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The effects of sapropterin on urinary monoamine metabolites in phenylketonuria

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ABSTRACT

Background: Sapropterin dihydrochloride (BH4, tetrahydrobiopterin) can lower plasma phenylalanine (Phe) concentrations for a subset of patients with phenylketonuria (PKU), an inborn error of metabolism. Studies suggest that monoamine neurotransmitter concentrations are low in PKU patients. Sapropterin functions as a cofactor for hydroxylases specific to Phe, tyrosine, and tryptophan metabolism, pathways essential for catecholamine and serotonin synthesis.
Objective: The objective of this study is to determine the impact of sapropterin on monoamine neurotransmitter status in patients with PKU.
Design: 58 PKU subjects were provided 20 mg/kg of sapropterin for 1 month. Those who responded with at least a 15% decrease in plasma Phe received sapropterin for 1 year, while Non-responders discontinued it. After an additional 3 months, Responders who demonstrated increased Phe tolerance and decreased medical food dependence were classified as Definitive, whereas Responders unable to liberalize their diet without compromising plasma Phe control were identified as Provisional. At study visits, patients provided blood for plasma amino acids, 3-day diet records, and 12-hour urine samples analyzed for epinephrine (E), dopamine (DA), dihydroxyphenylacetate (DOPAC), homovanillic acid (HVA), 3-methoxytyramine (3MT), serotonin (5HT), and 5-hydroxyindole acetic acid (5HIAA) using HPLC with electrochemical detection.
Results: Compared with healthy non-PKU controls, subjects with PKU had significantly lower baseline concentrations of DA, HVA, 3MT, 5HT, and 5HIAA ($p < 0.001$ for all). Medical food protein intake had a direct association with DA, HVA, 5HT, and 5HIAA during the study ($p < 0.05$ for all), while plasma Phe had an inverse association with these markers ($p < 0.01$ for all). DOPAC was also associated with plasma Phe throughout the year ($p = 0.035$), although not at baseline. Patients with PKU had a significant increase in HVA ($p = 0.015$) after 1 month of sapropterin. When stratifying by Responder and Non-Responder status, significance of HVA increase in Non-responders ($p = 0.041$) was confirmed, but not in Responders ($p = 0.081$). A declining trend in urinary 5HIAA, significant only after controlling for plasma Phe ($p = 0.019$), occurred for Definitive Responders during the 1-year study.
Conclusion: Urinary monoamine concentrations are low in patients with PKU and are influenced by oral sapropterin and medical food intake, highlighting the importance of these therapies to neurotransmitter metabolism in phenylketonuria.

1. Introduction

In phenylketonuria (PKU), disruption of phenylalanine hydroxylase (PAH) function leads to abnormally high concentrations of phenylalanine (Phe) and phenylketones. If untreated, severe neurological damage occurs as early as infancy [1,2]. Standard treatment for PKU consists of a specialized low-Phe diet, along with medically prescribed Phe-free amino acid-rich medical food as a primary source of protein [3]. This diet, which involves regular monitoring of medical food and Phe intake, enables patients with PKU to maintain plasma Phe concentrations within a therapeutic range of 100–360 μmol/L [4]. Newborn screening has enabled prompt diagnosis and dietary intervention for

Abbreviations: PKU, Phenylketonuria; PAH, Phenylalanine hydroxylase; TH, Tyrosine hydroxylase; LNAA, Large neutral amino acids; MA, Monoamines; HPLC, High-Performance Liquid Chromatography; DA, Dopamine; DOPAC, Dihydroxyphenylacetate; E, Epinephrine; 5HIAA, 5-hydroxyindole acetic acid; HVA, Homovanillic acid; 5HT, 5-hydroxy tryptophan (serotonin); 3MT, 3-methoxytyramine.

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PKU infants, thereby helping to avoid irreparable brain damage. However, older children and adults with PKU are still reported to have a higher frequency of psychiatric and behavioral disorders [5,6], as well as other neurological complications [7–9] that are most pronounced when compliance with medical diet wanes and Phe concentrations exceed the therapeutic threshold [10–12].

Cognitive and neurologic ramifications can be attributed at least in part to disruptions in monoamine neurotransmitter metabolism. For example, high Phe concentrations inhibit metabolism and transport of tyrosine and other large neutral amino acid (LNAA) essential for monoamine synthesis [13–17]. Low monoamine metabolites in PKU patients have been documented in urine, blood, cerebral spinal fluid (CSF), and brain tissue samples [16,18–21], indicating a global physiologic inhibition of monoamine synthesis in patients with PKU. Nevertheless, even when PKU patients are treated early [22,23], monoamine deficiency persists and has been noted in PKU mouse models as well [13,24]. Further exploration of the impact of low monoamine concentrations on neurologic outcomes in patients with treated PKU is of prime importance.

Sapropterin dihydrochloride (Kuvan®) is a pharmaceutical form of tetrahydrobiopterin (BH4) essential in the metabolism of catecholamines and serotonin due to its cofactor activity for PAH, tyrosine hydroxylase, and tryptophan hydroxylase (Fig. 1). Though BH4 concentrations are normal for patients with PKU, several studies have shown that supplemental BH4 in the form of sapropterin can enhance residual hydroxylase activity [25], particularly PAH [26,27], by improving the efficiency of Phe turnover and lowering plasma Phe concentrations [28,29]. Only a fraction of patients with PKU have enough residual PAH activity, however, to allow sapropterin cofactor binding and stabilization [30]. Hence only 30–50% of patients with PKU respond to sapropterin with a plasma Phe decrease of at least 20% after one month of treatment [31,32].

The primary objective of this study was to determine whether sapropterin, along with corresponding changes in medical diet and plasma Phe, impact monoamine status, either short-term or long-term, for an age- and gender-diverse PKU cohort.

2. Materials and methods

2.1. Study design and patient enrollment

Patients 4 years of age and older with PKU who were planning to try sapropterin dihydrochloride (Kuvan, BioMarin Pharmaceutical Inc.) to improve plasma Phe control were asked to volunteer for a 1-year prospective cohort study. Urinary monoamines were evaluated at 5 time points: at baseline prior to sapropterin treatment; after a 1-month sapropterin trial, when initial response (Responder or Non-responder) was determined to assess short-term impact; at 4 months to differentiate Definitive and Provisional Responders; and at 8 and 12 months after baseline to assess long-term impact. Non-PKU healthy controls were recruited for a single study visit at baseline. Enrollment lasted from October 2008 through October 2009 at the Emory University Department of Human Genetics Clinic, Emory School of Medicine. Participants were asked to provide a 12-hour overnight urine sample and 3-day diet record at each study visit. Fasting blood samples were collected from PKU subjects for evaluation of plasma amino acids (Biochrom 30 HPLC Amino Acid Analyzer) at Emory Genetics Lab. Exclusion criteria for all subjects included pregnancy and breastfeeding and taking sapropterin within the past 8 weeks. Control subjects had to be healthy, with no psychiatric, cognitive, behavioral, or neurological diagnosis in their history or among first-degree relatives.

Study subjects provided written informed consent, or the legal guardian's written informed consent with age-appropriate verbal or written assent, prior to study participation. The study protocol and informed consent procedures were approved by Emory University's Institutional Review Board (IRB).

All PKU study patients were provided sapropterin (20 mg/kg/day) for 1 month to determine plasma Phe response. Patients were instructed by a registered dietitian to continue baseline medical food intake, Phe intake, and other dietary practices, during the 1-month sapropterin challenge. If plasma Phe decreased by at least 15% after 1 month on sapropterin, the patient was classified as an initial Responder and continued sapropterin therapy. Patients with less than 15% decrease in plasma Phe were classified as Non-responders and sapropterin was discontinued, although they were encouraged to continue with study participation. Over the next 3 months, initial Responders were provided with a Phe challenge to determine sapropterin-dependent changes to Phe tolerance (mg of food Phe per day), followed by assessment of decreases in medical food dependence (g of medical food per day). Responders were then classified as "Definitive" if able to increase Phe tolerance and decrease medical food according to previously published criteria [32,33] while maintaining plasma Phe within the therapeutic range. Initial Responders were classified as "Provisional" if unable to increase Phe tolerance according to published criteria [32,33]. At all study visits, PKU subjects met with a registered dietitian who reviewed 3-day food records and assisted with clinical diet needs. Three-day food records were analyzed using

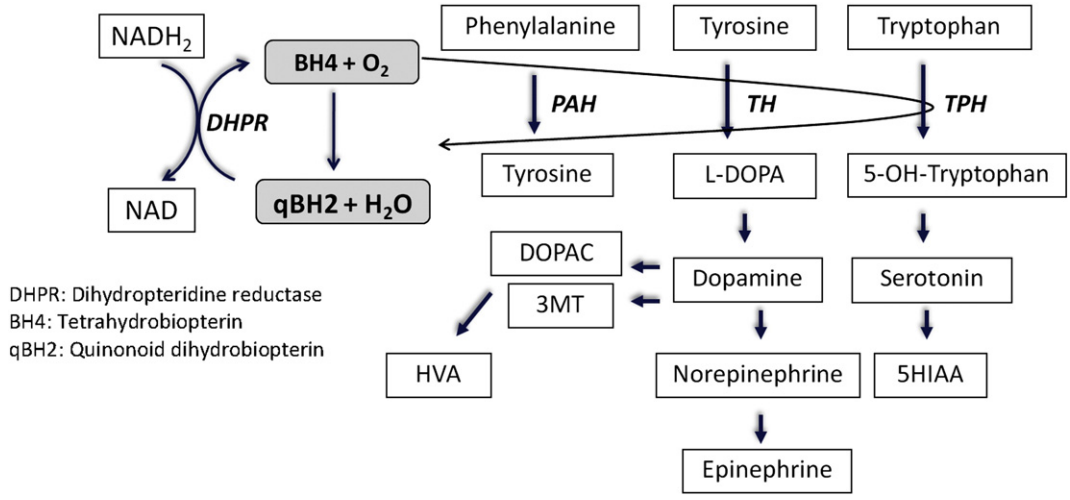

Fig. 1. BH4 within the MA metabolic pathway.
Adapted from prior source [60]