Neonatal epileptic encephalopathy: role of vitamin B-6 vitamers in diagnosis and therapy¹,²

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The term “vitamin B-6” refers to a group of naturally occurring pyridine derivatives represented by pyridoxine, pyridoxal, and pyridoxamine and their phosphorylated derivatives with similar physiologic actions. These 6 compounds are referred to as vitamin B-6 vitamers. The free forms of the vitamers could be converted to the key coenzymatic form pyridoxal phosphate (PLP)³ by the action of 2 enzymes, a kinase and an oxidase. Pyridoxal is irreversibly oxidized to 4-pyridoxic acid (PA) by an FAD-dependent aldehyde oxidase in the human liver. PA is excreted in the urine. As such, it is a measure of the total vitamin B-6 metabolized by the body. Phosphorylated pyridoxal and pyridoxamine, the primary animal-derived forms of vitamin B-6 vitamers, are converted to the free bases by intestinal alkaline phosphatase and absorbed by a carrier-mediated system. Pyridoxine phosphate (PNP) and pyridoxamine phosphate (PMP) are oxidized by pyridox(am)ine-5′-phosphate oxidase (PNPO) to form PLP. Only free bases can cross the blood-brain barrier. PLP is cleaved by nonspecific membrane-associated alkaline phosphatase to pyridoxal and transported to cerebrospinal fluid (CSF). Uptake of the free vitamers from CSF to brain follows a similar mechanism.

The versatility of reactions catalyzed by PLP has been recognized because there are >140 enzymatic reactions catalyzed by PLP-dependent enzymes and these are found in all organisms. They are involved in linking carbon and nitrogen metabolism, replenishing the pool of one-carbon units, and forming biogenic amines (1, 2). The crucial role played by vitamin B-6 in the nervous system is evident from the fact that the putative neurotransmitters dopamine, norepinephrine, serotonin (5-HT), and γ-aminobutyric acid (GABA) as well as sphingolipids and polyamines are synthesized by PLP-dependent enzymes (3, 4). The concept of the regulatory role of the hypothalamus primarily through neurotransmitters dopamine and 5-HT is generally accepted. Under conditions of vitamin B-6 deficiency, the hypothalamo-pituitary-end organ systems are affected. Vitamin B-6 status has a significant effect on 5-HT and GABA, neurotransmitters that control pain perception, anxiety, and depression (2). Vitamin B-6 also has a significant role in the cardiovascular system through its role in the metabolism of methionine/homocysteine and more directly through the inhibitory effect of PLP on both major calcium channels, the L-type as well as the ATP-mediated channels (2). The role of pyridoxamine as an inhibitor of pathogenic glycation and oxidative damage in preventing age-related aortic stiffening and vascular resistance places it in the category of a multifunctional pharmaceutical agent (5, 6). The use of PLP in the treatment of autoimmunity and in transplant rejection has been indicated (7).

The PLP-dependent enzyme glutamic acid decarboxylase (GAD) decarboxylates the excitatory neurotransmitter glutamate to produce GABA, an inhibitory neurotransmitter. Thus, vitamin B-6 status is a major contributor to the balance between neuronal excitatory and inhibitory states. The induction of congenital vitamin B-6 deficiency in rats was shown to result in pups with spontaneous neonatal convulsions (8). In addition, the effect of vitamin B-6 deficiency during the critical period in the development of the central nervous system in the rat had significant effects on various electrophysiologic variables. The bursts of high-voltage spikes during spontaneous electroencephalographic activity as well as the spontaneous convulsions observed, reflected the decrease in cerebral GABA concentration in the brain of the deficient rats (9). Domoic acid, a rigid structural analog of glutamate, is a neuroexcitant. Acute hippocampal administration of picomole amounts of domoic acid led to electroencephalography epileptiform seizure discharge activity (10, 11). In other experiments, electroencephalography recordings in the cerebral cortex of adult mice given a single subconvulsive dose of domoic acid exhibited typical spike and wave discharges. The administration of sodium valproate, nimodipine, or pyridoxine simultaneously with or after domoic acid administration resulted in significantly less spike and wave activity. Thus, pyridoxine has a significant neuroprotective activity. The inhibitory effect of PLP on calcium transport is also crucial to its neuroprotective action (12).
The use of pyridoxine in the treatment of neonatal and infantile epileptic encephalopathy (NEE) has been in vogue for decades since the recognition of pyridoxine-dependent seizures (PDSs) by Hunt et al (13). Since then, a variety of inborn errors of metabolism relating to the role of vitamin B-6 in the nervous system have been recognized. They can be categorized as pyridoxine-dependent (PDS), pyridoxine-responsive, and pyri- doxal phosphate–responsive conditions. In PDS, the onset of epileptic encephalopathy is within hours of birth with severe seizures that are not responsive to common anticonvulsant drugs but responding to pyridoxine. Mutations in the ALDH7A1 gene, which encodes the protein antiquitin, an aldehyde dehydrogenase that functions in the cerebral lysine catabolic pathway, are responsible for the biochemical abnormalities. Affected patients have elevations of piperolic acid in plasma taken before treatment and elevations of aminoadipic semialdehyde in CSF, plasma, and urine, a very specific biochemical confirmation of this inborn error of metabolism (14). Aminoadipic semialdehyde is in equilibrium with piperidine-6-carboxylic acid, which condenses with PLP, thereby inactivating it and causing a deficiency of PLP in the brain. There is no impairment of PLP synthesis in this condition. Withdrawal of pyridoxine treatment and recurrence of seizures was used to clinically verify this disorder (PDS). This is no longer necessary because testing the ALDH7A1 gene for mutations provides confirmation of PDS. A defect in proline metabolism due to a defect in proline-5- carboxylate dehydrogenase leads to an accumulation of proline and pyrroline-5-carboxylate, which condenses with PLP, thus producing a deficiency of PLP. This condition (PDS) responds to pyridoxine because there is no impairment in the synthesis of PLP. A subset of patients with PDS who respond to pyridoxine and in whom seizures did not recur after withdrawal of pyridoxine treatment are referred to those with pyridoxine-responsive seizures. The reason for this response is not understood.

An NEE unresponsive to both anticonvulsants and pyridoxine but responding to PLP has been recognized (15). The biochemical changes in CSF and plasma are indicative of reduced activities of several PLP-dependent enzymes. Elevated CSF concentrations of L-dihydrophenylalanine and 3-methoxy tyrosine and reduced concentrations of CSF homovanillic acid and 5-hydroxy indole acetic acid indicate impaired function of aromatic amino acid decarboxylase. Increased CSF and plasma concentrations of threonine due to decreased activity of threonine dehydratase and the increased CSF and plasma concentrations of glycine due to decreased activity of the glycine cleavage enzyme are indicative of a cellular deficit of PLP. It was shown that this disorder was secondary to mutations in the PNPO gene encoding PNP/PMP oxidase, resulting in the deficiency of PNPO oxidase, the salvage enzyme responsible for converting PNP and PMP to PLP intracellularly. This phenotype of NEE does not respond to the usual anticonvulsants or to pyridoxine but does respond to PLP.

In patients with neonatal seizures (NEE) not responding to anticonvulsants and in whom structural, traumatic, or cardiovascular causes or infection is not acknowledged as a causative mechanism, prompt treatment with PLP is recommended practice (14). NEEs responding to pyridoxine also respond to PLP. The clinical phenotype could be established after biochemical analysis of CSF, plasma, urine, and genetic testing.

In this issue of the Journal, Albersen et al (16) report on the determination of vitamin B-6 vitamers and their metabolite PA in plasma and CSF samples taken simultaneously from human subjects by using ultra HPLC–tandem mass spectrometry. Such procedures provide a reference set of vitamin B-6 vitamer concentrations in plasma and CSF samples. Although in terms of treatment the recommendation is to start treatment of patients with NEE with PLP without waiting for laboratory data, the confirmation of PLP-dependent PNPO-deficient NEE would require a pretreatment sample of CSF to indicate a decrease in PLP along with the determination of CSF concentrations of neurotransmitter/precursor metabolites as well as gene testing. Such simultaneous measurement of vitamin B-6 vitamers in plasma and in CSF might offer insight into as yet unrecognized alterations in the metabolism of vitamin B-6 vitamers.

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REFERENCES


