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Triple therapy with pyridoxine, arginine supplementation and dietary lysine restriction in pyridoxine-dependent epilepsy: Neurodevelopmental outcome

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ABSTRACT

Pyridoxine-dependent epilepsy (PDE) is an epileptic encephalopathy characterized by response to pharmacologic doses of pyridoxine. PDE is caused by deficiency of α-aminoadipic semialdehyde dehydrogenase resulting in impaired lysine degradation and subsequent accumulation of α-aminoadipic semialdehyde. Despite adequate seizure control with pyridoxine monotherapy, 75% of individuals with PDE have significant developmental delay and intellectual disability. We describe a new combined therapeutic approach to reduce putative toxic metabolites from impaired lysine metabolism. This approach utilizes pyridoxine, a lysine-restricted diet to limit the substrate that leads to neurotoxic metabolite accumulation and L-arginine to compete for brain lysine influx and liver mitochondrial import. We report the developmental and biochemical outcome of six subjects who were treated with this triple therapy. Triple therapy reduced CSF, plasma, and urine biomarkers associated with neurotoxicity in PDE. The addition of arginine supplementation to children already treated with dietary lysine restriction and pyridoxine further reduced toxic metabolites, and in some subjects appeared to improve neurodevelopmental outcome. Dietary lysine restriction was associated with improved seizure control in one subject, and the addition of arginine supplementation increased the objective motor outcome scale in two twin siblings, illustrating the contribution of each component of this treatment combination. Optimal results were noted in the individual treated with triple therapy early in the course of the disease. Residual disease symptoms could be related to early injury suggested by initial MR imaging prior to initiation of treatment or from severe epilepsy prior to diagnosis. This observational study reports the use of triple therapy, which combines three effective components in this rare condition, and suggests that early diagnosis and treatment with this new triple therapy may ameliorate the cognitive impairment in PDE.

1. Introduction

Pyridoxine-dependent epilepsy (PDE) is an early infantile epileptic encephalopathy characterized by a positive response to pharmacologic doses of pyridoxine. PDE is an autosomal recessive condition caused by mutations in *ALDH7A1*. PDE results from the deficiency of α-aminoadipic semialdehyde dehydrogenase with subsequent accumulation of α-aminoadipic semialdehyde (α-AASA) and Δ^1-piperideine-6-

Abbreviations: 5HIAA, 5-hydroxyindolacetic acid; α-AASA, α-aminoadipic semialdehyde; ABAS II, Adaptive Behavioral Assessment System-II; AIMS, Alberta Infant Motor Score; CSF, cerebral spinal fluid; DOL, days of life; EEG, electroencephalography; FMOC, fluorenylmethoxycarbonyl; GA I, glutaric aciduria type I; HVA, homovanilic acid; P6C, Δ^1-piperideine-6-carboxylate; PDE, pyridoxine dependent epilepsy; PLP, pyridoxal 5′-phosphate.

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carboxylate (P6C), which are linear and cyclic molecules, respectively, that are in equilibrium. The accumulated P6C inactivates the active vitamer of B_6 (pyridoxal 5′-phosphate; PLP) by forming a Knoevenagel condensation product [1]. PLP is a cofactor for over 140 enzymatic reactions including those involved in the synthesis and degradation of amino acids and neurotransmitters [2,3], and decreased availability of PLP for neurotransmitter synthesis is believed to be important to the pathogenesis of PDE, and is the basis of treatment with pyridoxine. Low cerebrospinal fluid (CSF) concentrations of PLP have been noted in vitamin B_6 responsive seizure disorders, although are not always identified in affected individuals with PDE [4,5].

The classic presentation of PDE is the neonatal onset of treatment-refractory seizures that have a dramatic response to pyridoxine supplementation, although onset of seizures may occur in childhood, multiple seizure types may be observed, and the clinical response to pyridoxine may be delayed [6,7]. Treatment with pharmacologic doses of pyridoxine provides sufficient pyridoxine to overcome the apparent sequestration of PLP due to condensation with P6C, although rarely patients have responded to physiological doses of pyridoxine [8]. The majority of patients are reported to achieve seizure control with pyridoxine alone, although additional antiepileptic drugs may be required in some patients for optimal seizure management [9]. The identification that folinic acid-responsive seizures are also a result of deficiency of α-aminoadipic semialdehyde dehydrogenase has led to concomitant treatment with folinic acid in some individuals [10]. The mechanism of response to folinic acid is not understood, and the benefit of folinic acid in the treatment of PDE has not been established.

Despite adequate seizure control, 75% of individuals with PDE have significant developmental delay and intellectual disability on pyridoxine monotherapy [9,11]. Even with early diagnosis and optimal seizure control, significant cognitive impairment has been noted in children and adults suggesting that pyridoxine supplementation alone is not sufficient to treat all neurologic aspects of the disease [11,12]. The degree of intellectual disability does not correlate with the age of seizure onset, seizure type, age at diagnosis, or biochemical findings at presentation [9,11].

Identification of alpha-aminoadipic semialdehyde dehydrogenase deficiency as the cause of PDE, an enzyme within the cerebral lysine degradation pathway, suggested that patients may benefit from dietary limitation of lysine [1,13]. The impaired lysine metabolism in PDE results in significant accumulation of α-AASA and P6C, which are likely neurotoxic and may contribute to the pathogenesis of PDE. In a previously reported case series, implementation of a lysine-restricted diet resulted in decreased plasma and urinary α-AASA levels and an improved developmental outcome [14]. As a result, a lysine-restricted diet has been recommended as adjunct therapy in PDE, and dietary guidelines have been developed by an international working group [15].

Glutaric aciduria type I (GA I) is another disorder of lysine catabolism, and results in elevated neurotoxic metabolites glutaric acid and 3-hydroxyglutaric acid. A lysine-restricted diet is a crucial component of treatment of GA I [16], and has a demonstrated neuroprotective effect [17,18], although dietary therapy alone is not sufficient to prevent all neurologic sequelae [19]. In a mouse model of GA I, dietary lysine restriction reduced the glutaric acid concentrations in the brain, although the 3-hydroxyglurtaric acid concentration remained unchanged [20]. Lysine is a dibasic amino acid that is transported at epithelia of the intestine, the kidney and the blood–brain barrier by the cationic transporters that also transport the dibasic amino acids arginine and ornithine [21–23]. As a result, the use of arginine to compete with lysine for transport has been suggested. Both arginine supplementation and a low ratio of dietary lysine to dietary arginine have been recommended in the treatment of individuals with GA I [24]. Experimental evidence for the efficacy of this treatment was demonstrated in the mouse model of GA I, in which, arginine supplementation alone mimicked the results of the lysine-restricted diet in the reduction of neurotoxic brain metabolites [20]. Arginine supplementation combined with a lysine-restricted diet was able to further reduce the brain glutaric acid concentration, compared to monotherapy with either lysine restriction or arginine, as well as the brain 3-hydroxyglutaric acid concentration [20]. This supports the hypothesis that supplemental arginine can decrease brain lysine metabolism through competitive inhibition, and suggests an additive benefit of both a lysine-restricted diet and arginine fortification in cerebral lysine disorders. Recently arginine supplementation was used as an alternative to the lysine-restricted diet in a child with PDE with a decrease in cerebral lysine and urine and CSF α-AASA levels as well as neurologic improvement [25].

We present the clinical outcome of six subjects with PDE who were treated with a novel treatment combination of pyridoxine supplementation, a lysine restricted diet, and arginine supplementation. We refer to this treatment as “triple therapy” for PDE.

2. Methods

The study is a retrospective review of available medical records from three centers in North America. For subjects 1–4, treatment for PDE was performed as part of clinical care. For subjects 5 and 6 the British Columbia Children's Hospital Review Board approved the study as an “innovative treatment protocol.” Consent for participation and publication of study results was obtained from each subject's parent or legal guardian. Medical records were reviewed for six subjects diagnosed with PDE through biochemical testing and mutations in *ALDH7A1*. Subjects were initially treated with pyridoxine supplementation and both a lysine restricted diet and arginine supplementation were added to the treatment regimen either sequentially (in five subjects) or concurrently (1 subject). Summary clinical and genetic information and information regarding treatment and outcome are listed in Table 1. Information regarding the effect of a lysine-restricted diet alone in subjects 1, 5 and 6 were reported previously [14,26]. Data extracted from medical records included plasma or CSF pipecolic acid, AASA, P6C, lysine, arginine, 5-hydroxyindolacetic acid (5HIAA) and homovanilic acid (HVA). Brain MR imaging findings and clinical evaluation of cognitive and motor development were also recorded.

2.1. Subjects

Subject 1 was the first child of non-consanguineous parents of European ancestry born after an unremarkable pregnancy. The subject presented at nine days of life (DOL) with hypoglycemia, acidosis, and episodes of stiffening with brief jerking and twitching movements of her extremities. Electroencephalography (EEG) was indicative of epileptic encephalopathy with mild burst suppression. She received 100 mg of IV pyridoxine at 11 DOL with no immediate changes noted on EEG. Pyridoxine supplementation was continued at 30 mg/kg/day and improvement in the subject's clinical status and EEG was noted 24 h after initial treatment. The diagnosis of PDE was established through elevated plasma levels of pipecolic acid, α-AASA and P6C. The subject had complete resolution of clinical seizures at 11 DOL with a normal EEG at 20 DOL. Brain MR imaging performed at 12 DOL noted numerous foci of restricted diffusion scattered throughout the bilateral anterior and posterior periventricular white matter (Fig. 1A, 1B). A lysine-restricted diet was initiated at 1 month of life through the addition of metabolic formula thereby limiting lysine from natural sources. Arginine supplementation was added to her treatment regimen at 3 months of age at 150 mg/kg/day and increased to 200 mg/kg/day at two years of age (Table 1).

Subject 2 was the second child of a consanguineous union of parents originating from El Salvador. Her pregnancy history was unremarkable, and she presented to care with seizure like activity at 13 DOL and an EEG noted multifocal onset short electrographic seizures with the clinical correlate of apnea. Brain MR imaging, at that time, showed mild diffuse cerebral swelling without evidence of ischemia. Pyridoxine

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