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Pyridoxal Phosphate Supplementation in Neuropediatric Disorders

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Pyridoxal phosphate (PLP) is the active form of vitamin B_6 and a cofactor in many enzyme reactions including neurotransmitter metabolism. PLP metabolism disturbances may mostly lead to refractory seizures. In this report, we review the main pathophysiological factors related with PLP deficiency and our experience in PLP treatment in pediatric patients with low-normal cerebrospinal fluid PLP values who presented epilepsy. Only one case had a definite diagnosis (Phelan-McDermid syndrome). The results of extensive metabolic workups and targeted genetic studies were normal for all patients. In 5 cases, the response to PLP supplementation (10-30 mg/kg/d) was initially positive. PLP adverse reactions were noticed in 4 patients and PLP was discontinued; however, one of the most noticeable symptoms was an asymptomatic increase in liver enzymes. These negative results with PLP supplementation are worth reporting, to improve the information we use to treat our patients.
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Introduction

Pyridoxal 5′-phosphate (PLP) is the active form of vitamin B_6, which is the essential cofactor for more than 100 metabolic reactions, including enzymes involved in the metabolism of the neurotransmitters glutamate, gamma hydroxybutyric acid (GABA), glycine, D-serine, dopamine, serotonin, and noradrenaline, among other biomolecules.[1] PLP is synthesized via phosphorylation through the action of a ubiquitous kinase and oxidation by pyridoxamine 5-prime-phosphate oxidase (PNPO; EC 1.4.3.5). PLP chaperone activity has been described in the folding of some enzymes.[2] Furthermore, its participation in some actions beyond its coenzymatic function has been extensively investigated[3] in regard to L-amino acid decarboxylase (AADC; EC 4.1.1.28) activity. It has been demonstrated that PLP interacts with transcription factors, is involved in AADC folding, dimerization, and splicing and may prevent the degradation of some PLP-dependent enzymes.

PLP-responsive seizures are caused by mutations in *PNPO* gene (OMIM#610090). This inborn error was initially described as a cause of neonatal vitamin–responsive epileptic encephalopathy.[4,5] As PNPO is the main enzyme involved in the synthesis of PLP, it is thought that mutations in the *PNPO* gene would explain most disorders with decreased levels of PLP in cerebrospinal fluid (CSF). However, beyond those *PNPO* mutations, there are patients already described with no mutations in this gene that exhibit low CSF PLP levels.[6] PLP

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deficiency may be related to other genetic conditions, such as pyridoxine-dependent epilepsy (OMIM#266100), hyperprolinemia type II (OMIM#239510), hypophosphatasia (OMIM#241500), and sulfite oxidase deficiency (OMIM#272300),[1,7] but also to environmental factors like the presence of seizures, the use of antiepileptics, and other drugs as well as malabsorption syndromes.

Our aim was to report our experience with a small sample of 10 patients with low-normal levels of PLP in CSF and who underwent supplementation with PLP. We also review the main pathophysiological factors associated with PLP deficiency and the possible side effects related with PLP supplementation after 2-year follow-up.

Methods

Study Setting and Inclusion Criteria

Over the past 10 years, we have biochemically studied CSF samples from 1380 neuropediatric patients. Details of this study have been reported elsewhere.[9] We analyzed PLP in a subset of 146 CSF samples owing to clinical picture (mainly epilepsy of unknown origin), or a biochemical profile suggestive of PLP deficiency, such as impaired CSF biogenic amines or amino acid profiles. From this subset, we selected a series of 10 cases with different phenotypes presenting epilepsy as a common clinical feature for PLP supplementation. These 10 cases presented low CSF PLP values or values close to the lowest limit of our reference intervals, and were selected for supplementation with PLP owing to the complex phenotype exhibited, the lack of an alternative therapy, and the need to ameliorate the clinical picture. Based on previous reports from patients with *PNPO* deficiency supplemented with PLP, and the low frequency of adverse effects reported, we decided to supplement our patients with this vitamin. All patients' biochemical results were compared with our reference values, and established in a control group, as previously reported.[8,10-12] This is a retrospective report of our experience from the past 2 years after the supplementation was initiated. All clinical records from these 10 patients were reviewed.

Biochemical Methods

Plasma and CSF amino acids were analyzed using ion exchange chromatography with ninhydrin detection (Biochrom 30, Pharmacia Biotech, Biochrom, Cambridge, Science Park, England). Alkaline phosphatase was measured with standard automated spectrometric procedures. Total urine sulfites were analyzed by semiquantitative test strips according to the manufacturer's protocol (Merck Millipore, MA, USA). CSF neurotransmitter analysis (3-ortho-methyldopa [3-OMD], 5-hydroxytryptophan [5-HT], 5-hydroxyindoleacetic acid [5-HIAA], and homovanillic acids [HVA]) and PLP were conducted with high-performance liquid chromatography using electrochemical (Coulochem II, ESA, Chelmsford, USA) and fluorescence (Perkin Elmer, serie 200, Norwalk, CT) detection according to previously reported procedures.[8,10]

Molecular Studies

Genomic DNA was isolated from venous whole blood from all patients except for one who died at 30 days of age. We amplified and sequenced the coding regions of *PNPO* gene in 9 patients using a set of 7 primer pairs. Patients #4 and #10 were studied by next-generation sequencing technology (Sure Select XT kit, Agilent Technology) and HiSeq 2000 system (Illumina, USA) to analyze 58 genes associated with neonatal or infantile epilepsy (early epileptic encephalopathy [EEE] panel).

PLP Supplementation

All patients were supplemented with PLP according to the existing recommendations. Oral doses ranged from 10-30 mg/kg/d.[1,13] We started with 10 mg/kg/d, scaling doses every week up to 30 mg/kg/d. In 2 patients who reported gastrointestinal symptoms (abdominal pain, diarrhea, and nausea) omeprazol was added to the treatment. The treatment was discontinued when no response after 6 months of therapy was observed, or when important adverse effects were reported. The clinical response was based both on parental subjective reports and the objective evaluation of the pediatric neurologist during the follow-up period. When possible, a second electroencephalogram (EEG) after treatment initiation was practiced.

Caregivers' Consent

All caregivers were informed of analysis results and consulted with about supplementary treatment with PLP. All gave their written consent as per clinical procedure for this investigation. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000.

Patients and Results

Patients' Characteristics

Patients' main clinical and biochemical data are summarized on Table 1. Although epilepsy was the most common clinical sign, different phenotypes were observed. Patient #5 did not have clinical seizures, and underwent lumbar puncture (LP) at 4 years after an extensive metabolic work-up for diagnosis and treatment of dystonic movements, mainly in the perioral region, together with mild motor and intellectual delay. No antiepileptic medication was taken by the patient at that time. Patients #1, 4, 6, 7, and 10 presented seizures in the neonatal period with varying clinical evolution. Patient #1 presented focal clonic seizures at a few days old. He was initially treated with valproic acid, and later switched to carbamazepine and phenobarbital (PB). This was the current treatment when the LP was done. Patient #10 started having seizures at 17 hours of life, being the most precocious patient with symptoms, exhibited an early myoclonic epileptic encephalopathy. In patients #4 and 10, EEE genetic panel was analyzed, and after the parental segregation studies they had no relevant change in