

Potential Role of Tetrahydrobiopterin in the Treatment of Maternal Phenylketonuria

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ABSTRACT. *Objective.* To evaluate the clinical relevance of tetrahydrobiopterin (BH₄) supplementation for pregnant women with phenylketonuria (PKU)/hyperphenylalaninemia (HPA) and the possibility of treating these patients with BH₄ instead of a phenylalanine (Phe)-restricted diet.

Methods. Genotyping was performed on 41 patients with PKU/HPA identified by newborn screening. Evaluating the genotype according to their BH₄ responsiveness is published. Follow-up of 3 patients with mild PKU treated with BH₄ is evaluated. Discussion of the transfer of these experiences to the possibility of treating mothers at risk for maternal PKU is presented.

Results. In 41 patients with PKU/HPA, we found 17 (41%) bearing at least 1 allele with a mutation described as being responsive to BH₄. In 8 of the patients, BH₄ loading had been performed in the newborn period, in 6 of whom the test showed a clear decrease of blood Phe 4 and 8 hours after loading. One of the nonresponders was reinvestigated at 3 years of age, showing a clear response (genotype Y414C/R408W): BH₄ supplementation resulted in a much higher Phe tolerance (500 instead of 250 mg/day) with blood Phe levels <200 μmol/L. Two children (genotype E390G/IVS10-11g>a and L48S/L48S, respectively) were treated with BH₄ only (15–20 mg/kg body weight/day), one from birth, the other from 2 years of age. Blood Phe decreased from >800 μmol/L to a mean of 321.4 and 331.7 μmol/L, respectively (range: 141–718 μmol/L) under a normal diet (total observation time: 4 years). Development was normal with no adverse reactions.

Conclusions. BH₄ supplementation seems to be a promising alternative treatment in some patients with mild PKU. Because blood Phe levels in maternal PKU should be maintained at 120 to 360 μmol/L, clinical relevance may be even greater than for treatment of children with PKU/HPA. BH₄ supplementation may also be combined with a Phe-restricted diet, allowing higher Phe intake and protecting mothers from high Phe blood peaks. However, additional studies are necessary to prove the safety and economy of such an alternative treatment in patients with PKU/HPA, especially during pregnancy. *Pediatrics* 2003;112:1566–1569; *maternal PKU, diet, hyperphenylalaninemia, tetrahydrobiopterin.*

ABBREVIATIONS, PKU, phenylketonuria; Phe, phenylalanine; HPA, hyperphenylalaninemia; BH₄, tetrahydrobiopterin; PAH, phenylalanine hydroxylase.

Treatment of classical and mild phenylketonuria (PKU) involves a restricted phenylalanine (Phe) diet supplemented by a Phe-free amino acid mixture. Recently, it has been shown that in some patients with non-PKU hyperphenylalaninemia (HPA; blood Phe usually <600 μmol/L), blood Phe responded to high dosages of tetrahydrobiopterin (BH₄), the natural co-factor of the Phe hydroxylase (PAH) enzyme.¹ In these patients, BH₄ deficiency had been excluded and mutational analysis of the *PAH* gene revealed various mild mutations. It was speculated that the mutated enzyme is a K_m variant, which in the presence of high BH₄ concentrations increases residual enzyme activity.² Because patients with these mild mutations resulting in blood Phe <600 μmol/L usually do not need dietary treatment, the practical relevance of BH₄ responsiveness did not seem to be high. However, in women who are at risk for maternal PKU, this may be different, because the International Maternal PKU Collaborative Study has shown that blood Phe levels >360 μmol/L may be harmful to the fetus. There are also reports showing that patients with mild PKU (blood Phe 360–1200 μmol/L) may respond to BH₄ supplementation.^{3–8}

We investigated the possible treatment of maternal PKU with BH₄ supplementation. We first looked into the frequencies of BH₄-sensitive alleles in a sample of 41 patients with PKU/HPA. In addition, we treated 3 patients with mild PKU with BH₄ supplementation to demonstrate the possibility of this treatment.

METHODS

Genotyping of the *PAH* gene was performed in 41 patients with persistent HPA (blood Phe >180 μmol/L) found in the newborn screening program. Allele frequencies were determined for mutations of the *PAH* gene reported in the literature to be responsive to BH₄ loading. Retrospective data for BH₄ loading in the newborn (20 mg/kg body weight; blood Phe measurement at 0, 4, and 8 hours postloading) were available for 8 patients in whom the genotype was indicative of being BH₄ responsive. Response to BH₄ loading was defined as a decrease of blood Phe by at least 30% within 8 hours. Quantitative blood Phe measurements were performed using dried filter spots and tandem mass spectrometry. PKU phenotype was defined as classical PKU (persistent blood Phe >1200 μmol/L), mild PKU (blood Phe between 600 and 1200 μmol/L), and mild HPA (blood Phe <600 μmol/L) under normal nutrition. BH₄ was provided by Schirck's Laboratories (Jona, Switzerland). In all responders BH₄ deficiency was excluded by urinary analysis of biopterin metabolites.

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RESULTS

Table 1 gives an overview of the 15 mutations described to be BH₄ responsive. In 41 patients with PKU/HPA, we found 17 (41%) with at least 1 allele with a mutation described as being responsive to BH₄. In 8 of these, BH₄ loading had been performed in the newborn period, of which 6 showed a clear response of blood Phe 4 and 8 hours after loading and 2 patients showed no response. One of the non-responders (ID 494), who is on a Phe-restricted diet since birth, was reinvestigated at 3.5 years of age and showed a clear Phe decrease even on a more relaxed diet (Fig 1). Details of BH₄ loading in this patient and 2 others in the newborn period are shown in Table 2. Although there was a clear decline of blood Phe in patients ID 230 and 445, patient ID 494 showed no response in the newborn period.

Patient ID 230 was treated from birth with BH₄ supplementation after his favorable response (Fig 2). Blood Phe increased when BH₄ supplementation was stopped at 6 months of age and decreased again when BH₄ was reintroduced at a dosage of 15 mg/kg/day. Since then, the patient is being treated with BH₄, last dosage at 2.5 years of age is 3 times 50 mg/day (~10 mg/kg/day). Blood Phe is being monitored at least 2 times per month. Mean blood Phe level was 321.4 μmol/L (range: 141–718 μmol/L; n = 26). Routine clinical examinations and routine blood chemistry were normal. No adverse effects have been observed. The patient is now 2.6 years of age and developing normally.

In Turkish patient ID 445, who is homozygous for the L48S mutation in the regulatory domain of the PAH gene, as recently published,⁷ parents had problems giving a Phe-restricted diet in the second year of life and stopped the diet. Because blood Phe levels constantly exceeded 600 μmol/L, we decided to introduce BH₄ at a dosage of 3 times 50 mg/day at 2.5 years of age (~8 mg/kg body weight/day). Since

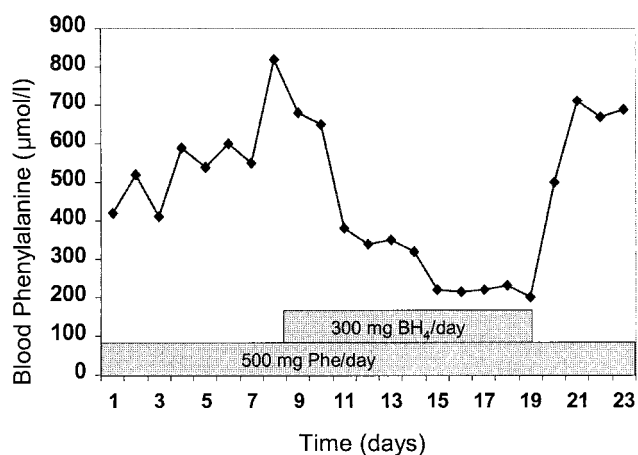


Fig 1. Effect of BH₄ supplementation on blood Phe levels in a patient with mild PKU (genotype Y414C/R408W). The patient was first allowed to relax the Phe-restricted diet to a Phe intake of 500 mg/day from 250, after which 300 mg of BH₄ was given for 5 days while continuing the diet.

then, blood Phe was monitored 1 to 2 times per month; mean blood Phe was 331.7 μmol/L (range: 183–600 μmol/L; n = 17). Routine clinical examinations and routine blood chemistry were normal. No adverse effects have been observed. The patient is now 3.5 years of age and developing normally.

DISCUSSION

In our sample of 41 patients, 41% are BH₄ responsive according to their genotype, indicating the high frequency of BH₄-responsive mutations in HPA/PKU patients. A similar observation was noted in a large retrospective study indicating that >60% of mild PKU and HPA patients may respond to BH₄ administration.⁹ In contrast, Weglage et al¹⁰ in a sample of 87 patients who underwent a BH₄ loading test in the newborn period, found only 3 responding

TABLE 1. List of Mutations in the PAH Gene Found in Patients With BH₄-Responsive PKU/HPA

Allele 1	PAH Enzyme Activity*	Allele 2	PAH Enzyme Activity*	Reference
L48S	39	L48S	39	Blau and Trefz (3)
V190A	nd	R243X	0	Saapen et al (5)
A313T	nd	1099insC	0	Saapen et al (5)
A300C	nd	A403V	32	Saapen et al (5)
R241C	25	A403V	32	Saapen et al (5)
E390G	70	IVS10-11g>a	0	Trefz et al (7)
R241C	25	R111X	0	Kure et al (1)
P407S	nd	R252W	<1	Kure et al (1)
P407S	nd	R111X	0	Kure et al (1)
A373T	nd	IVS4-1g>a	0	Kure et al (1)
R241C	25	R413P	<3	Kure et al (1)
Y414C	28	R408W	<3	Lindner et al (4)
A395P	nd	IVS12+1g>a	0	Lässker et al (4)
R261Q	27	165T	26	Lässker et al (6)
D129G	nd	R408W	0	Hennerman et al (13)
P211T	72	P211T	72	Hennerman et al (13)
S110C	nd	P281L	<1	Hennerman et al (13)
A104D	0	K320N	nd	Steinfeld et al (8)
Y414C	28	Y414C	28	Steinfeld et al (8)
IVS3-22G>a	nd	Y414C	28	Weglage et al (10)
A104D	nd	Y414C	28	Weglage et al (10)

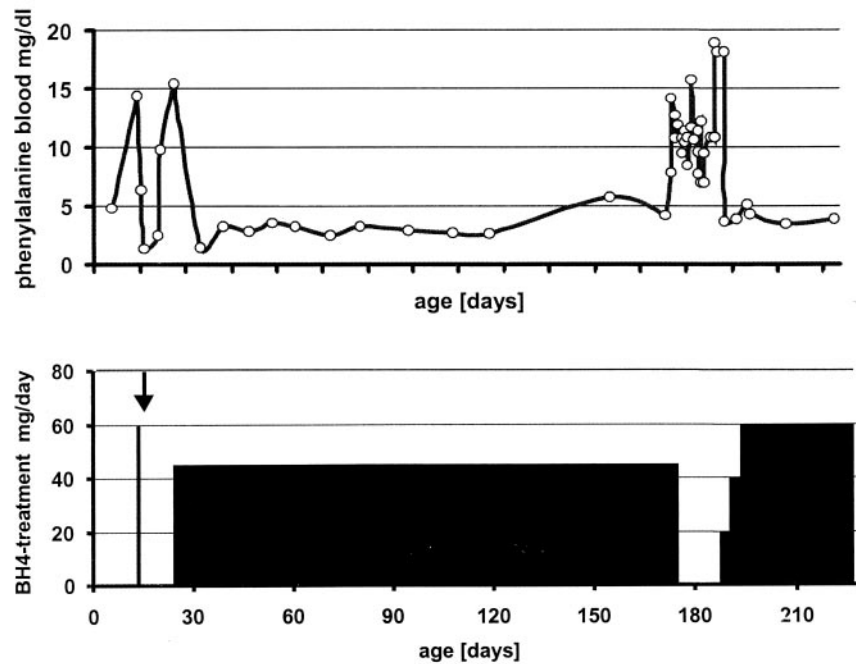
Underlined mutations are proposed to be causative for BH₄ responsiveness.

* of the wild-type activity.

TABLE 2. Summary of Data in 3 Patients With Mild PKU (Blood Phe Levels 600–1200 $\mu\text{mol/L}$) Treated With BH_4 Supplementation

	Patient ID		
	230	445	494
Genotype	E390G/VS10-11g>a	L48S/48S	Y414C/R408W
Phenotype	Mild PKU	Mild PKU	Mild PKU
Ethnic origin	Italian	Turkish	German
Maximal blood Phe ($\mu\text{mol/L}$)	1132	829	1068
Time on BH_4 treatment (10–20 mg/kg/body weight)	1 d–3 y	2.6–3.6 y	5 d–3.5 y
Phe-restricted diet	No	First year	Yes, 250 mg/day
BH_4 loading (newborn) 20 mg/kg			
Phe ($\mu\text{mol/L}$), 0 h	739	707	912
Phe ($\mu\text{mol/L}$), 4 h	584	522	894
Phe ($\mu\text{mol/L}$), 8 h	178	285	1068

Fig 2. Profile of blood Phe in a patient with mild PKU (genotype E390G/IVS10-11g>a) under BH_4 . BH_4 loading after birth and treatment up to 6 months. At 6 months of age, treatment was stopped and reintroduced after 1 week.



to BH_4 . As discussed at a recent BH_4 workshop,¹¹ such retrospective analyses must be interpreted with some caution. In Germany, BH_4 was provided exclusively by Dr Schircks (Jona, Switzerland). Until October 1999, this BH_4 was a mixture of the 6R and 6S forms, thus being only partially biologically active. Using a dosage of 20 mg of fully active 6R- BH_4 per kg body weight, 63%, 64%, 33%, 15%, and 1% of patients with plasma Phe levels of 200 to 400, 400 to 800, 800 to 1200, 1200 to 1600, and >1600 $\mu\text{mol/L}$, respectively, responded significantly by lowering Phe levels by at least 30%.⁹ In addition, the BH_4 test was performed over a period of only 8 hours because this test was originally designed to exclude BH_4 deficiency. Recently, Blau and Muntau¹¹ reported that in some patients with PAH deficiency, effects of BH_4 on blood Phe levels may be observed only over an observation time of up to 24 hours postloading. Also, in 1 of our patients (ID 494), no response to BH_4 was observed in the newborn period, whereas on the same dosage of 20 mg/kg, a clear drop of blood Phe could be demonstrated (Fig 2).

Our single case studies (now almost 5 treatment years) demonstrated that BH_4 provides an alterna-

tive treatment in mild PKU. However, treatment cost may be ~3 to 4 times higher beyond the first year of life than with the conventional Phe-restricted diet with amino acid supplementation.¹¹

Use of BH_4 in the treatment of maternal PKU may be in principle a possibility. However, so far, there is only 1 study available showing that in a case of maternal BH_4 deficiency, BH_4 administration is without any adverse effect on the developing fetus.¹² As demonstrated in patient ID 494, in patients with mild PKU, combining a Phe-restricted diet and BH_4 supplementation is an option. This may increase Phe tolerance to allow an intake of, for example, 500 mg of Phe per day together with 10 to 20 mg/kg/day BH_4 as demonstrated in the above patient. In addition, BH_4 supplementation may “stabilize” the blood Phe profile so that high blood Phe “peaks” (eg, during infections or catabolic status) are avoided. This may play an important role especially during early pregnancy (Trefz et al, this supplement). However, additional studies are necessary to find the optimal dosages in adults, taking into account the high costs of BH_4 .

The exact mechanism of how exogenous BH_4 af-

fects blood Phe in some patients with PAH deficiency is under discussion. Possibly BH₄ supplementation influences the promoter activity of the gene rather than enhancing residual enzyme activity by improving affinity for BH₄.⁷ Differences of this influence on promoter activity in newborns compared with children might also explain the different effects of BH₄ in the same patient.

There is no doubt that the BH₄ loading test should be performed routinely in the newborn with HPA to exclude BH₄ deficiency. To establish BH₄ responsiveness in PKU/HPA patients, this test should be extended to 15 to 24 hours postloading.

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