Assessment of Tetrahydrobiopterin (BH₄) Responsiveness in Phenylketonuria

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Objective  To determine the prevalence of and identify subjects with phenylketonuria (PKU; phenylalanine hydroxylase deficiency) responsive to 6R-tetrahydrobiopterin (BH₄) and to establish selection criteria for potential treatment with BH₄.

Study design  Blood phenylalanine levels from 557 newborns and children with various degrees of PKU (blood phenylalanine, 301 to 4743 μmol/L) challenged with BH₄ (20 mg/kg of body weight) were analyzed at 8 and 24 hours after BH₄ administration. The 2 modalities were compared in terms of phenylalanine reduction.

Results  The overall prevalence of BH₄ responsiveness within patients with PKU for blood phenylalanine reductions of 20%, 30%, 40%, and 50% was 48%, 38%, 31%, and 24%, respectively, using the 8-hour modus and 55%, 46%, 41%, and 33%, respectively, using the 24-hour modus. Using the 30% cutoff, BH₄ responsiveness was similar regardless of the modality in patients with mild hyperphenylalaninemia (79% to 83% responders), mild PKU (49% to 60% responders), and classical PKU (7% to 10% responders).

Conclusions  BH₄ responsiveness is more prevalent than was previously assumed, particularly in patients with mild hyperphenylalaninemia and mild PKU. Depending on the severity of hyperphenylalaninemia, selection criteria for the potential treatment with BH₄ may range from 20% to 40% blood phenylalanine reduction after 24 hours. (J Pediatr 2007;150:627-30)

Phenylketonuria (PKU), the most common inborn error in amino acid metabolism, is caused by mutations in the phenylalanine hydroxylase (PAH) gene. Blood phenylalanine concentration during childhood is the major determinant of cognitive outcome in these patients. Particularly, adolescents and young adults generally do not comply with recommendations for the monitoring and control of phenylalanine concentrations, and 2/3 of pregnant women with PKU in the United States do not follow the diet before becoming pregnant. Recently, it was shown that some patients with PKU respond to the 6R-tetrahydrobiopterin (BH₄)-loading test with decreased plasma phenylalanine concentrations, and that these patients can be treated with BH₄. We found that >60% of patients with plasma phenylalanine concentrations between 400 and 800 μmol/L responded to a BH₄ challenge with decreased blood phenylalanine levels by 30% 8 hours after administration, and that the responsiveness was dose-dependent. Keep in mind, however, that this study used data obtained with both the old 33% less active formulation of BH₄ and the new fully active compound.

Various authors have suggested different criteria for the definition of BH₄ responsiveness using 10 mg BH₄/kg, 6-8 20 mg BH₄/kg, 9-15 or a combined phenylalanine (100 mg/kg) and BH₄ (20 mg/kg) 16-19 challenge over 24 hours. Numerous patients with PKU have currently been on BH₄ therapy for more than 3 years, and BH₄ administration has been shown to increase phenylalanine tolerance in both mild and moderate phenotypes. Thus, replacement of the low-phenylalanine diet with the commercially available BH₄ may significantly improve compliance in patients with PKU and may be an option for better control of blood phenylalanine levels in pregnant women with PKU.

Although most laboratories use a 30% phenylalanine reduction cutoff after a 24-hour challenge as a definition of responsiveness, some clinicians suggest lower cutoffs to assess additional patients. The aim of the present study was to compare 2 loading test modalities (8 hours and 24 hours) to assess the best criteria for the selection of BH₄-responsive patients, based on the BH₄ loading tests (20 mg/kg) performed in 557 newborns and infants with PKU.
METHODS

Patients

A total of 557 patients from Switzerland, Germany, Italy, Austria, Slovenia, Hungary, Turkey, and Israel (age range, 1 week to 7 years; median, 2 weeks) diagnosed with hyperphenylalaninemia (HPA) (blood phenylalanine 301 to 4743 μmol/L) between 2000 and 2006 were investigated for possible BH₄ deficiency. There was an almost equal distribution between females (43%) and males (57%). BH₄ deficiency was excluded in all patients by measuring urinary pterins and dried blood dihydropteridine reductase activity. In all 557 patients, a loading test with 6R-BH₄ (20 mg/kg) was performed over a period of 8 hours. In 293 of the 557 patients, the same loading test was extended to 24 hours. Patients were divided into 3 phenotype groups (mild HPA with phenylalanine levels <600 μmol/L, mild PKU with phenylalanine levels 600 to 1200 μmol/L, and classical PKU with blood phenylalanine levels >1200 μmol/L). This classification is based on phenylalanine levels before the test and may differ from the actual phenotype. PAH gene mutation analysis was done in only a few patients and was not included in this study. All tests were performed as a part of routine investigations at the time of diagnosis and before introducing the diet.

BH₄-Loading Test

Plasma or blood phenylalanine was measured before and 4, 8, and 24 hours after oral administration of 20 mg/kg BH₄ (6R-BH₄; Schircks Laboratories, Jona, Switzerland) as described previously.²⁶ Responsiveness to BH₄ was calculated as a percentage of blood phenylalanine reduction 8 or 24 hours after administration. Phenylalanine concentrations were measured using an amino acid analyzer or tandem-mass spectrometry.

Statistical Analyses

WinSTAT for Excel, version 2003.1 (R. Fitch Software, Bad Krozingen, Germany) was used for descriptive statistics.

RESULTS

The outcome of the 8- and 24-hour loading tests with 20 mg/kg BH₄ in patients with PKU is summarized in the Table (according to initial blood phenylalanine levels) and the Figure (according to phenotype groups). Of 557 patients with various degrees of PKU (301 to 4743 μmol/L), 48% responded to BH₄ administration (20 mg/kg) with at least a 20% decrease in their initial blood phenylalanine levels after 8 hours. Increasing the cutoff for phenylalanine reduction to 30%, 40%, and 50% reduced the sensitivity of the 8-hour loading test to 38%, 31%, and 24%, respectively, in the overall population with PKU.

In 293 of the total of 557 patients, the loading test was performed over 24 hours; the overall responsiveness was higher than in the 8-hour test (55%, 46%, 41%, and 33% of patients with 20%, 30%, 40%, and 50% reduction, respectively). The patients with mild HPA (phenylalanine <600 μmol/L) responded better than those with mild PKU (phenylalanine 600 to 1200 μmol/L) or classical PKU (phenylalanine >1200 μmol/L), regardless of the selected cutoff (20% to 50%) or test modality (8 or 24 hours) (Figure).
Using the currently accepted cutoff of 30% phenylalanine reduction in the 24-hour test, the highest incidence of responders was in patients with blood phenylalanine levels between 301 and 1100 μmol/L (55% to 89%) (Table). About 1/3 of patients with blood phenylalanine levels between 1100 and 1300 μmol/L responded to the same protocol, and only 1% to 15% of patients with blood phenylalanine levels >1300 μmol/L responded to BH4 administration according to the same protocol. Of the 27 patients with blood phenylalanine levels between 1500 and 1800 μmol/L, 4 patients responded to BH4 administration with a 50% decrease in their initial phenylalanine levels.

Although there was an overall consistency in responsiveness between the 8-hour and 24-hour loading test, 8 of the 295 patients in whom the loading test was performed over 24 hours were defined as responders only at 8 hours. In addition, 26 of the 295 patients were responders at 24 hours, but not at 8 hours. All other patients were either responders at both 8 and 24 hours or nonresponders (data not shown). In about 15% of all patients who responded to BH4 with a 20% decrease in initial blood phenylalanine level, the blood phenylalanine level increased by at least 10% from 8 to 24 hours.

**DISCUSSION**

Between 2000 and 2006, a total of 557 patients with PKU were challenged with BH4 (20 mg/kg) and evaluated for possible BH4 deficiency. Although BH4 deficiency was excluded in all of these patients, 38% of the patients responded to BH4 administration with at least a 30% decrease in their initial blood phenylalanine levels after 8 hours, regardless of the phenotype. This prevalence was even higher (46%) when using a 24-hour loading test. Patients with a milder phenotype (phenylalanine levels <1200 μmol/L) were the best responders (49% to 79%). These figures are much higher than previously reported, and reducing the cutoff for responsiveness at 24 hours from 30% to 20% produced 93% of responders within the subgroup of patients with mild HPA (phenylalanine 400 to 500 μmol/L). Although recommendations for treating HPA and PKU differ among countries, patients with mild HPA are not a main target for BH4 treatment.

There remains no consensus as to the gold standard protocol for the diagnosis of BH4-responsive HPA/PKU and the point at which a patient should be defined as a BH4 responder. A 24-hour protocol with 20 mg/kg BH4 is the most commonly used method, and multiple administrations of BH4 and extension of the test up to 1 week may detect additional “slow” responders. Results from different studies are difficult to compare because some used lower doses of BH4 (10 mg/kg) or a combined phenylalanine (100 mg/kg) and BH4 (20 mg/kg) challenge. Some authors use a 20% cutoff, some a 30% cutoff, and some a 50% cutoff at 8, 15, or 24 hours after a single administration of BH4.

Several factors hamper the interpretation of published reports. The first such factor concerns the use of different methods for determining the phenylalanine reduction. Muntz, tau et al, and Okano et al investigated the therapeutic efficacy of BH4 by measuring in vivo rates of [14C]phenylalanine oxidation. Leuzzi et al considered daily fluctuations of basal phenylalanine levels and defined BH4 responsiveness as variations exceeding the individual variability of blood phenylalanine levels. Second, although under normal conditions and in most cases newborns are tested under a normal protein intake, some authors tested patients who were already on a low-phenylalanine diet with or without additional phenylalanine supplementation. The total amount of phenylalanine intake and the resulting blood phenylalanine concentrations seem to be determining factors for BH4 responsiveness. Pay and Martinez demonstrated in an in vitro system that a 5-fold increase in phenylalanine concentrations doubles the Km values for BH4 in the PAH assay. Thus, the higher the blood phenylalanine level, the more BH4 is needed to activate the mutated protein.

Our results demonstrate that a considerable number of patients with classical PKU are responsive to the BH4 loading test, particularly if the cutoff is reduced to 20% (see the Table and Figure). Other authors excluded classical PKU from responsiveness to oral BH4 administration when different criteria were applied. Thus, selection criteria for the phenotype classification may be responsible for the relatively high number of classical PKU responders. Most authors use newborn screening phenylalanine values or fasting phenylalanine concentrations in basal conditions (before the loading test) for the phenotype classification. Tolerance to dietary phenylalanine intake and genotype analysis might be better methods for phenotype classification than blood phenylalanine levels, but even then there is not always a good correlation among these 3 variables.

Evaluation of responsiveness at 8 and 24 hours after BH4 administration revealed a greater number of responders at 24 hours than at 8 hours (see the Table and Figure); for example, 8.9% of patients were responsive at 24 hours but not at 8 hours after BH4 administration. Extension of the BH4 loading test up to 24 hours seems to be fundamental for detecting possible BH4-responsive patients. Nevertheless, some patients (2.7%) were responsive at 8 hours but not at 24 hours after BH4 administration. Based on the present knowledge of BH4 pharmacokinetics, a second administration of BH4 12 hours after the first might further improve the sensitivity of the test and detect slow responders. Although most patients on BH4 receive doses between 5 and 20 mg/kg, and 20 mg is given almost exclusively during the BH4 loading test, another rather high dose of BH4 may enhance enzyme catalytic activity. Accordingly, we suggest modifying the BH4-loading test to include a second administration of 10 to 20 mg/kg BH4 at 12 hours, with assessment of plasma phenylalanine levels at 0, 8, 12, and 24 hours after BH4 administration.

In summary, the cutoff level for BH4 responsiveness depends on the patient's clinical phenotype, being lower for patients with classical PKU (>20% phenylalanine reduction) and higher for those with mild or moderate PKU (>30%...
phenylalanine reduction). Patients with mild PKU may replace a phenylalanine-restricted diet with BH₄ monotherapy, whereas those with moderate or classical PKU may combine BH₄ with a less-strict diet regimen. A 20% to 30% reduction in plasma phenylalanine can be significant in a patient with classical PKU, because it may allow the patient to relax his or her strict dietetic regimen in association with BH₄ therapy. Well-designed long-term studies are needed to compare short-term (BH₄ loading test) and long-term (BH₄ therapy) responsiveness.

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REFERENCES