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ORIGINAL ARTICLE

Efficacy and safety of BH4 before the age of 4 years in patients with mild phenylketonuria

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Abstract
Background Sapropterin dihydrochloride, an EMEA-approved synthetic formulation of BH4, has been available in Europe since 2009 for PKU patients older than 4 years, but its use with younger children is allowed in France based on an expert recommendation. We report the cases of 15 patients treated under the age of 4 years and demonstrate the safety and efficacy of this treatment for patients in this age group.
Patients and method We report the use of BH4 in 15 PKU patients treated before the age of 4 years.
Results Fifteen patients were enrolled in this retrospective study. Mean phenylalaninemia at diagnosis was 542±164 μM and all patients had mild PKU (maximal phenylalaninemia: 600-1200 μM). BH4 responsiveness was assessed using a 24-hour BH4 loading test (20 mg/kg), performed during the neonatal period (n=11) or before 18 months of age (n=4). During the test, these patients exhibited an 80±12% decrease in phenylalaninemia. Long-term BH4 therapy was initiated during the neonatal period (n=7) or at the age of 13±12 months (n=8). The median duration of treatment was 23 months [min

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References to electronic databases: Phenylketonuria, OMIM#261600

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7; max 80]. BH4 therapy drastically improved dietary phenylalanine tolerance (456±181 vs 1683±627 mg/day, p<0.0001) and allowed a phenylalanine-free amino acid mixture to be discontinued or not introduced in 14 patients. Additionally, in the eight patients treated after a few months of diet therapy, BH4 treatment significantly decreased mean phenylalaninemia (352±85 vs 254±64μM, p<0.05), raised the percentage of phenylalaninemia tests within therapeutic targets [120-300 μM] (35±25 vs 64±16%, p<0.05), and reduced phenylalaninemia variance (130±21 vs 93±27μM, p<0.05). No side effects were reported.

Conclusion BH4-therapy is efficient and safe before the age of 4 years in mild PKU, BH4-responsive patients.

List of abbreviations

PKU Phenylketonuria
Phe Phenylalanine

Introduction

Phenylketonuria (PKU, MIM#261600) is an autosomal recessive disorder characterized by the defective conversion of phenylalanine (Phe) to tyrosine, responsible for a toxic accumulation of Phe in the blood and brain leading to severe mental retardation. Its prognosis has considerably changed since newborn screening and early diet therapy were introduced in the 1970s. The goal of PKU treatment is to maintain Phe levels within the recommended target range for age, notably during the first decade of life (Feillet et al 2010, Haute Autorité de Santé 2010). This is classically achieved using a Phe-restricted diet, associating natural foods (for the Phe intake), a mixture of amino acids, and low-protein foods. However, dietary treatment is very restrictive and may be associated with nutritional deficiencies or poor compliance. Furthermore, the outcome for treated patients remains suboptimal, with some degree of cognitive impairment, difficulty in executive functions, psychological and psychiatric problems, and an increased incidence of attention deficit-hyperactivity disorder, thus confirming the need to optimize metabolic control (Antshel 2010; Brumm et al 2010; Christ et al 2010).

Sapropterin dihydrochloride (Kuvan®, Merck Serono®), a synthetic formulation of BH4 approved by the EMEA in 2008, offers a new therapeutic option for the management of PKU patients. Instead of reducing precursors of "toxic" Phe, i.e. natural protein intake, the aim of this treatment is to increase residual activity of Phe hydroxylase, presumably by a pharmacological chaperone action, and thus to improve Phe tolerance (Muntau and Gersting 2010). In BH4-responsive patients, this treatment allows natural protein consumption to be increased, improving therapy compliance and metabolic control (Burton et al 2010). Before 2009, a chemical form of BH4 (Schircks® product) was available. Since 2009, a soluble tablet formulation of BH4 has been available in Europe. However, its utilization is restricted to PKU patients aged over 4 years, because the initial clinical trials used for the EMEA approval included only subjects aged 4 years and older. The major difference between the European and the US label (obtained from the FDA in 2008) is that there are no age restrictions for the use of BH4 in the United States. Despite this authorization, its utilization remained limited in younger patients, particularly during the first months of life (Burton et al 2011). As BH4 has been used for many years in France for patients younger than 4 years old, and in view of the US label, the use of BH4 from the neonatal period was added to the recent French guidelines published in 2010 (Feillet et al 2010, Haute Autorité de Santé 2010). We report here our experience of BH4 therapy with 15 PKU patients under the age of 4 years, demonstrating its beneficial effects and the safety of this treatment for patients in this age group.

Patients and method

Patient screening and collected data

We conducted a retrospective study of PKU patients treated with BH4 before the age of 4 years. Patients were screened in eight PKU centres. A questionnaire was sent to each centre to identify PKU patients treated with BH4 before the age of 4 years and to collect retrospective data of these patients. All the PKU patients included in the study were found to be BH4-responsive on a 24 h BH4 loading test and were treated with BH4 before the age of 4 years. BH4-responsive patients treated above this age or treated for a primary BH4 deficiency were excluded. BH4 responsiveness was assessed using a 24 h BH4 loading test (single dose of 20 mg/kg); the patient was declared responsive when the Phe level decreased by 30% or more during the test. Collected data included: (i) patient characteristics, (ii) PKU genotyping when available, (iii) results of the 24 h BH4 loading test (20 mg/kg), (iv) plasma Phe levels from neonatal screening and during follow-up (before and during BH4 therapy) and plasma amino acid profile, (v) data from long-term BH4 therapy, including age of initiation, dose and any side effects, (vi) dietary records including Phe tolerance, the use of a Phe-free amino acid mixture, and any additional treatment. Safety and efficacy of long-term BH4 therapy were evaluated by collecting data on side effects related to treatment, and by comparing Phe intake tolerance and metabolic control before and during BH4 therapy. As eight patients were treated by conventional diet therapy before BH4 therapy was initiated, we compared their Phe tolerance and metabolic control under conventional diet therapy and under BH4 treatment.

Statistical analyses

Results are expressed as means ± SD, median [min-max], or frequencies and percentages when appropriate. The variance of Phe levels before and during BH4 treatment was estimated from the standard deviations for Phe levels of each patient during the corresponding periods. Statistical analyses were performed using GraphPad Prism version 4.0 (GraphPad Software, San Diego, CA, USA). Wilcoxon matched pairs test and repeated measures ANOVA were applied for continuous variables, and categorical variables were analyzed using the Chi-square test. A p-value <0.05 was considered statistically significant.

Results

We identified 15 PKU children born between 2004 and 2010 and treated with BH4 before the age of 4 years. Patient characteristics are summarized in Table 1. All were diagnosed by neonatal screening and had mild PKU, defined by maximal phenylalaninemia between 600 and 1200 μM reached before the age of three months.

BH4 responsiveness defined by BH4 loading test

A positive response to BH4 was assessed in all the patients by a 24-h BH4 loading test, using a single oral dose of 20 mg/kg. Responsiveness was defined as a reduction of more than 30% in blood Phe levels, in line with current recommendations (Blau et al 2009). In fact, Phe levels in all these patients reached therapeutic targets, i.e. ≤300 μM, in spite of normal Phe intake. This test was performed during the neonatal period (median age 21 days [min 8; max 30]) prior to initiation of diet therapy for 11 patients. For the other four patients, the 24-h BH4 loading test was performed at the age of 7, 12 (n=2) and 18 months, three days after starting an oral Phe load designed to increase basal phenylalaninemia prior to treatment. The Phe load was performed by increasing either the natural protein intake (3 g/kg/d) or the Phe intake alone (Phe capsules, 100 mg/kg/d) and was maintained throughout the 24-hr BH4 loading test. Compiled results from these tests are summarized in Fig. 1. The basal level of phenylalaninemia was above 480 μM for all the patients (H0: 638 ± 176 μM). The BH4 loading test induced a drastic decrease in phenylalaninemia of more than 30% at H8 for all the patients. This decrease occured at H4 for 12 patients. At H12, blood Phe level was always lower than 230 μM. Compared to H0, the maximal decrease in phenylalaninemia was about 80 ± 12%. BH4 responsiveness was also confirmed by PKU genotyping performed in ten patients (Table 2), demonstrating the presence of either one (n=5) or two (n=5) mutations previously reported to be associated with mild PKU phenotype and BH4 responsiveness (taken from BIOPKU database, http://www.biopku.org).

Table 1 Characteristics of the PKU patients treated with BH4 before the age of 4 years

Characteristics of the patients	
N	15
Gender male (%)	7 (47%)
Age at enrolment (months)	39 ± 27
Phenylalaninemia at neonatal screening (μM)	354 ± 151
Phenylalaninemia at diagnosis (μM)	542 ± 164
Maximal phenylalaninemia (μM)	825 ± 205
Long-term therapy with BH4	
Age at initiation (months)	4.6 [0.3-35]
Mean duration of treatment with BH4 (months)	23 [7-80]
Daily dose of BH4 (mg/kg/day)	20 [8-24]

Results are expressed as number of patients (%), mean ± SD or median [min-max]

Long-term therapy with BH4

Long-term therapy with BH4 was initiated either during the neonatal period (n=7), or later (n=8, mean age 13 ± 12 months [min 5; max 35]). In four patients tested during the neonatal period, long term BH4 therapy was started later, as soon as it was available in the form of medication (i.e. Kuvan®) for two patients and because of poor compliance and metabolic control with diet alone in the other two patients. For the other four patients who were also treated later, BH4 testing and long-term treatment were proposed because of unsatisfactory metabolic control with diet alone and presumably poor regimen compliance. Initial treatment used for the oldest patients was

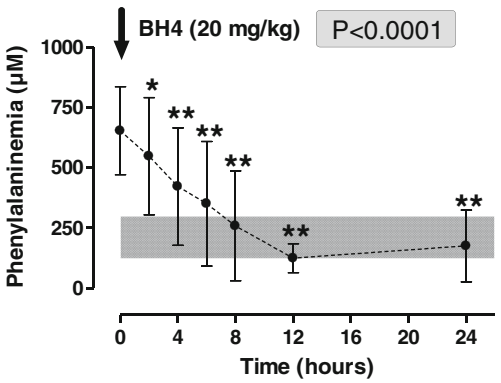

Fig. 1 BH4 responsiveness defined by 24-h BH4 loading test. Positive response to BH4 was assessed using a 24-h BH4 loading test, using a single oral dose of 20 mg/kg, performed during the neonatal period (n=11) or later (7, 12, 12 and 18 months respectively). Recommended target range for age (120-300 μM) is represented by the grey area. Repeated measures ANOVA: p<0.0001, Bonferroni post test: *p< 0.05, **p<0.001 versus H0

Table 2 Genotype and phenotype of the PKU patients treated with BH4 before the age of 4 years

Patient	Allele 1	Allele 2	[Phe] at diagnosis (μM)	Maximal [Phe] (μM)	Maximal decrease of [Phe] (%)	Phe tolerance before BH4 therapy (mg/d)	Phe tolerance during BH4 therapy (mg/d)
P1	L48S*	L48S*	401	1117	54%	600	2400
P2	L48S*	L48S*	497	1188	84%	780	2400
P3	E390G*	G272X*	815	876	79%	500	1600
P4	E390G*	V388M*	654	732	88%	450	1950
P5	E390G*	S349P	696	696	82%	220	2200
P6	A300S*	S349P	361	660	69%	600	2200
P7	V177M*	W187X	446	711	60%	800	2200
P8	A403V*	R252W*	744	1068	89%	480	1800
P9	E390G*	S349P	317	654	78%	300	800
P10	Y414C*	IVS10-11G>A	780	1152	95%	200	2400

Data about PKU genotype were available for ten patients. Long-term therapy with BH4 was initiated after several months of conventional diet therapy for the first seven patients, or during the neonatal period for the last three patients. The maximal decrease in phenylalaninemia during the 24-h BH4 loading test is expressed as a percentage of the basal phenylalaninemia value (prior to BH4 load). The level of phenylalanine tolerance before or during BH4 therapy was defined as the maximal phenylalanine intake (mg/d) associated with phenylalaninemia results within therapeutic target values. [Phe]: phenylalaninemia; Phe: phenylalanine; *mutations previously reported to be associated with BH4 responsiveness (taken from BIOPKU database, http://www.biopku.org)

BH4 from Schircks® laboratory (n=5); following its approval in 2008, all the patients were then treated with sapropterin dihydrochloride (Kuvan®, from Merck Serono®). Median daily dosage of BH4 was 20 mg/kg/d, with extreme values ranging from 8 to 24 mg/kg/d. The median duration of treatment was 23 months [min 7; max 80]. No side effects were reported in any patients.

BH4 increased phenylalanine tolerance

BH4 therapy drastically improved dietary Phe tolerance, with a four-fold increase in Phe intake, from 456 ± 181 mg/d to 1683 ± 627 mg/day (P<0.0001, Fig. 2). Concomitantly to the increase in natural protein intake, metabolic control remained acceptable, with a mean phenylalaninemia of 240 ± 72 μM and 71 ± 18% of Phe values within therapeutic targets (120 to 300 μM). Furthermore, the increase in natural protein intake allowed Phe-free amino acid mixture to be discontinued (n=7) or not introduced (n=7). The amino acid mixture was maintained in only one patient with a prescribed moderate Phe-restricted diet (Phe-intake 1000 mg/d), but whose therapeutic compliance was doubtful with a presumably more restricted natural protein intake. Only a few plasma amino acid profiles were available for each patient. Levels of branched chain amino acids and tyrosine did not differ significantly before and during BH4 therapy and remained within the normal range.

Compared to diet therapy, BH4 improved metabolic control

Eight patients were treated with BH4 after several months of conventional diet therapy (mean duration 13 ± 12 months). This allowed us to compare metabolic control before and after initiation of treatment with BH4. Apart from the large increase in oral Phe intake (from 550 ± 190 to 1754 ± 632 mg/d, p< 0.01), BH4 also improved metabolic control, as reflected by the decrease in mean phenylalaninemia (before BH4 352 ± 85 μM vs during BH4 therapy 254 ± 64 μM, p<0.05, Fig. 3). Indeed, the number of phenylalaninemia values within therapeutic targets increased significantly from 35 ± 25% to 64 ± 16% (P<0.05, Fig. 4), whereas the number of values higher than the therapeutic range decreased from 61 ± 30% to 29 ± 22% (p<0.05). Furthermore, BH4 therapy also increased the stability of blood Phe levels, as reflected by a significant 30% reduction in phenylalaninemia variance, from 130 ± 21 μM to 93 ± 27 μM (p<0.05). Taken together, these results suggest a better metabolic control of PKU with BH4 therapy than with conventional diet therapy.

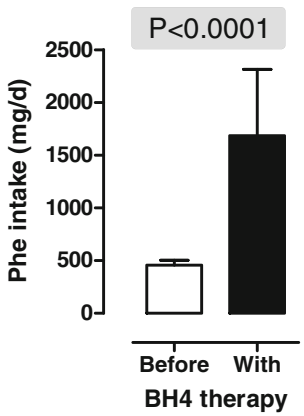

Fig. 2 Phenylalanine tolerance before and during BH4 therapy. Diet Phe tolerance calculated from the maximal oral Phe intake associated with adequate metabolic control before and during BH4 therapy in 15 PKU patients

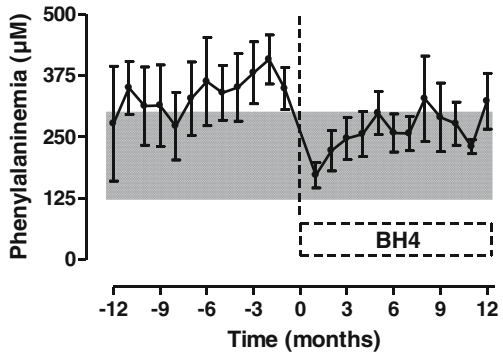

Fig. 3 Blood phenylalanine levels in eight PKU patients before and during BH4 therapy. Metabolic control of eight patients treated with BH4 after several months of conventional diet therapy. For each patient, data were averaged every month, from 12 months before to 12 months after initiation of BH4 therapy. The mean blood Phe level during BH4 therapy was significantly lower than during conventional diet therapy (243 ± 75 vs 331 ± 76 µM, $p<0.05$). Recommended target range for age (120-300 µM) is represented by the grey area

Discussion

Sapropterin dihydrochloride is a synthetic formulation of BH4, the naturally occurring phenylalanine hydroxylase cofactor, approved by the EMEA in 2008 for the treatment of PKU. According to EMEA label, its use is restricted to PKU patients older than 4 years, because the initial clinical trials on which EMEA approval was based were only conducted with subjects aged 4 years and older. Furthermore, its use in the United States also remains limited in younger patients, particularly during the first months of life. In fact, the US label authorizes the use of BH4 at any age, but its use in younger patients is restricted for practical reasons (Burton et al 2011). Conversely, maintaining Phe levels within therapeutic targets is critical during the first years of life for the normal development of neurocognitive and

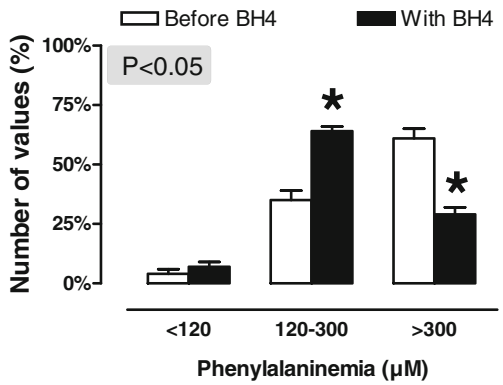

Fig. 4 Distribution of blood phenylalanine levels related to therapeutic targets in eight PKU patients before and during BH4 therapy. Blood phenylalanine levels of eight patients (see Fig. 3 for details) were distributed as within therapeutic targets (120-300 µM), low (<120 µM) or high (>300 µM) values, and expressed as a percentage of the number of values for each patient. Global Chi-square test: $p<0.05$, post test: *$P<0.05$, before versus during BH4 therapy

behavioural functions (Brumm and Grant 2010; Feillet et al 2010; Gassio et al 2005; Viau et al 2011). In France, the use of BH4 is allowed at any age, even during the neonatal period, on specialist recommendation (Feillet et al 2010, Haute Autorité de Santé 2010). We report here our experience of BH4 therapy before the age of 4 years, demonstrating the safety and efficacy of this treatment for patients in this age group. This retrospective study, performed in eight French PKU centres, identified 15 PKU patients treated with BH4 before the age of 4 years between 2004 and 2010. It should be noted that for older patients (n=5), responsiveness testing and long-term therapy were initiated with BH4 from Schircks® laboratory and later switched to sapropterin dihydrochloride (Kuvan®) from Merck Serono®.

BH4 responsiveness was assessed using a 24-h loading test using a single dose of 20 mg/kg of BH4 performed during normal diet (Blau et al 2009). This BH4 loading test procedure, in contrast to the FDA-approved algorithm which takes from one to four weeks, allowed us to perform this test during the neonatal period prior to starting diet therapy in most of the patients. In fact, this rapid neonatal test does not delay metabolic care by more than 24 hours and does not require an oral Phe load preceding BH4 administration, as is the case in later testing, thanks to the high levels of Phe in the neonatal period (Feillet et al 2008). This protocol is in line with the optimized BH4 test recently proposed in Europe (Blau et al 2009). It also allowed us to start long-term BH4 therapy during the neonatal period in seven patients, instead of a low-Phe diet. All our patients were found to be BH4-responsive. The mean decrease in phenylalaninemia was about 80 ± 12% and allowed all the patients to reach the therapeutic target Phe levels. This could represent total responsiveness, allowing all our patients to be treated solely with BH4 as recommended in recent French guidelines (Haute Autorité de Santé 2010). PKU genotyping was performed in ten patients and identified the presence of either one (n=5) or two (n=5) previously reported mutations associated with BH4 responsiveness, suggesting some correlation between genotype and BH4-responsive phenotype (Blau and Erlandsen 2004). However, it is currently assumed that response to BH4 is not limited to the presence of a single "BH4-responsive" mutation but results from the combination of the two allelic mutations (Blau and Erlandsen 2004; Karacic et al 2009). Genotyping alone cannot clearly identify BH4-responsive patients, and a clinical BH4 loading test is indispensable to assess responsiveness.

All the patients enrolled in this retrospective study were treated with BH4 before the age of 4 years and for a prolonged period, with a median duration of 23 months, range 7 to 80 months. Treatment was initiated in the neonatal period for half of the patients, and at a mean age of 13 months for the others. No adverse effects were reported for any of the patients, confirming the safety of BH4 therapy for patients in this age group. In fact, data in the literature about BH4 therapy in

young PKU patients is very limited, and is restricted to isolated case reports or small series of patients (Belanger-Quintana et al 2005; Boveda et al 2007; Burton et al 2011; Hennermann et al 2005; Lambruschini et al 2005; Shintaku et al 2004). For example, in the recent series reported by Burton and colleagues, only two patients were treated before the age of 3 years (Burton et al 2011). These reports support our findings of increased Phe tolerance and better metabolic control with BH4 therapy, with no severe side effects.

Long-term BH4 therapy was associated with significantly increased Phe tolerance, from 456 ± 181 to 1683 ± 627 mg/day ($P<0.0001$, Fig. 2), as previously shown in paediatric patients over the age of 4 years (Trefz et al 2009). In this area, previous studies have clearly demonstrated the nutritional advantages of increasing natural protein in the diet, notably for bone mineral density, which remains a major issue in adulthood (Adamczyk et al 2011; Modan-Moses et al 2007; Roato et al 2010). Furthermore, this increase in natural protein intake allowed Phe-free amino acid mixture to be discontinued or not initiated in all but one patient. Overall, increased natural protein consumption and the absence of amino acid mixture should simplify the regimen and improve dietary compliance and quality of life for patients and their family.

Besides increasing Phe tolerance, long-term therapy with BH4 also improved metabolic control, as demonstrated in the eight patients treated with BH4 after several months of conventional diet therapy. In these patients, the increase in Phe intake was associated with significantly lower mean phenylalaninemia with a greater number of values within the recommended target range with BH4 therapy, compared to metabolic control with diet therapy alone (Figs. 3 and 4). Maintaining control of Phe levels, especially during the first years of life, has clearly been shown to prevent neurocognitive impairments (Gassio et al 2005; Viau et al 2011). However, a recent meta-analysis reported suboptimal outcomes in patients with PKU treated early with conventional diet therapy alone, with persistent neurocognitive and psychosocial difficulties, bone and nutritional impairment, and decreased quality of life, suggesting that there is a need to optimize metabolic control (Enns et al 2010). Our data also shows that BH4 therapy increased the stability of blood Phe levels, as reflected by a significant 30% reduction in phenylalaninemia variance, from 130 ± 21 μM to 93 ± 27 μM ($p<0.05$). A recent study demonstrated that early and continuously diet-treated PKU children had suboptimal neurocognitive outcomes, as measured by full-scale IQ testing, in spite of well-controlled blood Phe levels (Anastasoaie et al 2008). These outcomes correlate with blood Phe varying within the recommended ranges. Attention should thus be given not only to maintenance of Phe levels within the recommended range but also to its variability (Viau et al 2011). Our results suggest that these goals may be achieved with the sole use of BH4 therapy in these fully responsive PKU patients. Some of these beneficial effects have been reported previously in older patients treated with BH4 (Burton et al 2010). However, this improvement in metabolic control is of greater importance in younger patients, given that it is clearly related to a better neurological and nutritional outcome (Anastasoaie et al 2008; Gassio et al 2005). This improved metabolic control with the use of BH4 can be explained by its proposed mode of action. In fact, the aim of conventional diet therapy is to limit Phe intake, i.e. natural protein intake, to compensate for the reduced ability to break down Phe related to Phe hydroxylase deficiency. In contrast, the aim of BH4 therapy is to restore Phe hydroxylase activity, and thus to improve the body's ability to metabolize Phe. In fact, in responsive patients, BH4 acts as a pharmacological chaperone, restoring enzyme function by improving protein folding and stabilizing the protein structure [for review, see Ref. (Muntau and Gersting 2010)]. Indeed, increasing Phe hydroxylase activity may improve Phe tolerance and tyrosine status, stabilize blood Phe levels and reduce episodes of high phenylalaninemia.

However, the results of this study should be interpreted with caution. Firstly, the beneficial effects reported with BH4 is limited to responsive patients, who were all mild PKU patients in our study, while metabolic control and long-term outcomes are more challenging for classic PKU patients. In fact, significant reductions in blood Phe levels in response to BH4 have been observed principally in patients with relatively mild hyperphenylalaninemia phenotypes, whereas most PKU patients do not respond to this treatment (Blau et al 2009). Nevertheless, substituting diet therapy with BH4 treatment remains a very attractive option, even if it is limited to patients with a mild phenotype. Secondly, we recognize that some minor side effects may have been missed, or that the levels of maximal Phe tolerance may have been underestimated, particularly before BH4 therapy, due to retrospective data collection. On the other hand, most of the patients, particularly the eight patients treated with BH4 after several months of conventional diet therapy, had numerous phenylalaninemia results above the therapeutic ranges prior to BH4 therapy, suggesting that the maximal Phe tolerance had been reached. Finally, we cannot rule out the possibility that some degree of liver immaturity, which resolves with age, contributed to the observed metabolic improvement, notably in patients treated from the neonatal period. In our study, BH4 therapy was temporarily discontinued in three patients after the age of 2 years, with a huge and immediate increase in blood Phe levels, confirming the efficacy of BH4 in these patients. Further studies should investigate the response to BH4 after the age of 2 or 3 years to confirm the severity of hyperphenylalaninemia and responsiveness to BH4.

In summary, this study demonstrated the safety and efficacy of BH4 therapy in mild PKU patients treated before the age of 4 years. BH4 responsiveness can be assessed using a 24-h BH4 loading test which can be performed during the neonatal period prior to starting Phe-restricted diet therapy. In

responsive patients, long-term treatment with BH4 increased Phe tolerance and improved metabolic control, with no adverse effect. Indeed, this treatment simplified the management of patients with milder forms of PKU and may improve their quality of life.

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