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ORIGINAL ARTICLE

Pyridoxal 5′-phosphate may be curative in early-onset epileptic encephalopathy

G. F. Hoffmann · B. Schmitt · M. Windfuhr · N. Wagner · H. Strehl · S. Bagci · A. R. Franz · P. B. Mills · P. T. Clayton · M. R. Baumgartner · B. Steinmann · T. Bast · N. I. Wolf · J. Zschocke

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Summary Neonatal epileptic encephalopathy can be caused by inborn errors of metabolism. These conditions are often unresponsive to treatment with conventional antiepileptic drugs. Six children with pyridox(am)ine-5′-phosphate oxidase (PNPO) deficiency presented with neonatal epileptic encephalopathy. Two were treated with pyridoxal 5′-phosphate (PLP) within the first month of life and showed normal development or moderate psychomotor retardation thereafter. Four children with late or no treatment died or showed severe mental handicap. All of the children showed atypical biochemical findings. Prompt treatment with PLP in all neonates and infants with epileptic encephalopathy should become mandatory, permitting normal development in at least some of those affected with PNPO deficiency.

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G. F. Hoffmann
Department of General Pediatrics, Ruprecht-Karls-University Heidelberg, Heidelberg, Germany

B. Schmitt
Division of Clinical Neurophysiology, University Children's Hospital Zürich, Zürich, Switzerland

M. Windfuhr · N. Wagner · H. Strehl
Childrens Hospital Dortmund, Dortmund, Germany

S. Bagci · A. R. Franz
Department of Neonatology, Center for Pediatrics, University of Bonn, Bonn, Germany

P. B. Mills · P. T. Clayton
University College London Institute of Child Health, London, UK

M. R. Baumgartner · B. Steinmann
Divison of Metabolism and Molecular Pediatrics, University Children's Hospital Zürich, Zürich, Switzerland

T. Bast · N. I. Wolf
Department of Neuropediatrics, Ruprecht-Karls-University Heidelberg, Heidelberg, Germany

J. Zschocke (✉)
Institute of Human Genetics, Ruprecht-Karls-University Heidelberg, Heidelberg, Germany
e-mail: johannes.zschocke@med.uni-heidelberg.de

Abbreviations

PLP	pyridoxal 5′-phosphate
PNPO	pyridox(am)ine-5′-phosphate oxidase

Introduction

Epileptic seizures represent a frequent and serious emergency in neonates. Rarely, severe early-onset seizures are the predominant or exclusive manifestation of an inborn error of metabolism. Such seizures are often refractory to conventional treatment and the outcome is grim, e.g. in GABA-transaminase deficiency or glycine encephalopathy (nonketotic hyperglycinaemia).

In 1954 Hunt and colleagues were the first to describe a patient with a seizure disorder that was successfully treated solely by the administration of pyridoxine (vitamin B_6) and coined the term 'pyridoxine dependency' (Hunt et al 1954). It became good clinical practice to test for pyridoxine dependency in every child with 'difficult-to treat' seizures starting before 2 years of age. Recently the enzymatic defect was pinpointed to a piperideine-6-carboxylate dehydrogenase located in the CNS degradation pathway of lysine, which resulted in the accumulation of an intermediate scavenging pyridoxal phosphate (Mills et al 2006). The diagnosis may be confirmed by measurement of urinary α-aminoadipic

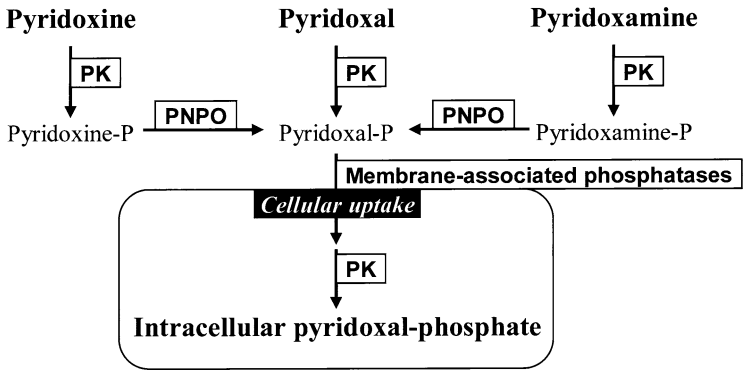

Fig. 1 Vitamin B_6 metabolism. Pyridoxal phosphate is synthesized from dietary pyridoxal, pyridoxamine and pyridoxine by pyridoxal kinase (PK) and pyridox(am)ine 5′-phosphate oxidase (PNPO). P = phosphate

semialdehyde. Elevated CSF and plasma pipecolic acid is also a marker for this inborn error of metabolism (Plecko et al 2000). A similar pathogenic mechanism is responsible for pyridoxal deficiency in hyperprolinaemia type II (Farrant et al 2001) and during treatment with the tuberculostatic drug isoniazid.

Very recently it was recognized that in some children epileptic encephalopathy may be resistant to pyridoxine but ceases with administration of pyridoxal 5′-phosphate (PLP) (Clayton et al 2003). It was shown that this condition is due to a genetic deficiency of pyridox(am)ine-5′-phosphate oxidase (PNPO) inherited as an autosomal recessive trait (Mills et al 2005). Without PNPO, pyridoxine and pyridoxamine cannot be converted into PLP, leaving exogenous pyridoxal/PLP as the only source of the active co-factor (Fig. 1). CSF and urine analyses in affected children show evidence of secondary deficiencies of several PLP-dependent enzymes including aromatic L-amino acid decarboxylase (resulting in decreased CSF concentrations of homovanillic acid, 5-hydroxyindoleacetic acid and 3-methoxy-4-hydroxyphenylglycol, and raised levels of L-dopa, 5-hydroxytryptophan and 3-*O*-methyldopa as well as increased urinary concentrations of vanillactic acid), threonine dehydratase (causing raised CSF threonine), glycine cleavage system (causing raised CSF glycine) and ornithine δ-aminotransferase (causing low CSF arginine). In addition, patients may display variable lactic acidaemia as well as a tendency to hypoglycaemia and raised CSF levels of histidine and taurine. All patients reported so far have been born prematurely (Clayton et al 2003; Mills et al 2005).

Unfortunately, PLP is not available as a licensed drug either in Europe or in the United States. Diagnosis in the past usually rested on the identification of the characteristic metabolic derangement in CSF, which so far has been found in all reported patients with PNPO deficiency. Nevertheless, in 2004 we started to use PLP in all infants with refractory epilepsy following a therapeutic trial of pyridoxine and folinic acid irrespective of diagnostic biochemical markers. This led to the identification of PNPO deficiency in six additional patients from three independent families, all with at least partly non-characteristic metabolic findings.

Patients

Patient 1, a girl, was born at 34 weeks of gestation, reportedly developed intractable seizures and died at 7 months of age. Detailed diagnostic efforts were unrevealing and Ohtahara syndrome was suspected. The father and mother were non-consanguineous of Lebanese and Iraqi origin, respectively; the father was not related to the third family described here.

Patient 2, sister of patient 1, was born at 37 + 5 weeks of gestation. Shortly after birth she developed repeated myoclonus and progressively more complex and frequent seizures with burst-suppression EEG that proved unresponsive to pyridoxine and various antiepileptic drugs. The child cried frequently as if in intractable severe pain, which was very straining for her carers. Detailed metabolic investigations were unremarkable except for an increase of threonine in CSF. CSF lactate was normal. A therapeutic breakthrough was achieved with PLP. The EEG immediately almost normalized, seizures ameliorated, and the girl awoke, stopped the frequent crying, started feeding, fixated and smiled and could be discharged home at 2 months of age. Molecular analysis in the girl revealed homozygosity for the mutation R95C (c.283C > T) in the *PNPO* gene and heterozygosity in the parents. This mutation has not been described before and affects a highly conserved amino acid. At the present age of 2 years 5 months she is developing well with moderate psychomotor delay and occasional seizures on 50 mg/kg/day PLP in six doses.

Patient 3, a boy, the first child of non-consanguineous parents, was born at 39 weeks of gestation. The mother reported rhythmic fetal movements in the 36th and 38th weeks of gestation. Family history was negative for epilepsy. At age 36 hours the otherwise normal newborn showed an episode of 15–20 minutes with eye deviation, intermittent prominent ocular convergence, grimaces, erratic movements and

multifocal myoclonic jerks associated with heavy crying, flushed appearance and diaphoresis. Similar episodes occurred over the next days and weeks. Additionally we observed long-lasting series of spasms, with crying and nasal eye deviation; and short episodes with abnormal eye movements, smiling, smacking or forehead frowning. Several long-term EEGs were normal, even during the spasms. Only one EEG showed subclinical left temporal spike wave discharges. MRI of the brain and metabolic studies in cerebrospinal fluid, plasma and urine including amino acids, organic acids and pipecolic acid were all normal. Phenobarbital, clonazepam, phenytoin and pyridoxine (up to 55 mg/kg) were not effective. Vigabatrin possibly suppressed the spasms. At age 28 days 200 mg/day PLP in four doses was introduced. In the next 24 hours short episodes of apnoea, sleepiness, muscle hypotonia and feeding difficulties occurred. Subsequently the boy normalized and antiepileptic medication could be discontinued without relapse. Withdrawal of PLP at age 7 months was followed by erratic movements, agitated eye movements, irritability and crying after 7 hours. The EEG, normal at first, showed generalized rhythmic fast activity over 2 minutes when the symptoms increased. After 200 mg PLP, symptoms resolved within about 20 minutes and the EEG normalized. At age 9 months testing by the Bayley Scales of Infant Development showed normal results (Psychomotor Developmental Index 101, reference range 100 ± 15). Because of rare breakthrough seizures (distress, agitation, rolling eyes) at the end of the 6 hours drug interval, PLP was increased from 60 to 100 mg/kg per day and divided into six doses. At the last visit (age 1.5 years) the boy was seizure free; only sleep was disturbed with frequent awakening. He was compound heterozygous for a missense mutation, D33V (c.98A > T), and a single base pair deletion, c.246delT, in the *PNPO* gene. CSF studies initially showed only increased levels of 3-methoxytyrosine and serine and slightly increased taurine and histidine. All of these parameters normalized after PLP substitution.

Patient 4's parents were first cousins of Lebanese origin. Two other children, a boy (*patient 5*, born at 36 weeks of gestation) and a girl (*patient 6*, born at 30 weeks of gestation), had died from neonatal-onset epileptic encephalopathy. Patient 4, a boy, was born spontaneously at 35 weeks of gestation with signs of fetal distress. He had treatment-resistant, pyridoxine-nonresponsive epilepsy with frequent seizures from the second day of life. Electroencephalograms showed generalized burst suppression. Cranial MR was normal at 6 weeks of age. Severe epilepsy persisted under various antiepileptic drug regimens and other treatments including ketogenic diet and ACTH, and his psychomotor development was severely retarded. He showed frequent attacks of painful crying which were interpreted as signifying abdominal pain by the parents but were associated with epileptic seizures. At the age of 3 years, a repeat MRI scan revealed severe diffuse cerebral atrophy. At this time, a therapeutic trial with PLP (35 mg/kg per day in 3 doses) was initiated. Twenty-three minutes after the first oral administration of PLP the EEG burst-suppression pattern changed to complete suppression for 90 minutes before alpha and theta activity resumed. PLP treatment resulted in markedly reduced seizure activity, albeit seizures did not stop completely. All three affected children in this family initially presented with lactic acidosis. Remarkably, detailed metabolic investigations including CSF analyses performed in patient 4 during infancy did not reveal any of the abnormalities reported as characteristic for PNPO deficiency. In patient 5, metabolic investigations including amino acids and metabolites of biogenic amines in CSF were also all normal. In patient 6, only a transient increase of threonine and glycine in CSF and a decrease of serotonin in plasma had been noted; homovanillic acid and 5-hydroxyindoleacetic acid in CSF were transiently increased but at no time decreased, as would have been expected. Mutation analysis in patients 4 and 6 identified homozygosity for the same mutation R95C found in the family of patients 1 and 2, and heterozygosity in the parents.

Discussion

In view of the rapid treatment success and in analogy to pyridoxine dependency we suggest calling this disorder pyridoxal phosphate dependency. Characteristic differences between these two conditions are listed in Table 1. We do not yet know the full clinical and biochemical spectrum of pyridoxal phosphate dependency, but it is much broader than initially suggested. Most likely, there are other children who are not born prematurely and have normal or inconclusive results in biochemical studies. Other prematurely born infants with PNPO deficiency will be misdiagnosed as hypoxic–ischaemic encephalopathy since an associated transient lactic acidosis would wrongly support this working hypothesis. Furthermore, biochemical features of reduced aromatic L-amino acid decarboxylase activity such as a decrease of serotonin in plasma or a decrease of homovanillic and 5-hydroxyindoleacetic acids in CSF, thought to be the most suggestive and characteristic findings (Brautigam et al 2002; Clayton et al 2003), may be only transiently present or absent altogether.

We suggest that in future, all neonates and infants with epileptic encephalopathy as well as prematurely born infants with hypoxic–ischaemic encephalopathy should receive a therapeutic trial with oral PLP (30 mg/kg per day in three doses for at least one day) in addition to pyridoxine (100 mg i.v. in a single dose, to be repeated and possibly increased to 500 mg in total) and folinic acid (3–5 mg/kg per day for two to three days; no therapeutic response was observed with pyridoxine or folinic acid in our patients). This test should be

Table 1 Comparison of characteristic clinical and biochemical features of pyridoxal phosphate dependency (PNPO deficiency) compared to pyridoxine dependency

	Pyridoxine dependency	Pyridoxal phosphate dependency
Pregnancy	Normal (*in utero* seizures)	Fetal distress frequent[a]
Prenatal seizures	Yes	Yes
Prematurity	Unusual	Frequent
Apgar score	Normal	Low
Perinatal complications	Mild distress possible	'Signs of asphyxia'
Lactate (blood, CSF)	Normal	May be elevated
Amino acids (blood, CSF)	Normal	Elevated glycine, threonine
Metabolite pattern suggestive of AADC deficiency[b]	No	Frequent (pseudo-AADC deficiency)
Pipecolic acid (CSF, blood)	Elevated	Normal
α-Aminoadipic semialdehyde (urine)	Elevated	Normal

[a]Fetal distress documented on the cardiotocogram in several of our patients is thought to represent PLP deficiency and intrauterine seizure activity rather than fetal hypoxia
[b]AADC deficiency = aromatic L-amino acid decarboxylase deficiency

carried out in conjunction with appropriate metabolic investigations in urine, blood and CSF but should not be delayed until the results of the biochemical tests have been returned. A single dose of PLP may not be conclusive as seizures can recur early and mask the positive response. The recommended dose of PLP may not be enough for some children, and to gain complete seizure control it may be necessary to increase PLP administration to four to six times per day. It is quite likely that PLP will also correct pyridoxine-dependent seizures due to piperideine-6-carboxylate dehydrogenase deficiency, and thus PLP could even be used as the first-line drug instead of pyridoxine in neonatal and infantile epileptic encephalopathy once it has become available as a licensed drug. Distinction between PNPO deficiency and piperideine-6-carboxylate dehydrogenase deficiency is possible by biochemical and molecular means in children who respond to PLP (Table 1).

Additional infants with epileptic encephalopathy as well as older infants and children with intractable seizures who responded to PLP but not to pyridoxine have already been described (Kuo and Wang 2002; Wang et al 2005). It remains to be seen whether these too are affected by PNPO deficiency. Further research will be necessary to investigate the full clinical and biochemical spectrum of this disorder and to optimize timing and dosage of therapy as well as prenatal therapy in families known to be at risk.

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