

Minireview

Response of patients with phenylketonuria in the US to tetrahydrobiopterin

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Abstract

Tetrahydrobiopterin (BH₄) responsive forms of phenylketonuria (PKU) have been recognized since 1999. Subsequent studies have shown that patients with PKU, especially those with mild mutations, respond with lower blood phenylalanine (Phe) concentrations following oral administration of 6-R-L-erythro-5,6,7,8-tetrahydrobiopterin (BH₄). To determine the incidence of BH₄ responding PKU patients in the United States and characterize their phenylalanine hydroxylase (PAH) mutations, a study was undertaken at UTMB in Galveston and the Children's Hospital of Los Angeles on 38 patients with PKU. Patients were screened by a single oral dose of BH₄, 10 mg/kg and blood Phe and tyrosine were determined at 0, 4, 8, and 24 h. Twenty-two individuals (58%) responded with marked decrease in blood Phe (>30%) at 24 h. Some of the patients that responded favourably were clinically described as having Classical PKU. Blood tyrosine concentrations did not change significantly. Twenty subjects with PKU, responsive and non-responsive to BH₄, were enrolled in a second study to evaluate blood Phe response to ascending single doses of BH₄ with 10, 20, and 40 mg/kg and to evaluate multiple daily doses, for 7 days each, with 10 and 20 mg/kg BH₄. The 7-day trial showed a sustained decrease in blood Phe in 14 of 20 patients taking 20 mg/kg BH₄ (70%). Of these 14 patients, 10 (71%) responded with a significant decrease in blood Phe following 10 mg/kg BH₄ daily. To understand the mechanism of response to BH₄, the kinetics and stability of mutant PAH were studied. We found that mutant PAH responds with increase in the residual enzyme activity following BH₄ administration. The increase in activity is multi-factorial caused by increased stability, chaperone effect, and correction of the mutant Km. These studies indicate that BH₄ can be of help to patients with PKU, including some considered to have Classical PKU. The PKU population in US is heterogeneous and mutations can be varied so mutations need to be characterized and response to BH₄ tested. It is more likely that mutations with residual activity should respond to BH₄, therefore the clinical definition of "Classical PKU" should be reconciled with the residual activity of PAH mutations.

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Introduction

Phenylketonuria (PKU) is caused by phenylalanine hydroxylase (PAH) deficiency [1,2]. The gene for PAH has

been cloned and over 400 mutations have been identified [3–5]. A diet restricted in phenylalanine (Phe) has become the standard of treatment since the discovery of Bickel et al. [6]. Studies show that the restricted Phe diet improves the cognitive function of patients with PKU even beyond childhood [7,8]. Therefore, diet for life has been advocated to prevent functional deficits observed in patients with

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PKU when blood Phe remains elevated. The NIH Conference report recognized that it is difficult to keep blood Phe treatment at 120–360 $\mu\text{mol/L}$ for life [9]. The NIH committee suggested that acceptable blood Phe concentrations after the age of 12 years of 120–900 $\mu\text{mol/L}$ would be acceptable, however, blood Phe concentrations less than 600 $\mu\text{mol/L}$ were encouraged. Improved methods of treatment are desirable in order to maintain blood Phe within the desired treatment range. PAH requires tetrahydrobiopterin (BH_4) as a cofactor. The cofactor BH_4 is synthesized in several cell types, such as liver and brain. Defects in the synthesis or regeneration of BH_4 lead to a severe disease that can be mistaken for PKU [10–14]. Patients with elevated blood Phe are screened to rule out defects in the synthesis or regeneration of BH_4 before they are diagnosed with PKU [15].

Kure et al. [16] in 1999 reported patients with mild PKU that responded with lowering of blood Phe after oral treatment with 6-R-L-erythro-5,6,7,8-tetrahydrobiopterin (BH_4). Other reports of PKU patients responding with lowering of blood Phe to oral BH_4 followed [17–20]. Lindner reported different responses to oral BH_4 in patients with similar genotypes [21]. Weglage described a large series of newborns with HPA given 20 mg/kg BH_4 and only three newborns had a positive response [22]. Matalon et al. reported on PKU subjects studied in the United States and found favourable response in patients, including some classified as classical PKU, in part due to genetic heterogeneity [23–25]. Muntau found that a large number of patients with mild PKU respond to oral BH_4 [26]. Comprehensive reviews of patients that respond to BH_4 are available [27,28]. The purpose of this study was to expand the report of patients with PKU in the United States who respond to BH_4 , characterize their mutations and study the mechanism of response. The heterogeneous population in the US seems to offer a higher response rate to BH_4 compared to other countries, because of the large number of mutations, many with residual PAH activity [29].

Methods

Subjects

The subjects for inclusion in the study required the diagnosis of hyperphenylalaninemia (HPA) and signed an IRB approved consent to join the study. Subjects were labelled as Classical PKU based on blood Phe levels greater than 1200 $\mu\text{mol/L}$ at diagnosis, Atypical PKU when blood Phe levels were >360 and <1200 $\mu\text{mol/L}$ and Mild HPA when blood Phe remained <360 $\mu\text{mol/L}$ while on normal dietary intake. Patients in the United States are often categorized by the blood Phe concentration as genotyping is not routinely available on all subjects with PKU in this Country.

There were 38 patients screened for blood Phe response to a 10 mg/kg oral dose of BH_4 . These subjects had a mean age of 16.5 years (6 months to 43 years). Subjects included 24 Classical PKU, all on dietary treatment, 11 Atypical PKU, seven on dietary treatment and four who discontinued the diet, and three Mild HPA that required no dietary treatment. The subjects had normal synthesis and regeneration of BH_4 and PAH. Genotypes were determined and mutant PAH that responded to BH_4 were studied.

All subjects and their parents were instructed to continue the same dietary Phe prescription as they were on at the initiation of the study. Subjects were screened using a loading dose of 10 mg/kg BH_4 (6-R-L-erythro-5,6,7,8-tetrahydrobiopterin, Schircks Laboratories, Jona, Switzerland). The BH_4 was given by mouth after an overnight fast. Blood Phe and tyrosine were determined at zero time, 4, 8, and 24 h.

Twenty patients with PKU were recruited to a second study regardless of previous response to BH_4 after signing an approved informed consent. There were 11 adults and 9 children with a mean age of 15.7 years (7–44 years). Subjects were counselled to follow their pre-study dietary Phe prescription. Blood Phe and tyrosine concentrations were measured before and 24 h after BH_4 dosing. Subjects were given ascending doses of BH_4 with 10, 20, and 40 mg/kg (part 1) and multiple daily doses, for 7 days each, of 10 and 20 mg/kg BH_4 . The BH_4 was given in a single dose in the morning. There was a one week interval between each loading dose of BH_4 and between the 1 week trial with 10 and 20 mg/kg BH_4 . The 40 mg/kg BH_4 was not given for the weekly trial.

Results

Twenty-two individuals (58%) responded with marked decrease in blood Phe (>30%) 24 h after 10 mg/kg BH_4 . The subjects that responded to BH_4 included 12 of 24 Classical, 8 of 11 Atypical and 2 of 3 Mild (benign) HPA. The mean decline in blood Phe following the 10 mg/kg BH_4 was 36.5% for the Classical PKU patients and greater than 50% decline in blood Phe for Atypical and Mild HPA patients. Table 1 shows the mean blood

Table 1
Response of patients with PKU to 10 mg/kg BH_4 orally^a

| Classical PKU, (n = 12) | | Atypical PKU, (n = 8) | | Mild PKU, (n = 2) | |
|----------------------------------|------|-----------------------|------|-------------------|------|
| Zero | 24 h | Zero | 24 h | Zero | 24 h |
| Mean blood Phe $\mu\text{mol/L}$ | | | | | |
| 955 | 607 | 464 | 205 | 263 | 125 |
| Percent decline at 24 h | | | | | |
| 36.5% | | 55.8% | | 52.5% | |

^a All subjects on a Phe restricted diet except two Atypical PKU subjects and two Mild PKU.

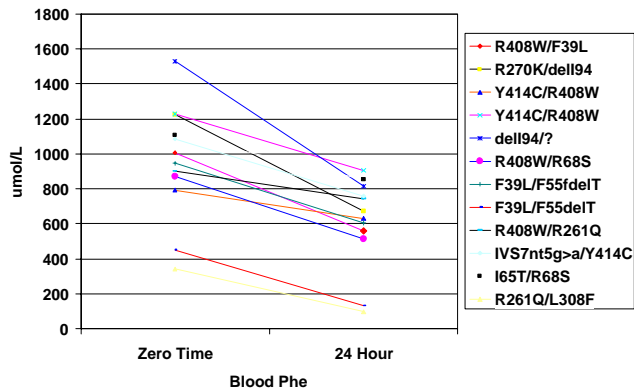


Fig. 1. Positive blood Phe response to BH₄ in patients with Classical PKU.

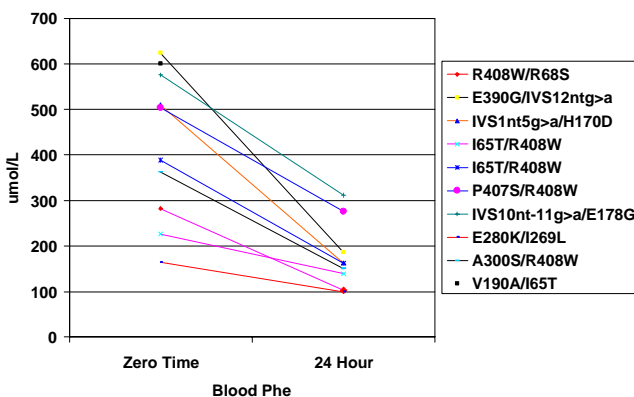


Fig. 2. Positive blood Phe response to BH₄ in patients with Atypical PKU and Mild HPA.

Phe at zero time and 24h for subjects with Classical PKU, Atypical PKU, and Mild HPA and the percent mean decline in blood Phe at 24h. The individual response to BH₄ along with the genotype of each of the responding subjects with Classical PKU is shown in Fig. 1. Fig. 2 shows the individual zero time and 24h blood Phe in Atypical and Mild PKU with the genotypes. Blood tyrosine concentrations did not change significantly.

There were 20 subjects enrolled in the second study regardless of previous response to BH₄. The subjects were given single screening doses of BH₄ at 10, 20, and 40 mg/kg. All 20 subjects (including non-responders) receiving a single dose of BH₄, 10 mg/kg, had a mean decline in blood Phe of $10 \pm 0.26\%$. The mean decline in blood Phe was $17 \pm 0.28\%$ following a single dose of 20 mg/kg BH₄ and $27 \pm 0.25\%$ following 40 mg/kg BH₄. These subjects also took 10 mg/kg BH₄ for 1 week, and after a wash out period, 20 mg/kg BH₄ for a week. Of the 20 subjects, 10 (50%) maintained >30% reduction in blood Phe concentration on 10 mg/kg BH₄ and 14 (70%) showed a similar response on 20 mg/kg BH₄ for the week. Fig. 3 illustrates the reduction in blood Phe at 10 and 20 mg/kg BH₄ in the same PKU subjects.

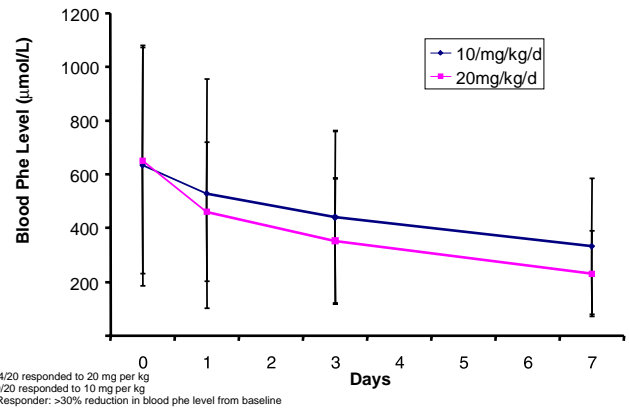


Fig. 3. Mean (SD) blood Phe change after 10 and 20 mg/kg BH₄ daily in BH₄-responders*.

The mutations responding to BH₄ were expressed and compared to the full length human PAH. The mutations were mapped on the PAH monomer and were found through-out the entire protein, including the catalytic domain, the BH₄ binding region and the regulatory domain (F39L, I65T, and R68S). The oligomerization domain had Y414C while the other mutations were in the catalytic domain. All these mutations yielded protein with residual activity above 30% while V388M had 23% residual activity.

Our study of the PAH mutations identified seven novel mutations responding to BH₄; R68S/R408W, F39L/F55fdeIT and F39L/R408W, E178G/IVS10nt-11g>a, L308/R261Q, H170D/IVS1nt5g>a and two subjects with Mild HPA (diet not indicated) had novel mutations A313T and A373T. The kinetic and crystal structure studies indicate that such a response is multifactorial. The Km mutations, which were initially thought to be the majority of the responsive mutations, were few, F39L, I65T, and L308F. A major effect of BH₄ was on the stability of the mutant proteins. Mutation A313T showed slight decrease in affinity to BH₄. The crystal structure study of mutant A313T showed change of BH₄ binding in relation to Asp 315 and Arg 252 and also Ala 309, which seems to increase the enzyme stability with added BH₄ [29].

Discussion and conclusions

This study with single loading and the 1 week open label study showed favourable response in over 50% of PKU patients with a significant decline (>30%) in blood Phe following oral BH₄. In the 1 week study we found more responders with 20 mg/kg BH₄ although 70% of these subjects responded favourable to 10 mg/kg. In future studies the dose of BH₄ needs to be adjusted for the individual patient. Most previous reports showed response to BH₄ in mild PKU phenotypes. In our study

50% of patients categorized as Classical PKU responded with a significant decline in their blood Phe level (>30%). Our data suggest that patients with a clinical definition of Classical PKU need to be challenged with BH₄. The determination of mutations can be of help if one allele has a mutation known to have residual activity. However, we did find a patient with R261Q, a mutant previously reported to respond to BH₄, that did not have a response with 10 mg/kg BH₄ or 20 mg/kg BH₄. Similar to the findings of Lindner et al. and others [21,24,28].

The loading study with BH₄ showed that 58% of the PKU patients responded to the oral BH₄ 10 mg/kg with a significant reduction in blood Phe of >30% of baseline. A decrease in blood Phe greater than 30% of baseline was used as agreed upon at the International Congress of Inborn Errors of Metabolism (ICIEM) in Brisbane, Australia (2002), although Blau et al. have more recently suggested that a significant drop in blood Phe is >50% of baseline. Most publications have used a decline of 30% from baseline as an indication of a significant drop. A decrease in blood Phe of 30% from baseline is a clinically significant level when treating subjects with PKU, although such a drop may indicate that dietary restriction of Phe is also required. The significance of the drop in blood Phe concentrations should ultimately be determined by the treating Physician and the guidelines followed. The NIH Conference guidelines have been useful in this Country to determine what blood Phe concentration should be maintained.

It is important to note that all subjects were following a restricted Phe diet except for four adults with Atypical PKU that had discontinued the diet and three cases of mild PKU, diet not indicated. The surprising finding was that 12 out of 24 (50%) of the patients responding had Classical PKU. The response rate was 73 % (8 of 11) of Atypical PKU subjects and 2 of 3 (66%) of Mild PKU.

This study showed an increased decline in 24 h blood Phe following a single dose of 10, 20 or 40 mg/kg BH₄ in the same 20 subjects. However, the majority of responders can be identified with a load of 10 mg/kg BH₄, 10 of 20 subjects on 10 mg/kg BH₄ compared to 14 of 20 subjects on 20 or 40 mg/kg BH₄. Using a dose of 10 mg/kg identifies most subjects and is more cost effective. The 10 mg/kg dose of BH₄ helped 10 of 20 subjects maintain a >30% decline in blood Phe for 1-week. Patients were instructed to remain on the same diet when enrolled in this open label study. Dietary intake was not collected and evaluated so it is possible that some subjects may have liberalized their diet while on the 1-week trial. Liberalization of diet may result in a less dramatic decrease in blood Phe to BH₄ because of higher dietary Phe intake.

At the molecular level, initial reports of BH₄ responsiveness suggested that such an affect was due primarily to K_m. However, recent studies show that only a few mutations have high K_m values for BH₄, for example,

F39L, I65T, and R68S [24,29]. In an investigation of several BH₄ responsive mutants, the half life of PAH was shorter in several mutant proteins studied than the wild type with the exception of R68S and A300S. A significant increase in half life with BH₄ was found in V388M, Y414C, and some stabilization of F39L and A373T and E390G. In order to further biophysically characterize BH₄ responsive mutants, A313T (Mild PKU) was crystallized and the crystal structure was compared to the wild type PAH. Some differences were found between the wild type and A313T PAH mutant [29] that might explain the increased residual activity of A313T and suggest a molecular explanation for the increased stability when BH₄ is given. Future work on the full characterization of PKU mutations however is needed to more fully understand this phenomenon at the molecular level.

These studies show that the response of patients with PKU to BH₄ is encouraging. A double blind efficacy study should help further our understanding of BH₄ responsiveness, which patients will need monotherapy only and what percentage of patients will see an improved blood Phe control with BH₄ and diet Phe restriction.

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