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RESEARCH REPORT

Long-Term Pharmacological Management of Phenylketonuria, Including Patients Below the Age of 4 Years

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Abstract BH4 therapy is an advancement in the treatment of phenylketonuria, reducing blood phenylalanine (phe) levels and increasing tolerance to natural proteins of responding patients. We report the results of 16 patients undergoing long-term BH4 treatment. Responding patients to BH4 was usually based on 24-h loading tests; a $\geq$30% decrease in blood phe was considered a positive response. Weekly loading made it possible to identify an additional "slow responder." The 16 responders constitute 24.6% of patients who completed the trial (87.5% of responders in mild hyperphenylalaninemia, 38.1% in mild PKU, and 2.8% in classical PKU).

Mean dose of BH4 used was 9.75 $\pm$ 0.9 mg/kg per day, during a mean of 62 months. Age at treatment start was below 4 years in seven patients; five of which begun treatment during their first month since birth. All but one patient showed good treatment compliance; six continue on BH4 monotherapy without dietary phe restriction; six showed an increase in phe tolerance of 24–55%; and in the five patients who received treatment since the neonatal period an increase in phe tolerance following the phase of maximum growth has persisted. None of the patients showed side effects except one whom vomiting at the beginning of the treatment.

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Testing at the time of diagnosis in the neonatal period is very appropriate, and if there is a positive response, the patient can be treated with BH4 from onset with the advantage of being able to continue breast-feeding.

Introduction

Phenylketonuria (PKU) is an autosomal recessive inborn error of metabolism resulting from a deficiency of phenylalanine hydroxylase (PAH:EC 1.14.16.1), the liver enzyme that catalyzes the hydroxylation of phenylalanine (Phe) to tyrosine. This enzyme deficiency leads to elevated levels of Phe in the blood and to other tissues, as well as to corresponding neurotoxic effects. Untreated patients are characterized by mental retardation, microcephaly, delayed speech, seizures, eczema, and behavior abnormalities.

There are different phenotypes of PKU resulting from a mutated PAH: mild hyperphenylalaninemia (HPA), mild PKU, and classical PKU, which are classified on the basis of plasma Phe levels at the time of diagnosis as well as protein tolerance.

Adherence to a low-Phe diet from birth is effective for preventing mental retardation; however, it is very restrictive and limits the quality of life of patients and their families. Since Kure's study in 1999 (Kure et al. 1999), a number of reports have demonstrated the existence of a subset of patients with mutations in the PAH gene who show a positive response to tetrahydrobiopterin (BH4), the natural cofactor of PAH, thus reducing their plasma Phe levels.

This has facilitated important new treatment possibilities for a significant proportion of HPA and PKU patients; in many cases, BH4 treatment (with a dose of 5–20 mg/kg per day) allows these patients to follow a near normal diet, or at least, a less rigid low-protein diet.

A tablet formulation of BH4 (dihydrochloride) has been available for three decades. Although this formulation has been used extensively in experimental studies, it has not been evaluated in formal clinical trials and was not recorded. A newer formulation of BH4 (sapropterin dihydrochloride, Kuvan®) for the treatment of PKU that is more stable at room temperature has been available in the USA and Europe (Burnett 2007) since 2008; in Spain, the Ministry of Health authorized this product for commercial use in 2009. Clinical studies suggest that treatment with sapropterin provides better Phe control and increases dietary Phe tolerance (Belanger-Quintana et al. 2005; Burlina and Blau 2009; Burton et al. 2011; Blau et al. 2010; Vernon et al. 2010; Trefz et al. 2010). No serious adverse events with BH4 treatment were reported in medical literature (Belanger-Quintana et al. 2005; Nielsen et al. 2010); headache, upper respiratory tract infections, and rhinorrhea were the side effects observed in sapropterin-treated patients with PKU in clinical trials (Kuvan® 2008; Trefz et al. 2009a).

In a previous work (Bóveda et al. 2007), we reported a study of response to oral BH4 loading in a group of 36 patients with PKU, and subsequent treatment and follow-up of patients who responded positively in this test. Here, we describe the evolution of patients in the PKU group, with different phenotypes, who responded to BH4 and are currently being treated with sapropterin (Kuvan®) in the Metabolic Disorders Unit of the University Clinical Hospital of Santiago de Compostela (Galicia, Spain).

Patients and Methods

Our Metabolic Disorders Unit is monitoring a total of 107 patients diagnosed with HPA and with different phenotypes: 36 classical PKU (Phe $>$ 1,200 μmol/L, tolerance $\leq$ 400 mg/day), 21 mild PKU (Phe between 600 and 1,200 μmol/L, tolerance $\leq$ 500 mg/day), 8 mild HPA (Phe between 360 and 600 μmol/L, tolerance up to 650 mg/day), and finally 42 benign HPA (Phe $<$ 360 μmol/L, free diet); the latter are subjected to Phe level monitoring, but are not subjected to dieting and, therefore, are not the object of this paper.

Of these patients, 16 responders are being treated with BH4: one classical PKU, eight mild PKU, and seven mild HPA. Table 1 lists the characteristics of the 16 patients who are currently being treated with sapropterin: phenotype, plasma Phe concentration at diagnosis, type of diagnosis (by newborn or late screening), genotype, 24-h BH4 loading (age at testing, % reduction at 24 h).

The screening of the group of patients who responded to BH4 was based on loading with cofactor: 24-h testing with 20 mg/kg of BH4 with previous loading of a dose of 100 mg/kg of phe and baseline Phe endpoints at 4, 8, 15, and 24 h. This screening was started in 2002, since then, BH4 loading is carried out in the neonatal period when the level of Phe is over 400 μmol/L, and there is no previous loading with this amino acid.

A decrease of $\geq$ 30% in blood Phe is considered to be a positive result.

Patients who showed a 25–30% response in the 24-h underwent to a weekly BH4 loading test. We carried out the following protocol: dose of 20 mg/kg per day of BH4, administered for 1 week, with daily Phe-level measurement. A 30% or higher reduction in blood Phe levels by the end of the period was considered to be a positive response.

Responsive patients were treated with BH4 since testing, including those under the age of 4 years who were administered the drug after requesting compassionate treatment. Informed consent was requested from all parents or patients of legal age. Response to BH4 treatment was measured, assessing dietary tolerance to phe and blood Phe levels in treated patients; this response was then correlated with its genotype. Side effects were assessed by asking about headache, vomiting, abdominal pain, rhinorrhea, or other symptoms after BH4 treatment.

Results

A total of 24.6% (16/65) of patients with HPA (excluding patients with benign HPA) who were treated in the Diagnosis and Treatment Unit at the University Clinical Hospital of Santiago de Compostela received BH4 pharmacological treatment (6R-BH4 from Dr. Schircks Laboratories, Jona, Switzerland) and since 2009, Sapropterin dihydrochloridre (Kuvan®) as the single treatment in addition to diet. Age range at the beginning of treatment varied from 1 month to 24 years since birth.

Phe levels decreased by $\geq$ 30% in these 16 patients after 24-h or weekly BH4 loading, except in patient no. 4, who is a sibling of responsive patient no. 3 with similar mutations and phenotypes and whom was administered treatment directly, without loading tests. Patient no. 14, who at 2 years and 5 months underwent combined Phe/BH4 loading test (Bóveda et al. 2007) with a 28% decrease of Phe level after 24 h, continued dietary treatment and at 8 years 6 months underwent a weekly BH4 loading test, with a 34% decrease in phe levels, since then, pharmacological treatment was added to dietary treatment.

Table 2 shows the evolution of the group of patients with PKU treated with sapropterin; the table shows, tolerance and mean Phe levels before and after treatment with sapropterin, cofactor dose in each case, and time to treatment evolution.

All of them began BH4 treatment at a dose of 5–10 mg/kg per day increasing to a maximum of 20 mg/kg per day; this dose

Table 1 Characteristics of patients treated with sapropterin: phenotype, type of diagnosis (newborn or late screening), genotype, 24-h BH4 loading (age at testing, % reduction at 24 h), current age, and psychomotor development (PDI/IQ)

Patient	Phenotype	Diagnosis	Genotype	Age at BH4 testing	% Phe reduction 24 h	Actual age	Current PDI/IQ
1	Mild HPA	NBS	p.R243Q(c.728 G>A) / p.E390G (c.1169 A>G)	5 y 3 m	64.8	12 y 6 m	74
2	Mild HPA	NBS	p.P211T(c.631 C>A) / IVS10nt-11 G>A(c.1066-11 G>A)	1st month	64.2	4 y 11 m	88
3	Mild HPA	late (4 y)	p.S303A(c.907 T>G) / p.G46S (c.136 G>A)	9 y 2 m	90.2	16 y 5 m	99
4	Mild HPA	NBS	p.S303A(c.907 T>G) / p.G46S (c.136 G>A)	n/a	n/a	12 y 7 m	105
5	Mild HPA	NBS	n/a	1st month	64.2	1 y 3 m	110
6	Mild HPA	NBS	p.R176L(c.527 G>T) / –	3 y 9 m	76	11 y	97
7	Mild HPA	NBS	p.Y277D(c.829 T>G) / p.L48S (c.143 T>C)	1st month	60	4 y 5 m	103
8	Mild PKU	NBS	p.R68S(c.204 A>T) / IVS10nt-11 G>A (c.1066-11 G>A)	1st month	60	2 y	93
9	Mild PKU	NBS	p.Y277D(c.829 T>G) / p.L48S (c.143 T>C)	1st month	57.1	4 y 5 m	97
10	Mild PKU	NBS	p.R158Q(c.473 G>A) / p.L48S (c.143 T>C)	6 y 10 m	43	14 y 4 m	106
11	Mild PKU	late (6 y)	p.Q304Q (c.912 G>A) / p.R176L (c.527 G>T)	16 y 5 m	80.7	22 y 4 m	120
12	Mild PKU	NBS	p.V388M(c.1162 G>A) / p.L48S (c.143 T>C)	18 y 5 m	33.6	24 y 9 m	115
13	Mild PKU	NBS	p.V388M(c.1162 G>A) / p.R243Q (c.728 G>A)	13 y 10 m	37.1	18 y 8 m	118
14	Mild PKU	NBS	p.I65T(c.194 T>C) / p.R243X (c.727 C>T)	2 y 5 m	28	9 y 6 m	120
15	Mild PKU	NBS	p.P244L(c.731 C>T) / p.R261Q (c.782 G>A)	9 y 7 m	31.9	16 y 6 m	122
16	Classical PKU	NBS	p.G46S(c.136 G>A) / p.R243Q (c.728 G>A)	16 y 4 m	40.3	22 y 11 m	97

n/a not analyzed*; y* years*; m* months

was adjusted based on later dietary tolerance and phe levels, thus a mean dose of 9.75 ± 0.9 mg/kg per day of sapropterin (range 8–12.5) was maintained in our patients. A few days after confirming positive response to BH4 loading, all patients began treatment; 31.2% (5/16) began treatment during their first month since birth. Mean treatment duration was 5 years 2 months; duration in each of the three patients who have been receiving treatment the longest was 7 years and 3 months.

The five patients who began treatment during the neonatal period (numbers 2, 5, 7, 8, and 9), three mild HPA and two mild PKU, with phe levels at the time of treatment between 403 and 1,066 μmol/L, showed a decrease of more than 30% after 8 h and more than 50% after 24 h since the 24-h BH4 loading test. Treatment with BH4 enabled tolerance to 550 mg/day of phe, and all patients maintained optimal levels of phe (mean 171 ± 98, medium 150, range 135–195 μmol/L). As far as their genotype, except for one patient who was not studied, the remaining patients are compound heterozygous with at least one BH4-responsive allele (Zurflüh et al. 2008). Anthropometric parameters remain between p50 and p90 in all of them with a normal nutritional status.

The 11 patients who began treatment after the neonatal period showed at least a 24% increase in phe tolerance, except for patient no. 12 who was not taking the drug regularly. This was clearly apparent in the four patients with mild HPA since they all moved to a near-normal diet with recommended maximal Phe intake of 100 mg/kg per day (i.e. "controlled free diet"), presenting a tolerance of Phe from 2,000 to 3,000 mg/day by age. In the seven patients with mild PKU and classical PKU, the increase in tolerance was more fluctuating, from 24% to more than 50%, one patient was even able to relax dietary restrictions with a 3,000 mg/day tolerance of Phe. In addition, phe levels in these patients remained slightly higher before BH4 (mean 412 ± 154, medium 303 μmol/L) than after BH4 (mean 403 ± 136, medium 394 μmol/L) and stayed within the appropriate range for their age, except in one patient with classical PKU whose levels remained within the highest

Table 2 Evolution of the 16 patients with different phenotypes treated with sapropterin

Patient	Phenotype	Phe level (μmol/L) and tolerance (mg/day) pre-BH4			Phe level (μmol/L) and tolerance (mg/day) post-BH4			BH4 dose mg/kg per day	Treatment duration
		Mean ± SD	Medium	Phe tolerance	Mean ± SD	Medium	Phe tolerance		
1	Mild HPA	254 ± 104	242	640	273 ± 65	279	2,000[a]	10	7 y
2	Mild HPA	n/a	n/a	n/a	192 ± 84	182	880	8.9	4 y 10 m
3	Mild HPA	208 ± 80	188	580	182 ± 35	182	3,000[a]	8	7 y 3 m
4	Mild HPA	222 ± 84	212	600	233 ± 63	212	2,200[a]	10	7 y 3 m
5	Mild HPA	n/a	n/a	n/a	135 ± 35	127	1,100[a]	10	1 y 2 m
6	Mild HPA	230 ± 127	194	650	164 ± 42	151	2,000[a]	9.4	7 y 3 m
7	Mild HPA	n/a	n/a	n/a	170 ± 111	139	800	10.4	4 y 4 m
8	Mild PKU	n/a	n/a	n/a	195 ± 142	179	565	10	1 y 11 m
9	Mild PKU	n/a	n/a	n/a	164 ± 118	127	750	10	4 y 4 m
10	Mild PKU	290 ± 145	273	430	310 ± 158	324	739	9.5	6 y 6 m
11	Mild PKU	418 ± 127	394	500	396 ± 127	376	3,000[a]	9.7	6 y
12	Mild PKU	356 ± 151	303	400	559 ± 64	539	500	9	6 y 9 m
13	Mild PKU	497 ± 139	509	350	409 ± 212	427	480	9.4	5 y 1 m
14	Mild PKU	241 ± 139	212	390	216 ± 81	194	513	12.5	1 y
15	Mild PKU	288 ± 105	285	470	269 ± 88	254	750	9.3	6 y 11 m
16	Classical PKU	794 ± 273	891	350	666 ± 224	648	780	10	6 y 7 m

n/a not analyzed; *y* years; *m* months

[a] Controlled free diet

recommended limit; however, in this patient mean values decreased after BH4 treatment. The genotype is quite varied; patients no. 10, 12, 13, and 15 have two different BH4-responsive alleles, the remaining patients have at least one BH4-responsive mutation. In our opinion, p.G46S mutation may be classified as responsive in light of the findings by Wang et al. (2007).

Patient no. 14, who was not responsive to the 24-h test but was responsive to the weekly test, with one year of evolution and BH4 treatment at 11.5 mg/kg per day, showed tolerance to 513 mg/day (31% more) with 10% lower mean phe endpoint values for that year. This patient has a BH4 responsive mutation (p.I65T) in one allele and a mutation that is classified as nonresponsive in the other (p.R243X).

One additional late-diagnosed adult with a severe phenotype and an E280K mutation in homozygosis was tested with a week-long protocol due to difficulties with dietary compliance. He experimented a reduction of 34% in Phe levels and has been treated with a low BH4 dose, showing mild improvement in his Phe tolerance but much greater dietary compliance and refers less mood disorders after a period of one year in treatment. As the long-term responsiveness of this patient is still under evaluation, we have not included his data on the tables.

None of the patients showed BH4 treatment-related secondary effects, except patient no. 12 whom vomiting at the beginning of the treatment.

Discussion

At this time, there is no single protocol for considering a patient to be BH4 responsive (Blau et al. 2010). The 24-h protocol with 20 mg/kg of BH4 with or without combination phe 100 mg/kg is most commonly used (Blau 2008); this is also the protocol we used in most of our patients; since 2003 we applied this test without Phe overload in the neonatal period, as many authors advise (Feillet et al. 2008). We know that BH4 only demonstrates a pharmacological chaperone effect at high Phe concentrations (Gersting et al. 2010), however, BH4 test conducted in our neonates with values of phe not very high (between 403 and 636 μmol/L) showed significant results.

We administered a weekly test with 20 mg/kg of BH4 in one patient with a response that was very close to positive (between 25% and 30% decrease in phe levels) and in this patient phe levels decreased by 34%; therefore, the patient was deemed responsive.

There are recent recommendations for carrying out baseline 48-h loading tests (Blau et al. 2009), but we are currently, in general, administering the 24-h test to all patients during the neonatal period with no previous phe loading; in our opinion, it is very important to adjust timepoints in this particular stage of life in order to prevent excessive treatment delays when there is no response.

However, if they are not administered during this period, their administration is, in our opinion, a good option.

One must take into account that patients with an early diagnosis of PKU (<15 days since birth) can show no decrease in phe levels with BH4, while at the same time being responsive at a later age. That is why, in the event of a negative response in the neonatal period and an indicative phenotype/genotype, repeating the study at later ages through a 7-day test with 20 mg/kg per day of BH4 is recommendable; this option also helps identify "slow responders."

The 16 patients in our study with a positive response to BH4 constitute 24.6% of patients who completed the trial (87.5% of responders in mild HPA, 38.1% in mild PKU, and 2.8% in classical PKU). This percentage is similar to that of other studies (Trefz et al. 2010). Other authors obtained a far superior rate of response, 62%, (Vernon et al. 2010); this may be because the loading test experiments are performed under dietary supplementation with a low-phe food.

All patients who underwent BH4 treatment showed good treatment compliance except patient no. 12, who also suffered from vomiting at some points when administration began, although it was transitory. In the 15 remaining patients, after a slight decrease in phe levels following 9.75 ± 0.9 mg/kg per day of BH4 for a mean time of 5 years, phe tolerance increased significantly. We did not use higher BH4 medium doses because we did not observe better protein tolerance. Five of the seven patients with mild HPA received BH4 monotherapy without dietary Phe restriction; in patients with mild PKU and classical PKU, one patient with mild PKU is receiving BH4 monotherapy and the other patients (who began treatment after the neonatal period) phe tolerance increased 24–55%; however, patients no.12–14 had a moderate improvement of the diet, which meant less burdensome for these patients and their families, although the cost benefit ratio, due to the high price of the drug, is an issue to take into account.

On the other hand, patients with a higher percentage of response to BH4 showed higher tolerance to phe; as a result, patient no. 11 with mild PKU and a decrease in phe levels of 51% and 80.7% at 8 and 24 h since BH4 loading, respectively, receives only monotherpy with BH4 without dietary restrictions.

Seven of the 16 patients who received BH4 treatment were below 4 years old at start of treatment, five of them had already begun treatment on their first month of life. With a mean evolution time of 53 months (12–87 months), phe tolerance increases have persisted following the phase of maximum growth after six months since birth, which constitutes a "honeymoon period," and protein requirement, including Phe intake, is easily assimilated (Burlina and Blau 2009) without any secondary effects resulting from the medication. Medical literature includes few published studies that use sapropterin at those ages (Spaapen et al. 2001; Shintaku et al. 2004; Hennermann et al. 2005; Burton et al. 2011), like us, all of them found the drug to be safe and no significant side effects were observed. Classical dietary treatment of PKU may cause several micronutrient deficiencies (Acosta and Yannicelli 1999); children treated with BH4 since neonatal period had a good somatic growth development and an adequate nutritional status, including selenium which was deficient in many patients treated with dietary treatment. However, comparative studies with more patients and longer period of study are needed.

Although this is not always the case, similarly to other studies, we usually found a correlation between genotype and response to BH4; some studies establish a 76% correlation, others deem there to be good correlations, particularly in homozygotes, for nonresponsive mutations (Daniele et al. 2009; Desviat et al. 2004; Trefz et al. 2009b; Zurflüh et al. 2008).

In our 5-year experience with 6-methyltetrahydrobiopterin and one year and a half with sapropterin, we assessed no difference between each during phe level decrease (unpublished data), and patients prefer to take sapropterin.

As a result, in light of our results we can conclude that the 24-h BH4 loading test is most commonly used and that tests carried out once a week or every three weeks can identify additional responders. The level of response to BH4 with BH4 loading is very important for later, long-term treatment. Test administration at the time of diagnosis is more practical, if detection is neonatal, Phe tolerance with BH4 treatment between the ages of 6 to 12 months will be reevaluated for false positives. To date we have not assessed any side effects with BH4 treatment, including children below the age of 4 years, so we consider that BH4 test should be made in the neonatal period and start treatment in those with positive response.

Synopsis

This study presents long-term evolution of 16 PKU patients undergoing BH4 treatment showing its efficacy. Furthermore, it highlights the usefulness of BH4 test at the time of diagnosis in the neonatal period and the early treatment if there is a positive response.

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