Pyridoxine or pyridoxal-5′-phosphate for neonatal epilepsy

The distinction just got murkier

Phillip L. Pearl, MD
Sidney M. Gospe, Jr., MD, PhD

Correspondence to Dr. Pearl: Phillip.Pearl@childrens.harvard.edu

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The discovery of the cause of pyridoxine-dependent epilepsy (PDE), a unique autosomal recessive and treatable cause of refractory neonatal seizures, came 50 years after its first description with the identification of *ALDH7A1* mutations leading to deficiency of the enzyme α-aminoadipic semialdehyde dehydrogenase (antiquitin).[1] Dysfunction of this enzyme interferes with lysine degradation and leads to accumulation of a carboxylate product that irreversibly inactivates pyridoxal-5′-phosphate (PLP), the biologically active form of vitamin B_6.

About the same time as the discovery of antiquitin deficiency, a related condition, pyridox(am)ine phosphate oxidase (PNPO) deficiency, was identified.[2] This took into account the knowledge that PNPO, phosphatases, and kinases are specifically required to synthesize PLP from dietary sources (i.e., pyridoxamine and pyridoxine from meat and vegetables, respectively), allow for transport across the blood–brain barrier, and rephosphorylate these substances into PLP (figure). While PDE was believed to be a rare condition, PNPO deficiency, treatable with PLP but not pyridoxine, was viewed as an even less common vitamin-responsive epileptic encephalopathy.

To differentiate these conditions, pyridoxine-dependent cases (i.e., PDE) were hypothesized to be associated with full-term births and PLP-dependent cases (i.e., PNPO deficiency) with prematurity,[3] and a classic burst-suppression EEG with PDE vs a more nonspecific and less paroxysmal pattern with PNPO deficiency.[4] Overlap cases, however, were reported, with partial pyridoxine responsiveness in patients later confirmed to have *PNPO* mutations.[5] In this issue of *Neurology*®, Plecko et al.[6] studied 31 patients with a clinical syndrome of neonatal seizures responsive to pyridoxine yet with normal levels of PDE biomarkers and normal *ALDH7A1* sequencing. Given the common phenotype of neonatal-onset epileptic encephalopathy, the investigators reasoned that there may be mutations leading to some residual PNPO enzymatic function that could be augmented with addition of more substrate (i.e., pyridoxine).

The authors identified 11 patients from 7 families with *PNPO* gene mutations; expression studies demonstrated reduced or absent PNPO activity, depending on the mutation studied. Overall, 3 novel mutations were sequenced: 2 missense mutations, both affecting arginine residues in codons interfering with the potential CpG DNA methylation site, and a deletion located in the β-strand S2 of the PNPO protein. All presented in the neonatorum with recurrent myoclonic and tonic seizures. Four of the 11 were born before 36 weeks' gestation. Pyridoxine administration led to prompt seizure cessation in 4 patients, delayed seizure reduction over several days in 2, EEG improvement only in 2, and had no effect in 2 (although a second dose at age 7 months was effective in one). Pyridoxine withdrawal in one patient at age 3 months led to seizure recurrence within 12 hours. Paradoxically, status epilepticus occurred in 2 when pyridoxine was replaced by an identical dose of PLP. Of 9 patients alive at the time of the report, 6 remain on pyridoxine monotherapy, and the other 3 also require treatment with antiepileptic drugs. While there was a range of outcomes, nonstandardized review of the clinical cases suggests earlier and continuous pyridoxine therapy was related to a better prognosis.

This study challenges the notion of exclusive PLP responsiveness in PNPO deficiency and even suggests potential deleterious effects of PLP in certain patients. The authors postulate that an inhibitory effect of PLP on PNPO activity may completely inhibit the enzyme in patients with certain *PNPO* genotypes, thereby resulting in status epilepticus. The study does not report CSF PLP levels, which can help to serve as a diagnostic, though nonspecific, marker.[7]

The observations by Plecko et al.[6] raise challenging new questions. Can pyridoxine-responsive PNPO-deficiency patients be distinguished on clinical grounds? The presentations of the 11 patients reported were not substantially different from those reported in other patients with PNPO deficiency, although 4 of 9 (44%) had a prompt initial pyridoxine response, in contrast to 85% of a larger series of patients

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From the Department of Neurology (P.L.P.), Children's National Medical Center, The George Washington University School of Medicine, Washington, DC; the Departments of Neurology and Pediatrics (S.M.G.), University of Washington, Seattle; and the Division of Neurology (S.M.G.), Seattle Children's Hospital, WA.

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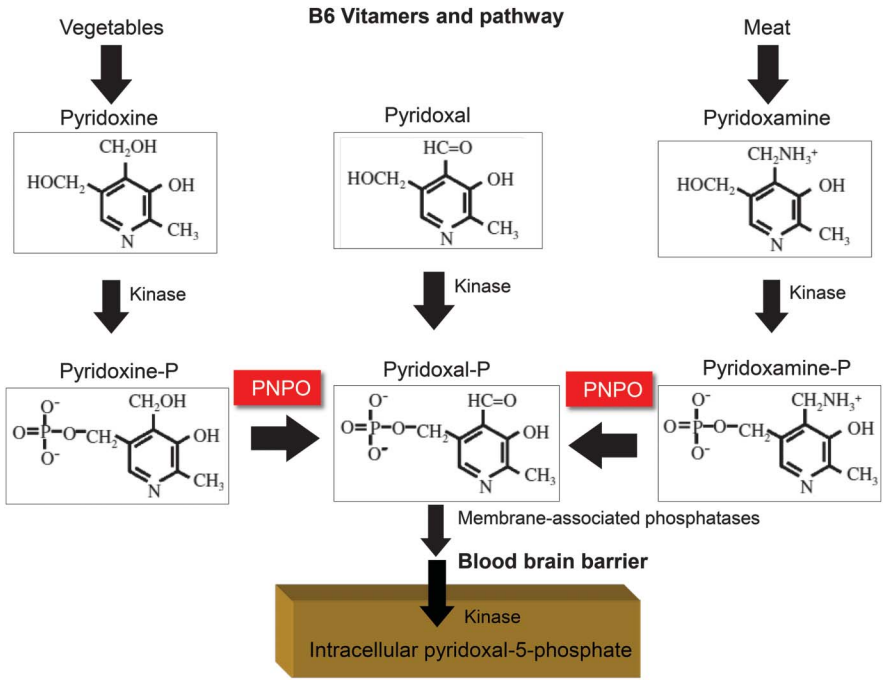
Figure B_6 vitamers and pathway



Pyridoxal-5-phosphate (P5P), the biologically active form of B_6, is formed via phosphorylation of pyridoxine and pyridoxamine, followed by oxidation using the enzyme PNPO (pyridox[am]ine phosphate oxidase). In order to gain access through the blood–brain barrier, P5P must be dephosphorylated and then rephosphorylated utilizing phosphatases and kinases, respectively.

with antiquitin deficiency.[8] If the *PNPO* mutations in this report were "leaky" or mild, why were the outcomes still at times poor, with severe handicap in the 2 patients with treatment delay and death from epileptic encephalopathy in 2 siblings without continuous pyridoxine treatment? What are the effects of the 3 mutations identified on PNPO function? None of the lost amino acids is actually involved in pyridoxine/pyridoxamine-5′-phosphate oxidation. Is there in vivo residual enzymatic activity? Is supplemental pyridoxine sufficient to provide additional substrate for adequate PLP formation? The primary role of PNPO is formation of PLP, a cofactor in more than 120 enzymatic reactions of neurotransmitter and amino acid metabolism, but other roles have been suggested, such as intracellular recycling of degraded enzymes[9] and channeling of PLP to its various apo-enzymes.[10] Why did one patient in the present series, and in some other reports, have elevated, not depressed, concentrations of plasma neurotransmitter biogenic amines?

Overall, it appears that the initial approach of PLP administration to cover the possibilities of both PDE and PNPO deficiency as causes of refractory neonatal and early infantile seizures must be modified to sequential testing of pyridoxine and PLP. Furthermore, PNPO mutations should be sought in patients with a positive pyridoxine response yet with normal PDE biomarkers and no mutations in *ALDH7A1*. As clinical and laboratory findings compatible with a diagnosis of birth asphyxia have been reported in a number of cases of confirmed antiquitin and PNPO deficiency, trials of pyridoxine and PLP should be performed in neonates with antiepileptic drug–resistant seizures regardless of birth history. And, with the identification of *PNPO* mutations in 11 patients out of a group of 31 with pyridoxine responsiveness without confirmed antiquitin deficiency, the most striking and inescapable conclusion is that PNPO deficiency may not be so rare and perhaps considerably more common than its historical predecessor.

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P. Pearl works on a scientific advisory board for the FDA; NIH (National Institute of Neurological Disorders and Stroke), R01HD58553, investigator, 2008–present; works on the editorial boards of *Pediatric Neurology*, *Journal of Child Neurology*, *Future Neurology*, *Faculty of 1000*, *Pediatric Health*, *International Journal of Clinical Practice*, and *Music and Medicine*; part of the Delman Family Fund for Pediatric Neurology Research, Investigator 2007–present; and serves on the Neurology RRC of the ACGME. S. Gospe is Senior Associate Editor of *Pediatric Neurology*. Go to Neurology.org for full disclosures.

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