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Sapropterin therapy increases stability of blood phenylalanine levels in patients with BH4-responsive phenylketonuria (PKU)

Barbara K. Burton [a,b,*], Heather Bausell [b], Rachel Katz [b], Holly LaDuca [c], Christine Sullivan [d]

[a] Department of Pediatrics, Northwestern University Feinberg School of Medicine, Chicago, IL, USA
[b] Division of Genetics, Birth Defects and Metabolism, Children's Memorial Hospital, Chicago, IL, USA
[c] Genetic Counseling Graduate Program, Northwestern University Feinberg School of Medicine, Chicago, IL, USA
[d] Children's Memorial Research Center, Chicago, IL, USA

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ABSTRACT

It has recently been demonstrated that variability in blood phenylalanine levels is inversely correlated with IQ and is a better predictor of IQ in early and continuously treated patients with phenylketonuria (PKU) than mean blood phenylalanine levels. This suggests that stability of blood phenylalanine should be a therapeutic goal in patients with PKU. The purpose of this study was to determine if treatment with sapropterin in patients with BH4-responsive PKU would increase the stability of blood phenylalanine levels. The records of all patients treated with sapropterin in the PKU Clinic at Children's Memorial Hospital in Chicago were examined retrospectively. Patients were included in the study if they were responsive to sapropterin during a 2- to 4-week challenge (reduction of blood phenylalanine level of at least 25% after 2 weeks of therapy or, in the case of patients with well-controlled blood phenylalanine at the time of testing, increased dietary phenylalanine tolerance by 4 weeks of treatment). A total of 37 subjects were eligible for inclusion (16 male; 21 female); the mean age was 12.6 years (range, 1.5–32.0). The total number of observations (phenylalanine levels) for all subjects was 1391 with a mean of 39 per subject (range, 13–96). Linear mixed modeling was utilized to estimate variances of the blood phenylalanine before (pre) and after (post) starting sapropterin. Likelihood ratio test was performed using SAS 9.1. Means and standard deviations for phenylalanine as estimated by the model were 6.67 mg/dl (4.20) and post 5.16 (3.78). The mean level post-sapropterin was significantly lower ($p = .0002$). The within-subject variances (mean and SD) of phenylalanine were: pre 6.897 (2.62) and post 4.799 (2.19). These two variances are significantly different with a $p = .0017$. We conclude that sapropterin therapy results in increased stability of blood phenylalanine levels. This effect is likely to improve cognitive outcome in BH4-responsive patients with PKU.

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1. Introduction

The prognosis for infants with phenylketonuria (PKU) has changed dramatically since newborn screening and early dietary treatment for this disorder were implemented in the United States in the mid to late 1960s. Severe mental retardation no longer occurs in this disorder yet there is increasing evidence that many patients continue to suffer from more subtle neurocognitive consequences of their disorder such as defects in executive functioning (1) or mood disorders (2). A meta-analysis of published literature describing IQ in patients with PKU treated continuously from birth with dietary phenylalanine restriction has demonstrated that IQ is inversely correlated with mean blood phenylalanine levels calculated both during the critical period before 12 years of age and throughout the lifetime of the individual [3]. This is true even when blood phenylalanine levels are near or within the accepted treatment range. More recently, it was demonstrated in a study of patients with PKU receiving care at a single clinic that IQ is also inversely correlated with the variability of blood phenylalanine levels [4]. Indeed, in this population, phenylalanine variability was a better predictor of IQ than mean blood phenylalanine level. This finding suggests that stabilization of blood phenylalanine (phe) levels should be a goal of treatment for PKU.

In December, 2007, the United States Food and Drug Administration approved sapropterin (Kuvan®, BioMarin Pharmaceutical, Novato, CA), a pharmaceutical form of tetrahydrobiopterin (BH4) for the treatment of BH4- responsive PKU. Clinical trials had previously demonstrated significant and sustained reductions in

* Corresponding author. Division of Genetics, Birth Defects and Metabolism, Children's Memorial Hospital, 2300 Children's Plaza, Box 59, Chicago, IL 60614, USA. Fax: +1 773 929 9565.
E-mail address: bburton@childrensmemorial.org (B.K. Burton).

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blood phe levels in patients with this disorder treated with sapropterin [5,6]. The goal of the study described here was to investigate the effect of sapropterin therapy on the variability of blood phe in patients with BH4- responsive PKU.

2. Materials and methods

The study was approved by the Institutional Review Board of Children's Memorial Hospital, Chicago, IL. The subjects in the study all had a diagnosis of PKU and were receiving care in the PKU Clinic at Children's Memorial Hospital. All were tested for responsiveness to sapropterin during a 2- to 4-week treatment trial as previously described [7]. Responsiveness was generally defined as a decline in blood phe of at least 30% after 2 weeks of treatment for those subjects with baseline blood phe of at least 3 mg/dl. However, one patient with a decline in blood phe of 25% and improvement in symptoms was also classified as responsive. In our experience, subjects who begin responsiveness testing with a blood phe level below 3 mg/dl often do not show a significant decline in the blood phe level even when they are later shown to have a significantly increased dietary phe tolerance. The reason for this may be suggested by the work of Gersting et al. who showed in a mouse model of BH4-responsive PKU that BH4 only demonstrates a pharmacological chaperone effect at high phe concentrations [8]. Subjects with baseline blood phenylalanine below 180 μmol/l (3 mg/dl)were considered responsive if they demonstrated an increase in dietary phenylalanine tolerance of at least 200 mg/day by 4 weeks of treatment. Patients were on sapropterin therapy for a minimum of 1 year at the time of data collection in September, 2009. All recorded blood phe levels between September 1, 2003, and September 1, 2009, for each subject were included in the analysis. Patients had to have a minimum of six blood phe levels available before and six after starting sapropterin therapy. The length of the pre-sapropterin and post-sapropterin time periods varied among the study subjects, as did the total number of blood phenylalanine determinations included in the analysis. Blood phe monitoring was recommended every 2 weeks for patients under 12 years of age and once a month for patients over 12 years of age (except during the course of responsiveness testing when samples were obtained at baseline, 1 day, 1 week and 2 weeks). Patients were variably compliant with this schedule. Five subjects were started on sapropterin during the course of clinical trials conducted prior to FDA approval of the drug; the remainder were started either during an “expanded access program” made available during FDA review or after FDA approval. All blood phenylalanine determinations were performed in a single laboratory on filter paper blood samples.

Compliance with dietary therapy was monitored by assessment of 3-day diet records submitted with blood samples. Patients were questioned at clinic visits and over the telephone regarding compliance with sapropterin but no pill counts or other formal measures of compliance were used. Most patients were on a phe-restricted diet but individual prescriptions varied widely. In the case of patients on sapropterin therapy, dietary phe intake was increased to the maximum level tolerated while maintaining blood phe level s in the desired range of less than 360 μmol/l (6 mg/dl).

A total of 37 patients (16 male; 21 female) were eligible for inclusion in the study. The mean age of the subjects was 12.6 years (range, 1.5–32.0). Twenty-two of the subjects had mild to moderate PKU; 17 had classical PKU. The mean sapropterin dose for study subjects was 20.1 mg/kg/day. Most subjects were on a single daily dose of 20 mg/kg/day rounded up to the next 100 mg increment.

Linear mixed modeling was utilized to estimate variances of phenylalanine before and after starting sapropterin therapy. Likelihood ratio test was used to evaluate the difference between variability pre- and post-initiation of treatment. SAS 9.1 was used for statistical analysis. Level of significance used was alpha = 0.05.

3. Results

The total number of observations (phe levels) for all patients was 1391 with a mean of 39 per subject (range, 13–96). Subjects were receiving sapropterin treatment for a mean of 19 months (range, 12–31 months) at the time of data collection.

Individual blood phe levels in the pre- and post-saproterin time periods are plotted in Fig. 1. Actual values in mg/dl on the Y axis are plotted against the time of the sample. In Fig. 1A, the time of the sample is days from the starting date of the analysis. In Fig. 1B, the days on the X axis refer to days from initiation of sapropterin therapy. Fig. 2 depicts the variability of each blood sample from the individual patient's own mean value both before initiation of sapropterin (Fig. 2A) and after initiation of sapropterin treatment (Fig. 2B).

The mean and standard deviations of blood phe for all subjects before staring sapropterin was 6.67 mg/dl (4.20); after sapropterin it was 5.16 (3.78). The mean phe level post-sapropterin was significantly lower ($p = .0002$) The within-subject variances of phenylalanine were 6.897 (0.43) pre-sapropterin and 4.799 (0.27) post-sapropterin. These two variances are significantly different with a $p = .0017$ (likelihood ratio test, chi-square = 12.7, $df = 2$).

The study subjects were older during the post-sapropterin period than in the pre-sapropterin period. To rule out the possibility that increased age was the explanation for the findings, the relationship between age and mean phe levels and phe variance was explored. In a model of phe observations as a function of age, phe levels were significantly associated with age, with older ages associated with higher levels of phe (for each 1 year increase in age, phe increases by 0.24 (0.05), $p < .0001$ after adjusting for repeated measurements). With regard to the variance of phe levels, a model which allows for separate variances for each of three age groups, both between patients and for the patients' repeated measurements, shows a clear increase in variance in older subjects (Table 1).

4. Discussion

Despite the remarkable success of newborn screening and early treatment in preventing the mental retardation previously associated with PKU, the outcome for treated patients with this disorder is still not comparable to that of their unaffected siblings or the general population. Mean IQ is decreased [9], problems with executive functioning are common [1], and there is an increased incidence of attention deficit-hyperactivity disorder [10]. In the adult, psychiatric disorders such as depression, anxiety and phobias are seen with increased frequency [2]. There is evidence of decreased autonomy and difficulty in forming stable social relationships [2]. All of these findings suggest the need to focus our attention on interventions that may improve the outcome for patients with this disorder.

To a large extent, the suboptimal outcomes observed in patients with PKU can be attributed to poor control of blood phe levels. As patients grow older, compliance with the phe-restricted diet typically becomes more difficult so that the majority of adolescents and adults have blood phe levels higher than the recommended target range [11]. A meta-analysis of all published literature including both phe levels and IQ measurements has documented an inverse relationship between IQ and mean blood phe levels when either the critical period of birth to 12 years or the lifetime of the individual is considered [1]. This observation underscores the need for improved blood phe control in patients with PKU.

Although mean blood phe level is clearly an important predictor of IQ in patients with PKU and the major goal of PKU treatment is lowering of the blood phe level, a recent study revealed that phe variability was even more highly correlated with IQ than mean blood phe in patients with this disorder [4]. In light of this observation,

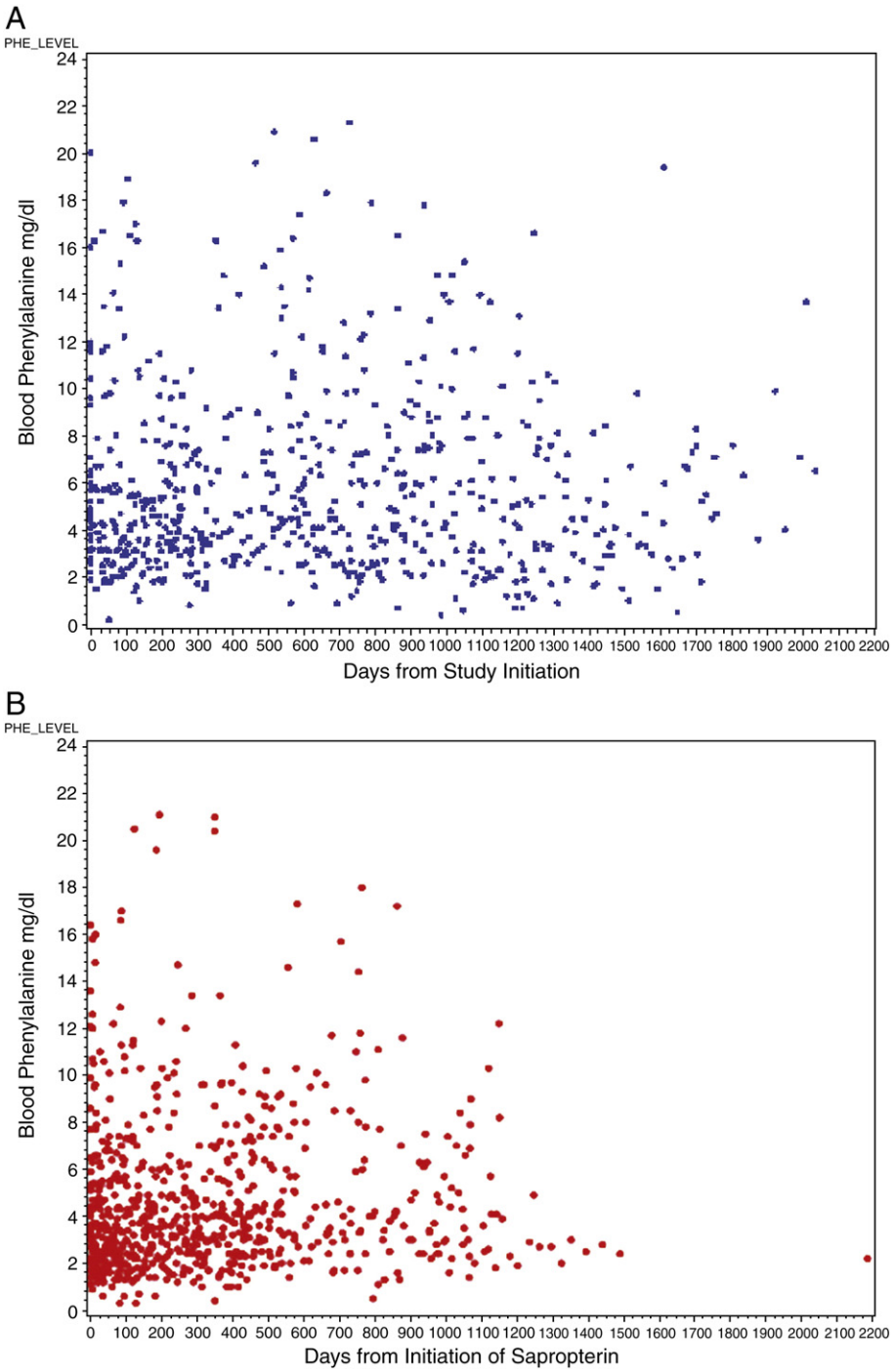

Fig. 1. A. Blood phenylalanine values in study subjects prior to treatment with sapropterin. Each point represents one determination in a single subject. Data for all 37 subjects are included. The X axis represents the date of the sample, expressed as days from the date of study initiation. B. Blood phenylalanine levels in study subjects on sapropterin therapy. Each point represents a single determination on a single subject. Data on all 37 subjects are included. The X axis reflects the date of the sample, expressed as days from initiation of sapropterin therapy.

stabilization of blood phe levels should be an additional goal of treatment.

The data presented here show that blood phe variability is decreased in patients with BH4-responsive PKU on sapropterin therapy. Coupled with the decrease in mean blood phe, this should be associated with improved outcome. The findings observed may actually represent an underestimate of the impact of sapropterin on phe variability since subjects were older in the post-sapropterin period and increasing age was shown to be associated with increasing phe variability.

In summary, treatment with sapropterin was shown to result in a statistically significant decrease in both mean blood phe level and phe variability in patients with BH4-responsive PKU. Both of these changes can be expected to result in improved outcome for sapropterin-treated patients with this disorder.

5. Disclosures

Dr. Barbara K. Burton has received grants for education and research, honoraria and consulting fees from BioMarin Pharmaceutical.

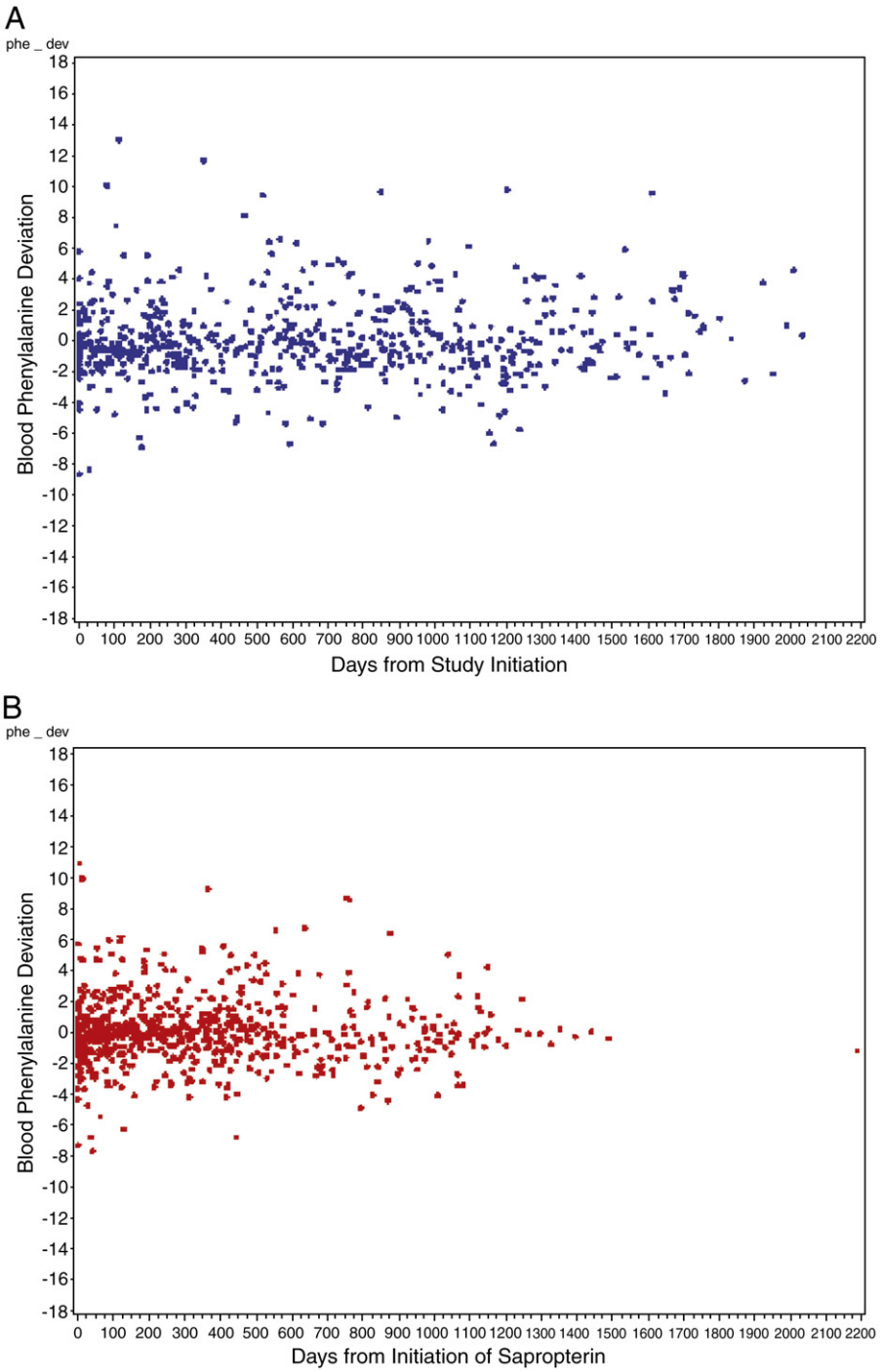

Fig. 2. A. Blood phenylalanine deviations prior to treatment with sapropterin. Each point represents the deviation of a single determination from the patient's own mean value. Data on all 37 subjects are included. The X axis reflects the date of the sample, expressed as days from the date of study initiation. B. Blood phenylalanine deviations on sapropterin therapy. Each point represents the deviation of a single determination from the patient's own mean value. Data on all 37 subjects are included. The X axis reflects the date of the sample, expressed as days from initiation of sapropterin therapy.

Table 1
Variances in blood phenylalanine levels as a function of age.

Comparison	Age group (years)	Estimate of variance
Between subjects	<3	1.4708
Between subjects	3–10	6.2798
Between subjects	>10	7.6354
Within subjects	<3	3.6962
Within subjects	3–10	8.7274
Within subjects	≥10	9.4995

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