ORIGINAL ARTICLE

Pyridoxine-dependent seizures in Dutch patients: diagnosis by elevated urinary alpha-aminoadipic semialdehyde levels

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Background: Pyridoxine-dependent seizures (PDS) is a rare, autosomal recessively inherited disorder. Recently α-aminoadipic semialdehyde (α-AASA) dehydrogenase deficiency was identified as a major cause of PDS, which causes accumulation of both α-AASA and pipecolic acid (PA) in body fluids.
Methods: We studied urinary and plasma α-AASA and PA levels in 12 Dutch clinically diagnosed patients with PDS.
Results: α-AASA was elevated in both urine and plasma in 10 patients. In these patients plasma PA levels were also elevated but urinary PA levels were normal.
Discussion: In all patients with clinically definite PDS, and in most patients with probable or possible PDS, the clinical diagnosis of PDS could be confirmed at the metabolite level. Non-invasive urinary screening for α-AASA accumulation provides a reliable tool to diagnose PDS and can save these patients from the classical and potentially dangerous pyridoxine withdrawal test to prove PDS.

Pyridoxine-dependent seizures (PDS) is a rare, autosomal recessively inherited disorder usually presenting very shortly after birth and in some cases in the womb. For 50 years PDS has been a clinical and biochemical conundrum which has puzzled physicians and scientists.[1] Plecko et al and Willemsen et al observed isolated pipecolic acid (PA) elevations in the plasma and cerebrospinal fluid of PDS patients, yet the biochemical relationship with pyridoxine metabolism remained unclear.[2–4] Recently, α-aminoadipic semialdehyde (α-AASA) dehydrogenase deficiency due to pathogenic mutations in the ALDH7A1 gene, was shown to be a major cause of PDS.[5] In mammals, the essential amino acid L-lysine is degraded via PA into the intermediate α-AASA, which is subsequently oxidised to L-2-aminoadipic acid, a reaction catalysed by the enzyme α-AASA dehydrogenase (EC 1.2.1.31, also named antiquitin) (fig 1). In PDS patients, the lack of α-AASA dehydrogenase leads to an accumulation of α-AASA and PA in body fluids. α-AASA is in spontaneous reversible equilibrium with piperideine-6-carboxylate (P6C) in the cytosol. Accumulated P6C irreversibly reacts with active pyridoxine, ie, pyridoxal-5-phosphate (P5P), by forming a Knoevenagel condensation product. This irreversible reaction causes a secondary deficit of P5P in affected children, which subsequently leads to epileptic seizures. Restoration of the P5P pool can easily be achieved by oral pyridoxine supplementation, which resolves the seizures.

Recently, we reported on the epidemiology and clinical features of PDS in the Netherlands in this journal.[6] In that paper the classical clinical criteria according to Baxter were used to establish the diagnosis of PDS. We therefore re-evaluated that series of PDS patients by measuring their levels of α-AASA and PA in urine and plasma.

METHODS

We re-evaluated all children (n = 11) with a diagnosis of definite, probable or possible PDS from a recently described Dutch cohort of 13 patients.[6] These patients and their parents were invited to visit our hospital and were informed of the novel insights into the pathophysiology of PDS. All except one patient (patient 11) underwent further diagnostic work-up by laboratory investigations of urine and blood. Furthermore, we were able to include a recently born sibling of patient 10 in the present study (patient 12).

α-AASA in urine and plasma was measured by liquid chromatography-tandem mass spectrometry as previously published.[5] Quantitative determination of PA in urine and plasma was performed by stable isotope dilution gas chromatography-mass spectrometry.[7]

RESULTS

Urine and/or blood samples were obtained from 11 patients. The results of α-AASA and PA measurements are given in table 1. α-AASA was elevated in the urine and plasma of 10 patients. PA in plasma was elevated in all patients with elevated (plasma and urine) α-AASA, while urinary PA concentrations were normal in all patients.

DISCUSSION

In this study, in all patients with a definite diagnosis of PDS according to the criteria published by Baxter,[8] α-AASA dehydrogenase deficiency could be proven at the metabolite level by demonstrating elevated concentrations of α-AASA (plasma and urine) and PA (plasma). The diagnosis was also confirmed in two out of three patients with probable PDS and in three out of four patients with possible PDS.

The diagnosis of probable PDS could not be confirmed in one patient (patient 6). She is a younger sister of a girl with a definite clinical diagnosis of PDS and a metabolically confirmed diagnosis of α-AASA dehydrogenase deficiency (patient 4). She had subtle neonatal seizures with only minimal epileptic discharges on a 24-h EEG, which responded to 100 mg of pyridoxine given intravenously. She is performing well at school. Her normal development makes a diagnosis of PDS unlikely since most PDS patients suffer from, at least a mild, encephalopathy with learning difficulties.[5] However, the nature of the neonatal seizure-like period remains unexplained. We advised a trial period of pyridoxine withdrawal but could not

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Abbreviations: α-AASA, α-aminoadipic semialdehyde; PA, pipecolic acid; PDS, pyridoxine-dependent seizures; P5P, pyridoxal-5-phosphate; P6C, piperideine-6-carboxylate
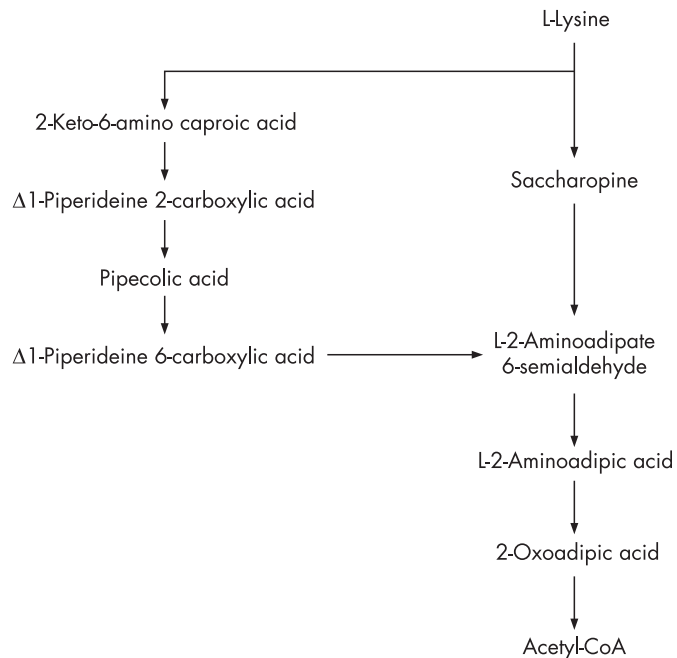
L-Lysine

Figure 1 Metabolic pathway of L-lysine.

convince the parents to stop treatment. DNA analysis of patients included in our study are pending.

In patient 11, originally diagnosed with possible PDS, pyridoxine was recently withdrawn without recurrence of seizures. We consider PDS a very unlikely diagnosis in this patient because of the above observation and the fact that the child is developing well. The parents did not want to cooperate with further metabolic investigations.

In our first report,[6] we described two patients (patients 12 and 13 in that paper) who did not meet the criteria for definite, probable or possible PDS. In both patients we have now demonstrated normal α-AASA concentrations in plasma and urine, as would be expected (data not shown).

This report is the first nationwide population-based study on metabolically confirmed PDS. Our results show that at least 10 children with PDS were born in the Netherlands between January 1991 and December 2004. As 2 764 697 children were born in the Netherlands during this time (adapted from http://statline.cbs.nl), the birth incidence of biochemically proven PDS in the Netherlands is at least 1: 276 000 children. This

study further shows that most patients, namely nine out of 11 (82%), were diagnosed correctly using the criteria proposed by Baxter. Thus, in circumstances where metabolic examination of α-AASA and/or PA is not possible, applying the clinical criteria proposed by Baxter seems a reliable method to establish a diagnosis of PDS.

The concentrations of α-AASA and PA, in urine as well as in plasma, vary considerably in patients with PDS. A remarkably wide range of α-AASA and PA levels in patients has also been found by Mills et al in their first report on α-AASA dehydrogenase deficiency in PDS.[5] We have no clear explanation for this wide range. Hypothetically it might reflect different levels of α-AASA dehydrogenase residual activity, dietary protein (L-lysine) intake, or the amount of supplemental pyridoxine. It is tempting to speculate that optimum treatment (ie, pyridoxine dosage) in PDS might be achieved by focusing on the concentrations of α-AASA and PA.

CONCLUSION

Metabolic investigations of urinary concentrations of α-AASA provide a reliable tool to prove PDS associated with α-AASA dehydrogenase deficiency at the metabolite level. The potentially dangerous trial of withdrawal of pyridoxine, classically used to prove PDS, can now be avoided. The novel insights into the pathophysiological processes that underlie PDS further provide us with tools to better estimate the true incidence of

Table 1 Results of α-AASA and PA measurements

Patient	Sibling	Birth year	PDS (Baxter criteria)	α-AASA urine (mmol/mol cr)	α-AASA plasma (μmol/l)	PA urine (mmol/mol cr)	PA plasma (μmol/l)	PDS (confirmed metabolically)
1	5	1991	Possible	16	8.0	0.11	6.5	Y
2		1992	Definite	4.7	0.9	0.27	5.8	Y
3	7	1992	Definite	29	5.7	0.00	4.6	Y
4	6	1992	Definite	4.0	1.1	0.02	7.0	Y
5	1	1993	Possible	24	5.0	0.11	5.0	Y
6	4	1994	Probable	0.2	<0.2	0.02	2.2	N
7	3	1995	Probable	20	5.8	0.01	5.1	Y
8		1998	Possible	12	2.4	0.07	5.5	Y
9		2001	Definite	9.6	0.8	2.0	22	Y
10	12	2003	Probable	39	6.1	0.08	7.8	Y
11		2003	Possible	NA	NA	NA	NA	N
12	10	2004		75	5.2	1.37	11	Y

N, no; NA, not available; Y, yes.
Control α-AASA concentrations are <0.2 μmol/l for plasma and <1 mmol/mol creatinine for urine.
Control values for PA in urine are 0.55–24.1 mmol/mol creatinine (<6 months of age) and 0.01–1.54 mmol/mol creatinine (>6 months of age).
For PA in plasma, control values are 3.75–10.8 μmol/l (<1 week of age), and 0.7–2.46 μmol/l (>1 week of age).

PDS (at least 1:276 000 newborns in The Netherlands according to this study) and will hopefully lead to an optimum treatment regime for this serious neurometabolic disorder.

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Epoprostenol for severe pulmonary hypertension

Average survival time with untreated idiopathic pulmonary hypertension is 2.8 years in adults and 10 months in children. Successful treatment with epoprostenol (prostacyclin) was reported for adults in 1996 and for children in 1999. Now a report from the Great Ormond Street Hospital for Children, London (*Heart*, published online 25 Oct 2006; doi 10.1136/hrt.2006.096412) has provided details of all 39 children treated there with continuous intravenous epoprostenol between 1997 and 2005.

The children (22 girls, 17 boys; age range 4–17 years, median 5.4 years) were all in WHO functional classes III and IV. Twenty five had idiopathic pulmonary arterial hypertension and 14 pulmonary hypertension associated with congenital heart disease (6), cardiomyopathy (3), connective tissue disease (2), chronic interstitial lung disease (2) and HIV infection (1). Epoprostenol was started if oral therapies had failed or there were severe symptoms at presentation. Parents were trained to prepare and administer the drug at home and training was given to community health staff.

Over a mean follow-up of 27 months, seven children died and eight underwent transplantation (four double lung, three heart and lung, one heart only). Cumulative survival at 1, 2, and 3 years was 94%, 90% and 84%, respectively. WHO functional class improved and the mean increase in 6-min walking distance was 77 m. Fourteen children needed additional drug therapy (bosentan in eight, sildenafil in five, and both in one) and 17 with syncope or pre-syncope had atrial septostomy. Epoprostenol therapy is effective in children.