ELSEVIER

Bone 40 (2007) 1655–1661

BONE

www.elsevier.com/locate/bone

Case Report

Pyridoxine-responsive seizures as the first symptom of infantile hypophosphatasia caused by two novel missense mutations (c.677T>C, p.M226T; c.1112C>T, p.T371I) of the tissue-nonspecific alkaline phosphatase gene

Sara Baumgartner-Sigl [a], Edda Haberlandt [a], Steven Mumm [b], Sabine Scholl-Bürgi [a], Consolato Sergi [c], Lawrence Ryan [d], Karen L. Ericson [e], Michael P. Whyte [b], Wolfgang Högler [a,*]

[a] *Department of Pediatrics, Medical University Innsbruck, Austria*
[b] *Center for Metabolic Bone Disease and Molecular Research, Shriners Hospitals for Children, and Division of Bone and Mineral Diseases, Washington University School of Medicine, St. Louis, MO, USA*
[c] *Institute of Pathology, Medical University Innsbruck, Austria*
[d] *Division of Rheumatology, Department of Medicine, Medical College of Wisconsin, Milwaukee, WI, USA*
[e] *Department of Chemistry, Indiana University-Purdue University, Fort Wayne, IN, USA*

Received 22 September 2006; revised 30 January 2007; accepted 31 January 2007
Available online 14 February 2007

Abstract

Pyridoxine-responsive seizures (PRS) and the role of pyridoxine (PN, vitamin B_6) in hypophosphatasia (HPP) are incompletely understood. Typically, PRS and HPP are rare, independent, metabolic disorders. In PRS, seizures resist standard anticonvulsants apart from PN, yet have a good prognosis. In HPP, inactivation of the tissue nonspecific isoenzyme of alkaline phosphatase (TNSALP) impairs skeletal mineralization and causes rickets in infants that can be fatal. Here, we report a 7-month-old girl, newly diagnosed with infantile HPP, who presented as a neonate with PRS but without bony abnormalities. Analysis of biogenic amines in cerebrospinal fluid (CSF) suggested brain pyridoxal 5′-phosphate (PLP) deficiency, although PLP in CSF was not decreased. She had normal cognitive milestones but failure to thrive and rickets. Nearly undetectable serum ALP activity, elevated plasma PLP and urinary phosphoethanolamine (PEA) and inorganic pyrophosphate (PPi) levels, hypercalcemia, hypercalciuria and nephrocalcinosis were consistent with infantile HPP. Only prednisolone reduced serum calcium levels. Despite improved growth and weight gain, she developed rib fractures and died from respiratory failure at age 9 months. Sequence analysis of the TNSALP gene revealed novel missense mutations in exon 7 (c.677T>C, p.M226T) and exon 10 (c.1112C>T, p.T371I). Our patient demonstrated that PRS in neonates may not necessarily be "idiopathic"; instead, such seizures can be caused by severe HPP that becomes clinically apparent later in infancy. The pathophysiology of PRS in HPP differs from the three other genetic defects known to cause PRS, but all may lead to brain PLP deficiency reducing seizure thresholds. All reported HPP patients with neonatal seizures died within 18 months of birth, suggesting that PRS is an indicator of HPP severity and lethal prognosis. We recommend that assessment of any neonate with PRS should include measurement of serum ALP activity.

Keywords: Rickets; Vitamin B_6; Epilepsy; Phosphoethanolamine; Pyridoxal phosphate

Introduction

Pyridoxine-responsive seizures (PRS) has been recognized as a rare, autosomal recessive epilepsy for over 50 years [1,2]. Seizures usually occur within days after birth, are refractory to standard anticonvulsants and can be stopped only by pyridoxine (PN, vitamin B_6) therapy. Seizure reappearance upon PN withdrawal supports the diagnosis. The active metabolite (vitamer) of vitamin B_6, pyridoxal 5′-phosphate (PLP), is an essential coenzyme for synthesis of various neurotransmitters and biogenic amines. Therefore, the common explanation for PRS is that reduced PLP-dependent synthesis of neurotransmitters

* Corresponding author. Department of Pediatrics, Medical University Innsbruck, Anichstrasse 35, 6020 Innsbruck, Austria. Fax: +43 512 504 23484.
E-mail address: wolfgang.hoegler@i-med.ac.at (W. Högler).

8756-3282/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.bone.2007.01.020

lowers the seizure threshold. However, PN transport and metabolism appear normal in this heterogeneous disease [1,2].

Classical PRS maps to chromosomal locus 5q31.2–q31.3 (OMIM #266100) and is caused by deactivating mutations in *ALDH7A1*, the gene that encodes antiquitin, a dehydrogenase in the pipecolic acid pathway of lysine catabolism. Without adequate antiquitin activity, its substrate (Δ1 piperideine-6-carboxylate, P6C) accumulates and condenses with, and subsequently inactivates, PLP [3]. Two additional heritable defects in two other metabolic pathways cause PRS: (i) In hyperprolinemia type II, Δ1-pyrroline-5-carboxylate (P5C) accumulation inactivates PLP [4]. (ii) Deactivating mutations in *PNPO*, the gene that encodes pyridox(am)ine 5′-phosphate oxidase (PNPO), blocks conversion of PN-phosphate and pyridoxamine-phosphate to PLP. Therefore, intractable seizures in PNPO deficiency respond only to PLP, not to PN [5,6]. Deficiency of neurotransmitters, such as γ-amino butyric acid (GABA), due to reduced PLP availability probably explains the seizures in all three metabolic disorders. As PRS is rare and its presentation may be atypical, PN therapy is recommended in all cases of intractable seizures [7].

Hypophosphatasia (HPP) is a rare inborn error of metabolism featuring decreased activity of the tissue nonspecific isoenzyme of alkaline phosphatase (TNSALP) [8]. This disorder maps to chromosomal locus 1p36.1–p34 (OMIM #241500) and is caused by deactivating mutation(s) in *TNSALP*, the gene encoding TNSALP [8]. Characteristic laboratory findings in HPP include low serum alkaline phosphatase (ALP) activity (hypophosphatasemia) as well as increased urinary excretion of phosphoethanolamine (PEA) and inorganic pyrophosphate (PPi) [9–11]. Additionally, plasma PLP levels are elevated, sometimes to extraordinary concentrations [8]. These three phosphocompounds accumulate extracellularly in HPP because TNSALP functions as a cell surface hydrolase for each of them [8]. Endogenous excesses of PPi in HPP impair hydroxyapatite crystal formation, causing defective skeletal mineralization that manifests as rickets in children or osteomalacia in adults [11].

Depending on the age when HPP is diagnosed, five clinical subtypes are distinguished: perinatal, infantile, childhood, adult, and odontohypophosphatasia [8,9]. The prognosis for the infantile form is poor, with approximately 50% of patients dying during the first year of life from pulmonary complications of their skeletal disease [8,9,12]. There is no established therapy [8,12,13].

The association of PRS with perinatal HPP was first described in 1967 [14], with a few similar cases subsequently reported [10,15–19]. At least ten other HPP patients with neonatal seizures have been published (mostly as abstracts), including J.C. Rathbun's original case [20]. Whether seizures in these HPP patients were PN/PLP responsive/dependent is unclear as the anticonvulsive action of vitamin B_6 was not known at that time or it was not given.

We investigated a 7-month-old girl, newly diagnosed with infantile HPP, who had originally presented with PRS during the neonatal period and discuss the pathophysiology of neonatal seizures and PN responsiveness in HPP.

Case report

The patient was delivered spontaneously at term after an uneventful pregnancy. Birth weight was 3015 g (−0.64 SD), length 49 cm (−0.48 SD) and head circumference 32 cm (−1.4 SD). She adapted well postnatally and the initial physical examination was unremarkable. In particular, no skeletal abnormalities were noted. Her parents were Caucasian, non-consanguineous and well. The mother gave no history of stillbirth, and a 6-year-old sister was healthy.

On the patient's 7th day of life, a series of multifocal myoclonic jerks and tonic seizures became apparent. Upon hospitalization, there was no evidence of infection and an extensive metabolic work-up, including serum calcium of 2.73 mmol/l (nl 2.4–2.8), was unremarkable. Concentrations of amino acids in cerebrospinal fluid (CSF), plasma and urine were generally normal, as was serum pipecolic acid (3.4 mmol/l [nl 0.8–5.3]), the biochemical marker of classical PRS [2,3]. Analysis of biogenic amines in CSF showed essentially normal concentrations of 5-hydroxyindoleacetic acid (5HIAA; 821 nmol/l [nl 150–800]) and homovanillic acid (HVA; 939 nmol/l [nl 310–1100]) representing the end-products of serotonin and dopamine syntheses, respectively. However, the concentrations of 3-*ortho*-methyldopa (3-OMD; 669 nmol/l [nl <300]) and 5OH-tryptophan (5-HTP; 33.2 nmol/l [nl <10]), substrates of PLP-dependent enzymes upstream in respective synthesis pathways, were elevated. Hypophosphatasemia (serum ALP activity of <15 IU/l [nl 48–406]) was not investigated because she otherwise appeared healthy. Standard EEG showed a continuous burst suppression background, but cerebral ultrasound and magnetic resonance imaging were unremarkable.

Initially, seizures were interrupted using diazepam (0.1 mg/kg i.v.), but were then refractory to therapeutic doses of barbiturates (phenobarbital 5 mg/kg). Intravenous PN (Benadon®), 100 mg administered twice (60 mg/kg/day) on the 12th day of life, stopped the convulsions, and the burst suppression background on EEG normalized within a few days. A transient respiratory depression, necessitating short-term oxygen administration, was interpreted as a recognized side effect of PN [1]. Subsequently, she remained seizure-free while receiving oral PN therapy (7–10 mg/kg/day) and was discharged from hospital on the 21st day of life. During the following months, the patient continued PN treatment and showed nearly normal cognitive milestones. However, beginning at about 6 weeks of life, she developed progressive vomiting and severe failure to thrive.

Upon re-admission to the hospital at age 7 months, her weight was 4150 g (−4.33 SD), length 56 cm (−4.94 SD) and head circumference 39 cm (−3.45 SD). Physical examination showed muscle hypotonia as well as signs of rickets (e.g. craniotabes, a rachitic rosary, scoliosis and a Harrison-groove thoracic deformity). Laboratory studies were consistent with HPP, revealing serum ALP activity of 4 IU/l (nl 133–347), hypercalcemia (4.06 mmol/l) and hypercalciuria (calcium/creatinine ratio 4.98 mmol/mol [nl <2.2]) in the presence of physiologically suppressed serum parathyroid hormone (0.7 pg/ml [nl 10–55]), high-normal 25(OH)-vitamin D (111 ng/ml [nl 25–80 ng/ml])