Minireview

Tetrahydrobiopterin-responsive phenylalanine hydroxylase deficiency, state of the art

Leo J.M. Spaapen,a,* and M. Estela Rubio-Gozalbo,b

a Department of Biochemical Genetics, Academic Hospital, Maastricht, P.O. Box 1475, 6201 BL Maastricht, The Netherlands
b Department of Pediatrics, Academic Hospital, Maastricht, The Netherlands

Received 15 October 2002; received in revised form 10 December 2002; accepted 11 December 2002

Abstract

Since 1999 an increasing number of patients with phenylalanine hydroxylase (PAH) deficiency are reported to be able to decrease their plasma phenylalanine (Phe) concentrations after a 6R-tetrahydrobiopterin (BH4) challenge. The majority of these patients have mild PKU or MHP (mild hyperphenylalaninemia) and harbour at least one missense mutation in the PAH gene associated with this phenotype. The rate of decrease and the lowest achieved Phe level vary between patients with different genotypes but appears to be similar in patients with the same genotype. A number of the mutations associated with BH4-responsiveness have been studied in an ‘in vitro’ eukaryotic cell expression system leading to biosynthesis of a mutant PAH enzyme with some residual activity. Patients bearing mutations that cause severe structural distortion in the expressed protein (loss of function mutations), leading to undetectable PAH activity, are not responsive to BH4. These observations suggest that residual PAH activity (in vitro) is a prerequisite for BH4-responsiveness. However, an in vitro residual PAH activity is not a guarantee for in vivo BH4-responsiveness. Mechanisms behind this responsiveness could be relief of decreased binding affinity for BH4, BH4-mediated increase of PAH gene expression or stabilization of the mutant enzyme protein by BH4. BH4-responsive PAH-deficient patients have only been reported since 1999. For the western countries this is explained by the fact that the manufacturer changed the diastereomeric purity of the BH4 preparation from 69% of the natural 6R-BH4 (31% of 6S-BH4) to 99.5% 6R-BH4. The new findings on BH4-responsiveness may be of clinical relevance because these patients can be treated with BH4 with concomitant relief or withdrawal of the burdensome PKU diet. These observations warrant further clinical studies to assess efficacy, optimal dosage, and safety of BH4 treatment in this group. The data strongly emphasize the necessity of the BH4 loading test in patients detected in the newborn PKU screening.

© 2002 Elsevier Science (USA). All rights reserved.

Keywords: Phenylalanine hydroxylase gene mutations; Phenylketonuria; Hyperphenylalaninemia; BH4-responsiveness

Introduction

Phenylalanine hydroxylase (PAH, EC 1.14.16.1) deficiency leading to mild or severe phenylketonuria (PKU) is one of the most common autosomal recessive inborn errors of metabolism with an average incidence of 1/10,000 [1]. PAH is the rate-controlling enzyme of phenylalanine homeostasis. Phenylalanine, an essential amino acid, is converted to tyrosine in the liver by PAH requiring 6(R)-erythro-5,6,7,8-tetrahydrobiop- terin (BH4) as cofactor. Untreated PKU leads to mental retardation whilst early dietary treatment (phenylalanine restriction) prevents development of mental retardation. PAH deficiency (McKusick 261600) is associated with a wide range of phenotypes for which various classifications are used. Recently, Guldberg and co-workers [2] proposed a classification system for PKU patients based on the data of a European multi-centre study and assigned 105 pathologic mutations in the PAH gene to four arbitrary phenotype categories (Table 1). Otherwise, PKU can be classified on basis of serum Phe levels on unrestricted nutrition: classic PKU (Phe > 1200 µmol/L), mild PKU (Phe 600–1200 µmol/L), and mild HPA (Phe < 600 µmol/L) [3].

Detection of hyperphenylalaninemia (HPA) is included in the newborn screening programs of most western countries. Primary HPA can be either due to a
diminished activity of PAH or deficiency of its cofactor BH₄. The latter group comprises defects in either the biosynthesis or in the regeneration of BH₄. Screening for BH₄ deficiency is generally performed by analysis of pterins in urine and measurement of dihydropteridine reductase (DHPR) activity in erythrocytes or skin fibroblasts [4]. Additionally, in several countries, either a BH₄ loading test or a combined Phe/BH₄ loading test is included in the work-up of HPA [4,5]. So far, BH₄-responsiveness suggested a defect in the biosynthesis or regeneration of BH₄ and prompted further investigations. However, recently, several groups have reported BH₄-responsiveness in patients with defects in PAH, confirmed by detection of pathologic mutations in the PAH gene, and normal BH₄ biosynthesis and regeneration ([6–16], http://data.mch.mcgill.ca/pahdb_new/, Curators Page).

These findings have established the existence of a new sub-group of PAH-deficient patients who potentially can be treated with BH₄.

BH₄-responsive patient’s characteristics

At the EMG workshop in Zurich, June 1st, 2002, BH₄-responsiveness in PAH deficiency is arbitrarily defined as a decrease in plasma Phe of more than 30% of the value before the BH₄ challenge (20 mg/kg body weight) within maximally 24 h post-load [17]. BH₄ deficiency should be ruled out and pathologic mutations in the PAH gene should be established.

BH₄-responsiveness reported in the literature has been assessed in different ways. Newborns have usually been tested using the standard BH₄ loading test [4], however, different doses of BH₄ have been used (10 or 20 mg/kg). In some cases a treatment trial with BH₄ has been carried out. Table 2 lists the phenotype, genotype, way of testing, and percentage of decrease in Phe level in blood after BH₄ loading of BH₄-sensitive PAH-deficient patients known to us (our centre, personal communications and literature). With regard to the rate of Phe decrease and the lowest Phe level achieved, BH₄-responsiveness appears to vary between patients with different genotypes. Fig. 1 shows the time course of the plasma Phe concentration in combined Phe/BH₄ loading tests in 4 MHP patients (own data). These patients can be classified as complete responders because their Phe levels decreased rapidly to quite normal levels within 8–21 h after BH₄ administration. In some of the reported BH₄-responsive patients Phe levels appear to decrease significantly, but do not reach normal concentrations [6,9]. They can be classified as partial responders.

In combined Phe/BH₄ loading tests the possibility of spontaneous elimination of plasma Phe has to be considered. It was reported that after a Phe load of 100 mg/kg in two patients with non-PKU HPA plasma Phe levels returned to pre-load values within 24–48 h [18]. MHP patients who were investigated in our department by a combined Phe/BH₄ (69% 6R-BH₄, 31% 6S-BH₄) loading test in the years before 1999 did not show any or only a slight decrease of plasma Phe within 8 h after the BH₄ challenge. Furthermore, in a Phe challenge test (100 mg/kg) in our first BH₄-responsive patient no spontaneous decrease of plasma Phe was noticed within 6 h post-load [7]. Because these tests did not last more than 8 h, decreases of Phe in 24 h lasting combined Phe/BH₄ tests may be partly due to spontaneous Phe elimination.

Data on BH₄-responsiveness in genotypically identical patients are limited. Three patients with the genotype A300S; A403V (Fig. 1, 30), two patients with the genotype R241C; R413P [6] and two with E390G; IVS12nt18–a ([29]. Scrivener, personal communication) showed however, a similar BH₄ response. A different response in mild PKU patients with the same genotype has been published [9] but the patients appeared to be tested with different BH₄ preparations (see below). Another pitfall in interpretation of results may be caused by inadequate intestinal resorption of the administered BH₄. Most patients have mild or moderate phenotype and harbour at least one missense mutation associated with MHP, mild PKU or moderate PKU, though some classic PKU patients have been reported to be BH₄-responsive [15,16,19]. A number of PAH mutations have been studied by in vitro expression analysis, resulting in expression of mutant PAH protein with more or less enzymatic activity [21]; http://data.mch.mcgill.ca/pahdb_new/).

BH₄-responsiveness has not been found in any patient harbouring two null mutations in either homoallelic or heteroallelic state (Fig. 2). The observations suggest that some residual PAH activity (in vivo) is a prerequisite for BH₄-responsiveness and that mutations causing severe structural distortion (truncation) in the expressed protein, leading to undetectable PAH activity, are not likely to be stimulated by BH₄. However, in vitro expression of mutant PAH enzyme with residual activity does not guarantee BH₄-responsiveness. Two patients with moderate PKU and homozygous for the I65T mutation appeared to be non-responsive to BH₄ (Fig. 2; patient from our centre and patient from Dr. M.A. Vilaseca, Spain, personal communication). The I65T missense mutation, associated with an inconsistent
leads to a PAH enzyme with considerable residual activity (http://data.mch.mcgill.ca/pahdb_new/). Recently, the I65T mutation has been reported to be associated with BH4-sensitivity ([13], quoted in [12,19]). However, the BH4-responsive patient in this report was heterozygous for this mutation, the other being R261Q which most probably is associated with BH4-responsiveness (Tables 2 and 3). Other patients with mild PKU are reported to be non-sensitive [17,20]; one of them with the genotype: R176P; P281L.

Mild PKU A313T; L367fsinsC 20 n.s. (90) [7]
Mild PKU V190A; R243X 20 44 [7]
Mild PKU R158Q; A300S 100 20 57 (77) A
Mild PKU R68S; R68S 20 79 B
Mild PKU Y414C; E280K 20 64 C
Mild PKU R408Q; R408Q 100 20 41 C
Mild PKU Y414C; Del 194 20 88 [10]
Mild PKU E390G; IVS10 +1g->a 20 92 [8]
Mild PKU Y414C; R408W 20 55 [9]
Mild PKU L458S; L458S 20 83 [12]
Mild PKU E390G; IVS11+2g->a 0.7a n.s. [29]
Mild PKU A104D; K320N 5–10a n.s. [11]
Mild PKU Y414C; Y414C 10–20a n.s. [13]
Mild PKU A395P; IVS12nt 1g->a 10a n.s. [13]
Mild PKU D129G; R408W 10 a n.s. [15]
Mild PKU E390G; IVS11+2g->a 20 n.s. (70) E
Mild PKU Y414C; IVS3nt/C0 22g a 20 71 [27]
Mild PKU Y414C; A104D 20 58 [27]
Mild PKU R243Q; Y414C 20 36 [30]
Mild PKU R261Q; E390G 20 69 [30]
Mild PKU A403V; IVS10 +1g->a 20 87 [30]
Mild PKU A403V; IVS10 +1g->a 100 20 64 (85) [30]
Mild PKU R261Q; I65T 10 17 B
Mild PKU R261Q; R158Q 10 17 D
Classic PKU R261Q; E390G 20 17 D
Classic PKU R261Q; Y388M 10 17 D
Classic PKU P281S; P281S 10 17 D
Classic PKU Y414C; R408W 20 a n.s. [15]
Classic PKU Y414C; R252W 20 a n.s. [15]

MHP, mild hyperphenylalaninemia not requiring diet; PKU, phenylketonuria; n.s., not stated.
Sources: A, own patients; B, C, D, E, personal communications, respectively: R.C.A. Sengers, T.J. de Koning, M.A. Vilaseca, C.R. Scriver.
aDaily dose of BH4 in treatment trial.
bThe phenotype is either adopted from the reference or based on the pre-treatment plasma Phe concentration [3].
be positively classified as BH4-sensitive. The other 10 missense mutations have been found in heterozygous combinations (Table 3). These observations strongly suggest that BH4-responsiveness is primarily dependent on the nature of the PAH mutations but other factors may be involved as well.

**BH4-responsiveness: possible mechanisms**

The underlying mechanisms of BH4-sensitivity in PAH-deficient patients remain to be elucidated. Three possible mechanisms are proposed:

### BH4-responsiveness: possible mechanisms

1. **Alleviation of a disturbance in BH4 binding to the PAH enzyme**
   
   A diminished binding affinity for BH4 in the mutant enzymes as suggested by several authors [6–8] may play a role if mutations are located in or near the cofactor-binding regions (CBR). In their structural hypothesis for BH4-responsiveness Erlandsen and Stevens [22] suggested that BH4-sensitive mutations mapped onto the catalytic domain of the PAH gene may be located either in the CBRs or in regions that interact with secondary structures in the protein involved in cofactor binding. The mutations V245A, R261Q, E280K, and P281S are located in the reported CBR #1 and CBR #2. Mutation A300S is supposed to change the shape of the cofactor-binding site (CBR #1) probably leading to decreased binding affinity for BH4 [22]. These mutations would result in mutant enzymes that are Km variants that are still able to bind the cofactor to some extent. BH4 supplementation in these cases may augment the L-Phe hydroxylation reaction by the mutant PAH enzyme.

2. **Regulation of PAH gene expression**

   Blau and Trefz [12] suggested that the BH4-responsiveness in their patient, harbouring mutations (L48S;
L48S) in the N-terminal regulatory domain of the gene, might be caused by a BH4-mediated increase of PAH gene expression. It has been reported, recently, that BH4 regulates tyrosine hydroxylase and phenylalanine hydroxylase gene-expression in a GTP-cyclohydrolase/BH4-deficient hph-1 mouse [23].

Stabilization of the PAH enzyme

BH4 may enhance the stability of mutated homo- and heteropolymeric PAH enzyme molecules in cases of homozygous or hemizygous missense mutations of which one or both are located in the C-terminal tetramerization domain (e.g., Y414C). In patients with functionally hemizygous heteroallelic genotypes (missense/null) the null mutation may result in a severely truncated PAH monomer which has lost capacity of formation of homo- and heteropolymeric enzyme molecules. In this case only homopolymeric PAH molecules in which one amino acid is substituted will be formed. The stability and/or the activity of the latter mutated enzyme may be enhanced by BH4.

BH4-responsiveness: new 6R-BH4 versus old 6R,S-BH4 preparation

The recent finding of BH4-responsive PAH-deficient patients has raised an important question: why are BH4-responsive PAH-deficient patients detected only since 1999?

For the European patients the answer is most probably found in the purity of the BH4 preparation. Before 1999 the BH4 tablets of Dr. Schircks Laboratories (Jona, Switzerland) were composed of 69% of the natural 6R-BH4 and 31% of 6S-BH4. Since 1999 the diastereoisomeric purity of the BH4 preparation has been improved to 99.5% 6R-BH4. The (6R)-L-erythro-dihydroxypropyl side chain of the natural cofactor is critical for many aspects of the regulation of the PAH enzyme. The affinity of 6R-BH4 to rat liver PAH appears to be 2–3 times higher than of the unnatural 6S-BH4 [24]. It is reported however, that 6S-BH4 causes an irreversible inactivation of rat liver PAH [25,26]. Extrapolating these findings to the human PAH makes it quite conceivable that the mixture of the 6R- and 6S-epimer (69:31) would not have enhanced in vivo PAH activity in potentially 6R-BH4-responsive PAH-deficient patients.

This change in preparation might be the reason why Lindner et al. [9] found a different BH4-response in patients with the same genotype (two out of three patients were tested before 1999, personal communication).

Evaluating BH4-responsiveness retrospectively in a large series of PKU patients can lead to an underestimation of the number of BH4-responsive patients if this change in preparation is not taken into account and patients tested before 1999 with the less pure BH4 are included [27]. Already in 1992, 6R-BH4 (~100%, Suntory) was introduced in Japan and the first HPA-patient with a slow and incomplete response was reported at the annual meeting of the Japan Society of Inborn Errors of Metabolism (1992). No abnormalities in biopterin metabolism were found. Mutations in the PAH gene were only detected in 1998 (Prof. Y. Matsubara, personal communication; no abstract in English). The dose of 6R-BH4 used (7.5 mg/kg) may have been too small to detect most of the responsive patients (Dr. M. Yoshino, personal communication). The mentioned effect of the change in purity of the BH4 preparation should lead to consideration of (re-)testing patients diagnosed before 1999.

BH4-responsiveness and clinical relevance

The clinical implication of the discovery of BH4-sensitive PAH deficiency is the opening to a new treatment strategy for a sub-group of PKU patients. In principal this PKU variant is treatable with BH4 with concomitant relief or withdrawal of the burdensome PKU diet. Such a simplification of treatment will improve the quality of life for a considerable number of PKU patients. The group of BH4-responsive PKU patients seems to be quite large. Searching our database with genotyped MHP/PKU patients (partly published in [28]) revealed that 42 out of 85 PAH-deficient patients bear one or two of the mutations associated with BH4-sensitivity from Table 3. In a retrospective study of 1730 MHP/PKU patients 278 were tested with 6R-BH4, the others with 6R,S-BH4. Of the patients tested with 6R-BH4 responsiveness was noticed in approximately 70% of the MHP/mild PKU patients, 25% of moderate PKU patients and 10% of classic PKU patients. Among the others tested with 6R,S-BH4 the number of responsive patients appeared to be low [19]. The authors considered a decrease in plasma Phe of 5% between 0 and 4h and between 4 and 8h post-BH4 load to be positive and a decrease of at least 30% to be significant positive. The possibility that a 5% decrease in Phe concentration may well be within the analytical imprecision of the Phe measurement was not discussed by the authors.

With the present insights the clinical relevance of BH4-responsiveness is limited to PKU patients who require dietary treatment.

There is limited expertise about treatment of these patients with BH4. One of the first BH4-responsive patients has been treated with 5 mg BH4/kg/day for approximately 3 years now, without Phe restriction [7], T.J. de Koning, personal communication). Her plasma Phe levels are continuously around 130 μmol/L. No side effects have been noted. Reports on successful treatment of mild PKU patients appeared from Trefz et al. [8] who supplemented a mild PKU patient with 10 mg BH4/kg/day. Under this regimen the Phe levels dropped from...
934 µmol/L to values between 84 and 222 µmol/L. Their patient is developing normally. Bonafé et al. [14] reported on a mild PKU patient who presented with neurologic impairment. This patient showed both a clinical and a biochemical response on supplementation with 3.5 mg BH4/kg/day. Lindner et al. [9] succeeded in treating a mild PKU patient with 12−15 mg BH4/kg/day divided in three doses, resulting in blood Phe levels constantly between 240 and 360 µmol/L. Another German group has reported on two infants with mild PKU who were treated with 10 mg BH4/kg/day without protein restriction or PKU formulas. This treatment appeared sufficient to keep plasma Phe concentrations below 343 µmol/L. Both infants developed normally [11]. A Swiss patient with mild PKU is supplemented with 50 mg BH4/day leading to Phe levels remaining below 200 µmol/L [10]. Finally, a 25-year-old American woman with mild HPA, presenting with disabling depression and panic attacks appeared to be responsive to BH4 supplementation (100 mg/day). Without protein restriction her blood Phe levels decreased from 630 µmol/L to values between 360 and 480 µmol/L. Maintenance BH4 dosage of 100 mg/day has resulted in significant improvement of depression and panic attacks, with discontinuation of psychotropic medication [29]. However, Weglage et al. [27] reported three patients who were responsive after a loading test with BH4 but who did not respond on ongoing BH4 treatment.

In order to assess BH4-responsiveness the data strongly emphasize the necessity of a 24-h lasting BH4-loading test in patients detected in the newborn PKU screening. However, if a combined Phe/BH4 test is performed and found positive, an additional Phe-loading test (100 mg/kg) is strongly recommended in order to assess the spontaneous elimination of plasma Phe during 24 h after administration of Phe. The presented observations compel to further clinical studies to assess efficacy, optimal dosage and safety of BH4 supplementation in this group of patients.

Acknowledgments

We express our gratitude to Dr. T.J. de Koning, Dr. C.R. Scriver, Dr. R.C.A. Sengers, and Dr. M.A. Vilsenca, for making available their patient’s data. We are grateful to Dr. Matsunaka and Dr. Yoshino for providing information on their experience on BH4-responsiveness in Japan in the period before 1999. Further we thank Mr. W. Loots and Mrs. C. Velter for their excellent mutational analyses.

References


J. Zschocke, Correlation between genotype and BH4-responsivity in PAH deficiency. Phenylketonuria, Present Knowledge and Future Challenges, Elsinore Meeting II, Elsinore, Denmark, June 2002, abstract.


