Molecular Genetics and Metabolism 114 (2015) 409–414

Contents lists available at ScienceDirect

Molecular Genetics and Metabolism

journal homepage: www.elsevier.com/locate/ymgme

Individualized long-term outcomes in blood phenylalanine concentrations and dietary phenylalanine tolerance in 11 patients with primary phenylalanine hydroxylase (PAH) deficiency treated with Sapropterin-dihydrochloride ☆

Sylvia Stockler-Ipsiroglu *, Nataliya Yuskiv, Ramona Salvarinova, Delia Apatean, Gloria Ho, Barbara Cheng, Alette Giezen, Yolanda Lillquist, Keiko Ueda

Department of Pediatrics, University of British Columbia, Division of Biochemical Diseases, British Columbia Children's Hospital, Vancouver, Canada

ARTICLE INFO

Article history:
Received 2 October 2014
Received in revised form 20 November 2014
Accepted 20 November 2014
Available online 3 December 2014

Keywords:
Tetrahydrobiopterin
Cofactor
Medical nutrition therapy
Pharmacologic therapy
Outcome

ABSTRACT

We analyzed long-term sustainability of improved blood Phenylalanine (Phe) control and changes to dietary Phe tolerance in 11 patients (1 month to 16 years), with various forms of primary PAH deficiency (classic, moderate, severe phenylketonuria [PKU], mild hyperphenylalaninemia [HPA]), who were treated with 15–20 mg/kg/d Sapropterin-dihydrochloride during a period of 13–44 months.

7/11 patients had a sustainable, significant reduction of baseline blood Phe concentrations and 6 of them also had an increase in mg/kg/day Phe tolerance. In 2 patients with mild HPA, blood Phe concentrations remained in the physiologic range even after a 22 and 36% increase in mg/kg/day Phe tolerance and an achieved Phe intake at 105% and 268% of the dietary reference intake (DRI) for protein. 2 of these responders had classic PKU.

1 patient with mild HPA who started treatment at 2 months of life, had a significant and sustainable reduction in pretreatment blood Phe concentrations, but no increase in the mg/kg/day Phe tolerance. An increase in Phe tolerance could only be demonstrated when expressing the patient's daily Phe tolerance with the DRI for protein showing an increase from 58% at baseline to 78% of normal DRI at the end of the observation.

Long-term follow-up of patients with an initial response to treatment with Sapropterin is essential to determine clinically meaningful outcomes. Phenylalanine tolerance should be expressed in mg/kg/day and/or % of normal DRI to differentiate medical therapy related from physiologic growth related increase in daily Phe intake.

1. Introduction

Phenylketonuria (PKU; OMIM 261600) is an autosomal recessive deficiency of phenylalanine hydroxylase (PAH) resulting in an accumulation of phenylalanine (Phe) in blood and in the brain. Cognitive/behavioral deficiency is prevented by early institution of a Phe restricted medical nutrition therapy [1,2]. However, recommended blood Phe concentrations [3,4] are difficult to maintain over the long-term. Despite early diagnosis via newborn-screening programs, the prevalence of neuropsychiatric (ADHD, anxiety, depression) and executive functioning problems is high in this patient population [5,6]. Poor blood Phe control correlates with the severity of the PKU [7], and is of particular concern in adolescents and adults [8–11] and in children from families with insufficient psychosocial resources [12].

Sapropterin dihydrochloride (Sapropterin, Kuvan®) is a PAH cofactor [13] with the ability to reduce blood Phe concentration and to increase dietary Phe intake. The effect is believed to be due to Sapropterin's chaperon function and its ability to increase the residual enzyme activity. It is estimated that about 30–50% of patients respond to therapy with Sapropterin [14–18] with a decrease in blood Phe concentrations. Patients with milder forms of PKU and higher residual PAH activity seem to be particularly responsive to Sapropterin, but patients with severe/classic PKU may also respond [14,19].

Responsiveness to Sapropterin has been defined as 30% reduction of blood Phe concentrations after an initial Sapropterin trial in a variety of study protocols [17,20–24]. Recently less pronounced reductions have also been accepted [25]. Some authors have shown that a reduction

☆ Contributions of authors to this manuscript: SSI: designed study, wrote manuscript; NY: analyzed data, wrote manuscript; RS, BC, AG, KU, and YL: most responsible for patient care and clinical data monitoring; GH: collected data; and DA: coordinated research.

* Corresponding author at: Department of Pediatrics University of British Columbia, Division of Biochemical Diseases, British Columbia Children's Hospital, K3-204 4480 Oak Street, Vancouver, B.C., Canada, V6H3V4. Fax: +1 604 875 2349.
E-mail address: sstockler@cw.bc.ca (S. Stockler-Ipsiroglu).

http://dx.doi.org/10.1016/j.ymgme.2014.11.014

of blood Phe concentrations during the first 24 h of Sapropterin administration is predictive of a long-term response [26]; others suggest a one month trial to determine a response [27].

Despite an increasing number of outcome studies [18,28–32] information about long-term sustainability of reduced and absolute blood Phe concentrations and dietary Phe tolerance achieved upon treatment with Sapropterin is still limited.

Here we report long-term outcomes of blood Phe concentrations and dietary Phe tolerance in 11 patients in response to therapy with Sapropterin.

2. Patients & methods

2.1. Study protocol

We performed a chart review (Institutional Ethics Review board approval # H12-03598) in 11 patients diagnosed with primary PAH deficiency via newborn screening who had shown a >30% reduction of blood Phe concentrations after one month of treatment with 20 mg/kg/day Sapropterin. The initial response was determined by comparing mean blood Phe concentrations after 1 month of treatment with 1-month pretreatment values. To determine long-term sustainability we investigated whether the initial blood Phe reduction was sustainable, we compared mean 6-month treatment- with 6 month-pretreatment blood Phe concentrations. To determine whether treatment with Sapropterin resulted in a sustainable improvement of Phe tolerance, we compared the dietary Phe intake during the last 3 months of the entire observation period with the 6-month pretreatment Phe intake. The data were collected retrospectively on clinical observations made between 2009 and 2013.

2.2. Patient characteristics

For classification of the severity of the PKU phenotype, we applied generally used criteria based on pretreatment blood Phe concentrations: Classic PKU: >20 mg/dl (>1200 μmol/l); moderate PKU: 15–20 mg/dl (900–1200 μmol/l); mild PKU: 15–20 mg/dl (600–900 μmol/l); mild Hyperphenylalaninemia (mild HPA): <10 mg/dl (< 600 μmol/l); non-PKU HPA: 2–6 mg/dl (120–360 μmol/l) [33].

PAH genotype was not available in the patients included in this study, as mutation analysis historically has not been part of the diagnostic confirmation.

2.3. Standard treatment

Patients were treated according to a standard medical nutrition therapy protocol including a Phe restricted diet, medical Phe-free aminoacid formula and low protein foods with the aim to maintain therapeutic blood Phe concentrations of 2–6 mg/dl (120–360 μmol/l) and allowing for blood Phe concentrations of up to 10 mg/dl (600 μmol/l) in patients older than 12 years.

2.4. Sapropterin treatment

Sapropterin was administered orally once daily with a main meal. Initially all patients were started on 20 mg/kg/day. With increasing weight, dosages down to 15 mg/kg/day were allowed until new adjustments were made.

6/11 patients (P5, P6, P7, P8, P9, P10) received Sapropterin when enrolled in PKU-015 (Biomarin Pharmaceutical Inc), an ongoing open label trial to determine the safety and efficacy of Sapropterin dihydrochloride on blood Phe concentrations and neurocognitive outcomes in children with PKU between 0 and 6 years [34].

2/11 patients (P1, P3) were studied after completion of PKU-016 (Biomarin Pharmaceutical Inc), a randomized controlled trial to determine the safety and effect of Sapropterin on neuropsychiatric symptoms in PKU patients [35]. Because PKU-016 study started with a 13 week-randomized, double blinded treatment period, treatment start for the patients included here, was set arbitrarily at the beginning of the consecutive 13 week-open label phase. 3/11 patients (P2, P4, P11) received Sapropterin as part of clinical care. P11, whose treatment started at 1 month of age, only had a 1 month pre-treatment baseline mean Phe concentration.

2.5. Blood Phe measurements

The number of blood Phe measurements was defined according to the clinic's standard protocol for monitoring dietary control: blood Phe concentrations being measured with a minimum frequency of twice weekly in children younger than 6 months, once weekly in children between 6 months and 18 months, once every 2 weeks in children between 18 months and 12 years, and once monthly after completion of the 12th year of life. During the 6 month-treatment period, blood Phe concentrations were measured in weekly intervals during the first month of treatment with Sapropterin and afterwards according to the clinic's standard protocol.

Blood samples were collected after a 3–6 hour fasting period in children younger than 2 years and after a 6–10 hour overnight fast in older children. Blood Phe concentrations were determined in dried blood spots obtained during regular home-based monitoring, or in plasma from blood samples obtained during a clinic visit. Dry blood spot and plasma amino acid analysis was performed using liquid chromatography–mass spectrometry technology and an amino acid analyzer based on ion exchange chromatography, respectively.

As in our institution blood Phe concentrations are given in mg per deciliter (mg/dl) we will use this unit throughout the text. The conversion factor of mg/dl to μmol/l is 60.4.

2.6. Data analysis

We performed paired *t*-test comparing intra-individual mean blood Phe concentrations before and during treatment. Statistical significance was defined as $p < 0.05$.

2.7. Dietary Phe tolerance

To evaluate the long-term effects of treatment with Sapropterin on dietary Phe tolerance, we assessed dietary Phe intake achieved at the last 3 months of the individual observation treatment period and compared this value with 6-month pretreatment tolerance. We used prescribed dietary Phe intake as an indicator as diet records containing actual dietary Phe intake data was not systematically available.

We determined dietary Phe tolerance in 3 ways: total daily Phe intake expressed as milligrams Phe per day (mg/d); Phe intake per body weight expressed as milligrams per kilo per day (mg/kg/day); and DRI related Phe intake expressed as % normal DRI, comparing the patient's daily Phe intake with the Dietary Reference Intake (DRI) for age related normal peers. Phe norms were derived from the age specific norms of daily protein intake, multiplied by 47, given that 1 g protein = 47 mg Phe. Dietary Reference Intakes (DRIs) of Phe consumption were calculated from age specific recommended dietary allowance (RDA) tables from the Institute of Medicine DRI report [36].

3. Results

3.1. Patient characteristics

7 males and 4 females between 1 month and 16 years of age (median 5 years) were treated for 13–44 months (median 26). At the end of the individual treatment period, patients' age ranged from 2 to 18 years (median 9). 5 patients had classic, 1 had moderate, 1 had mild PKU, and 4 patients had mild HPA. 1 of the 4 patients with mild HPA (P8)