\alpha\)-AASA and/or PA are reliable markers to select PDE patients for molecular analysis of the ALDH7A or ATQ gene. To date more than 60 different mutations within the 18 exons of the ALDH7A or ATQ gene at chromosome 5q31 have been published\(^10–12\). Of these, 50–60% are missense mutations resulting in an altered amino acid in the protein sequence. A sequence analysis has shown that our patient is an apparent compound heterozygote for two mutations both of which are highly likely to be pathogenic. Furthermore, an intronic mutation, c.1566-1G > T, as that of our patient, may have an increased frequency among European patients\(^5\). Prenatal diagnosis by molecular analysis is feasible in forthcoming pregnancies.\(^3,10\)

If seizures subside after the administration of pyridoxine, instead of PA, the patients may have: folinic acid-responsive seizures (genetically identical to ATQ deficiency but not biochemically), pyridox(am)ine phosphate oxidase deficiency, neonatal/infantile hypophosphatasia (tissue-nonspecific alkaline phosphatase deficiency), familial hyperphosphatasia (phosphatidylinositol glycan anchor biosynthesis type V deficiency) and hyperplasminemia type II.\(^2,3\) Importantly, these affected patients with pyridoxine responsive seizures haven’t elevations in \(\alpha\)-AASA in plasma and/or urine. Detection of elevated levels of this organic acid is a sensitive marker for PDE. While \(\alpha\)-AASA was first thought to be a specific biomarker for PDE, recent research has demonstrated that \(\alpha\)-AASA is also elevated in patients with molybdenum cofactor deficiency (MoCoFa) and isolated sulfite oxidase deficiency (SOX)\(^14\). In patients with elevated levels of \(\alpha\)-AASA, these latter two conditions may be differentiated from PDE by measuring urinary sulfite/sulfo cysteine levels. MoCoFa and SOX deficiency are serious and often fatal diseases for which no effective therapy has been available until cyclopyranopterin was used successfully in a patient with MoCoFa\(^14,15\).

Conclusion

The diagnosis of PDE is not routine and requires significant involvement of medical personnel, so that the patient could receive appropriate treatment. Clinical diagnostic approach to PDE can now be replaced by measurement of reliable diagnostic marker. Our patient with possible PDE was diagnosed according to the elevated concentrations of urinary \(\alpha\)-AASA and did not undergo the risk of a diagnostic pyridoxine withdrawal. So far all published PDE patients with mutations of ATQ gene have had neonatal onset of seizures. Future studies are needed to investigate genotype-phenotype correlation of PDE.

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