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Tetrahydrobiopterin responsiveness of patients with phenylalanine hydroxylase deficiency

Luciana Giugliani,[1] Angela Sitta,[2] Carmen R. Vargas,[3] Luiz C. Santana-da-Silva,[4] Tatiéle Nalin,[5] Maria Luiza Saraiva-Pereira,[6] Roberto Giugliani,[7] Ida Vanessa D. Schwartz[8]

Abstract

Objective: To identify patients responsive to tetrahydrobiopterin (BH_4) in a sample of Brazilians with hyperphenylalaninemia due to phenylalanine hydroxylase deficiency (HPA-PAH).

Methods: Interventional study, convenience sampling. The inclusion criteria were: diagnosis of HPA-PAH; age ≥ 7 years; phenylalanine-restricted diet and phenylalanine (Phe) levels ≥ 6 mg/dL in all blood tests 1 year before inclusion. Blood samples were obtained the day before (day 1) and at 0, 4, 8 (day 2) and 24 h (day 3) after BH_4 intake. Phe levels were measured using tandem mass spectrometry. The criteria used to define responsiveness to BH_4 were: criterion 1- Phe reduction ≥ 30% 8 h after BH_4 administration; criterion 2 - Phe reduction ≥ 30% 24 h after BH_4 administration.

Results: Eighteen patients were enrolled (median age, 14 years; 12 boys). Five patients were responsive to BH_4, 3 according to both criteria (one classical PKU, two mild PKU); and two according to criterion 2 (one classical PKU; one indefinite PKU type). There were no differences between Phe serum levels on day 1 and at the other time points ($p = 0.523$). However, Phe levels on days 1 and 2 were significantly different ($p = 0.006$). The analysis of the phenotype-genotype association confirmed its multifactorial character.

Conclusion: A relevant number of Brazilian patients with HPA-PAH are responsive to BH_4, in agreement with other studies in the literature.

J Pediatr (Rio J). 2011;87(3):245-251: Phenylketonuria, phenylalanine hydroxylase, biopterin.

Introduction

Hyperphenylalaninemia due to phenylalanine hydroxylase deficiency (HPA-PAH), usually called phenylketonuria (PKU), is one of the most frequent inborn errors of metabolism and was the first to be treated using dietary therapy.[1] The classical treatment for this disease is the adoption of a low phenylalanine (Phe) diet. The dietary restriction of Phe protects the central nervous system against the toxic effects of the disease and prevents associated clinical

1. Mestre. Programa de Pós-Graduação em Saúde da Criança e do Adolescente, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brazil.
2. Mestre, Ciências Biológicas (Bioquímica). UFRGS, Porto Alegre, RS, Brazil.
3. Doutora, Ciências Biológicas (Bioquímica). Professora adjunta III, UFRGS, Porto Alegre, RS, Brazil.
4. Doutor, Ciências Biológicas (Bioquímica). UFRGS, Porto Alegre, RS, Brazil. Professor, Instituto de Ciências Biológicas, Universidade Federal do Pará, Belém, PA, Brazil.
5. Mestranda, Programa de Pós-Graduação em Medicina: Ciências Médicas, UFRGS, Porto Alegre, RS, Brazil.
6. Doutora, Genética Molecular, University of London (UL), London, UK, Professora associada, Departamento de Bioquímica, UFRGS, Porto Alegre, RS, Brazil.
7. Doutor, Genética. Universidade de São Paulo (USP), São Paulo, SP, Brazil. Professor titular, Departamento de Genética, UFRGS, Porto Alegre, RS, Brazil.
8. Doutora, Ciências: Genética. Professora adjunta, Departamento de Genética, UFRGS, Porto Alegre, RS, Brazil. Serviço de Genética Médica, Hospital de Clínicas de Porto Alegre (HCPA), UFRGS, Porto Alegre, RS, Brazil.

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manifestations. However, dietary treatments are complex and long. Low adherence to treatment is a frequent problem in the treatment of adolescents and adults because the dietary options are very limited.[2]

Since the publication of the study by Kure et al.,[3] who published the first report of patients with HPA-PAH who had a reduction in plasma Phe levels after the oral administration of tetrahydrobiopterin (BH_4), several other studies using different protocols and variable BH_4 doses found that Phe levels of patients with HPA-PAH may be better controlled by oral administration of BH_4.[4-7] Those studies showed that most individuals responsive to BH_4 belong to a group with mild phenylketonuria (mild PKU), and that 20 to 50% of the patients with HPA-PAH reach a ≥ 30% reduction of Phe levels in association with BH_4 use.[8] Data available suggest that the presence or absence of BH_4 responsiveness is multifactorial, and that genotype is one of the determinant factors.[9-11]

This study was the first, to our knowledge, to identify Brazilian patients responsive to BH_4 administered orally.

Methods

This interventional study was approved by the Ethics in Research Committee of Hospital de Clínicas de Porto Alegre (HCPA). The participation of the pharmaceutical industry was limited to the donation of BH_4 (sapropterin dihydrochloride, KUVAN®), necessary to conduct the study. Patients with HPA-PAH were seen in the Outpatient Metabolic Disorder Treatment clinics of the Medical Genetics Service of HCPA (ATDM-SGM/HCPA). All patients or their guardians signed a written informed consent form.

Patients included in the study were ≥ 7 years of age and had Phe serum level ≥ 6 mg/dL in the 12 months of life before the date of inclusion. Exclusion criteria were: pregnancy; symptomatic liver disease; use of levodopa; and irregular attendance to outpatient follow-up.

HPA-PAH type was classified according to plasma Phe level at the time of diagnosis (without treatment), which is the criterion adopted by the ATDM-SGM/HCPA[12]: classical PKU = Phe > 20 mg/dL; mild PKU - Phe = 6-20 mg/dL; and non-PKU HPA – Phe = 2-6 mg/dL. All patients with missing or unclear data were classified as indefinite PKU.

BH_4 loading test

Patients were asked to stay two days in the SGM/HCPA, where they received their usual diet, to undergo evaluations. On the first day (before BH_4, or day 1), blood was collected at 8 and 12 am and 4 pm, or at 9 am and 1 and 5 pm (time points 0, 1 and 2) to evaluate variations in Phe level. On the second day (day 2), a modified BH_4 loading test protocol was used: oral administration of a single 20 mg/kg dose of BH_4 for all patients.

On day 2, blood was collected at 0, 4 and 8 h after drug ingestion (time point 0 or baseline, time points 1 and 2). On day 3, blood was collected 24 h after the oral administration of the drug (time point 3). Phe level in the blood was measured using tandem mass spectrometry (MS/MS) in the Laboratory of Inborn Errors of Metabolism of SGM/HCPA. All measurements were made in duplicate, and the mean between the two values was the final result.

Patients were told to fast for about one hour before all blood collections. If a patient did not have a Phe level ≥ 6 mg/dL at time point 0 on day 2 (BH_4 loading day), the protocol had to be repeated at a later time, and the first test was not included in the study.

Responsiveness to BH_4

Two criteria were used to assess responsiveness to BH_4 because no consensus has been reached so far about the best criterion:

Criterion 1 - reduction of ≥ 30% in Phe level 8 h after drug administration[8];

Criterion 2 - reduction of ≥ 30% in Phe level 24 h after drug administration.[13]

Dietary intake of Phe

All patients were told to maintain the prescribed dietary intake of Phe used before the beginning of the study, that is, their usual Phe-restricted diet. Phe intake during the study was assessed using a 3-day dietary recall, applied from the day before the patients came to SGM/HCPA to the day of the BH_4 loading test, inclusive. Phe intake was calculated according to the dietary recall of each day using a computer nutrition program, Die twin Profissional® 2008 (DietWin, Porto Alegre, Brazil). The amount of prescribed Phe was confirmed on the patient's medical chart and recorded in a form developed for that purpose.

Genotype

Data about patient genotypes were collected from medical charts.

Statistical analysis

A Microsoft® Excel spreadsheet was used to store data. The Statistical Package for Social Sciences 14.0 (SPSS® Inc, Chicago, IL) was used for statistical calculations. Data were described according to absolute and relative frequencies. Continuous variables were expressed as mean ± standard deviation or median and interquartile range. Repeated measures ANOVA was used to compare Phe levels along time, and to compare Phe intake during the three days of the dietary recall. The Student *t* test for paired samples was used to compare Phe levels before and after BH_4 at each time point. The level of significance was set at 5%.

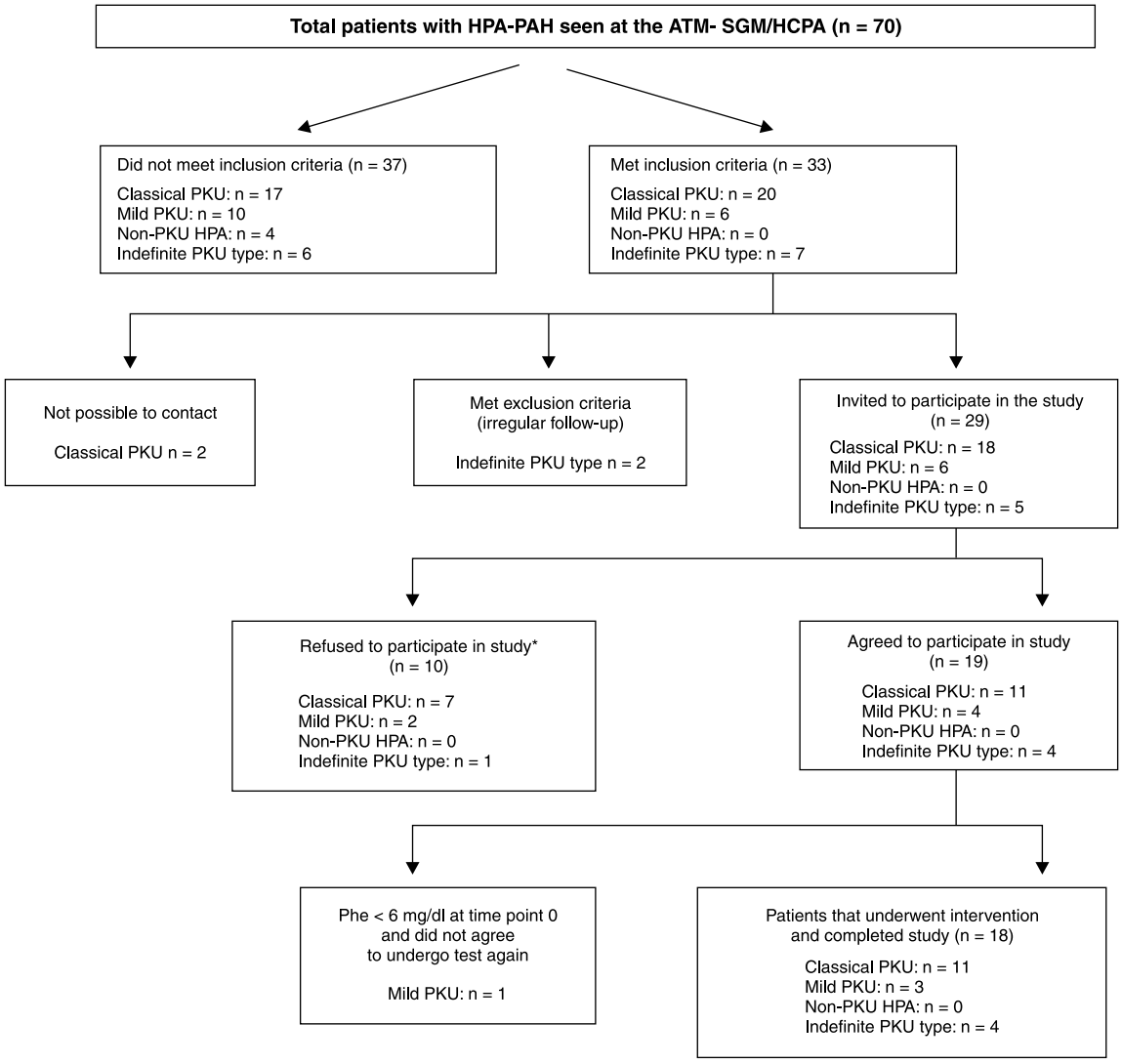
Results

Eighteen patients (12 boys, 66.7%) from 13 nonrelated families were included in the study (Figure 1). Median patient age (interquartile range) was 14 (11-21) years. No parental consanguinity was reported.

Phe plasma levels and the percentage of reduction at the time points after BH_4 administration are described in Table 1. Five patients were responsive to BH_4: three (one classical PKU; two mild PKU) met both criteria; and two (one classical PKU; one indefinite) met criterion 2. When only the index-case for each family (n = 13/18) was analyzed, four patients were responsive: three patients (one classical PKU; two mild PKU) met both criteria; and one patient (classical PKU) met criterion 2.

The analysis of dietary data revealed that there was no difference between Phe intake on the three days of the dietary recall (p = 0.059). Phe plasma levels on the day before the BH_4 loading test did not change at the other collection time points (p = 0.523). The comparisons of Phe levels at baseline time points on the day before and after BH_4 administration (9.44±3.12 mg/dL and 9.56±3.09 mg/dL) did not reveal any significant differences (p = 0.795). At time point 1, mean Phe values were 8.88±2.77 mg/dL and 7.73±2.79 mg/dL on the days before and after BH_4 (p = 0.025). At time point 2, mean Phe values were 9.09±3.24 mg/dL and 8.07±2.84 mg/dL on the days before and after BH_4 (p = 0.006).

In the analysis of genotype, the two mutant alleles of the PAH gene were known for 12 patients (Tables 1 and 2). The comparison of our results with reports in the literature (Table 2) confirms the multifactorial character of responsiveness to BH_4. In addition, of the three sibling pairs (patients 1 and 20, 7 and 8, and 14 and 17) and the sibling trio (patients



* Patients and/or their guardians did not accept to participate in the study because of lack of time and to avoid absence from work or school.

Figure 1 - Algorithm for sample selection in this study

Table 1 - Phenylalanine plasma level and reduction (%) after oral administration of 20 mg/kg BH_4 to patients with HPA-PAH (n=18)

Patient*	Phenotype	Genotype		Phe level (mg/dL)				Phe changes from time point 0 (%)		
		Allele 1	Allele 2	Point 0	Point 1 (4h)	Point 2 (8h)	Point 3 (24h)	Point 1 (4h)	Point 2 (8h)	Point 3 (24h)
1	Ind PKU	p.R158Q	p.R408W	10.5	9.4	9.3	7.9	-10.5	-11.4	-24.8
2	Cla PKU	p.I65T	p.R408W	9.8	7.3	7	6.7	-25.5	-28.6	-31.6
3	Mild PKU	p.L249F	p.V388M	6.5	4.9	5.8	5.7	-24.6	-10.8	-12.3
4	Cla PKU	ND	ND	13.9	12.3	11.5	11.6	-11.5	-17.3	-16.5
6	Cla PKU	p.V388M	IVS71 G>A	7.6	10.2	10	10.6	34.2	31.6	39.5
7	Mild PKU	p.L348V	p.R408W	6.2	4.1	3.4	3.2	-33.9	-45.2	-48.4
8	Ind PKU	p.L348V	p.R408W	6.5	4.8	5.6	7.4	-26.2	-13.8	13.7
10	Mild PKU	ND	ND	8.7	5.3	5.3	3.6	-39.1	-39.1	-58.6
12	Cla PKU	p.I65T	IVS2+5G>C	12.6	11.7	10.2	12	-7.1	-19.0	-4.8
13	Cla PKU	ND	ND	8.7	7	7.9	7.1	-19.5	-9.2	-18.4
14	Cla PKU	p.R261Q	IVS12+1G >A	6.7	6.4	6.4	5.4	-4.5	-4.5	-19.4
16	Cla PKU	ND	ND	16	11.7	13.1	12.1	-26.9	-18.1	-24.4
17	Cla PKU	p.R261Q	IVS12+1G >A	8.6	6.3	7.5	8.7	-26.7	-12.8	1.2
19	Ind PKU	ND	ND	14.1	10.6	14.5	14.1	-24.8	2.8	-7.1
20	Ind PKU	p.R158Q	p.R408W	13	9.6	11.1	8.6	-26.2	-14.6	-33.8
21	Cla PKU	ND	ND	10	8	8.9	10.2	-20.0	-11.0	2.0
22	Cla PKU	p.I65T	p.R261X	7.7	6.2	6.9	8.7	-19.5	-10.4	13.0
23	Cla PKU	p.I65T	p.R176X	6.2	3.5	4.2	3.3	-43.5	-32.3	-46.8

BH_4 = tetrahydrobiopterin; Cla = classical; HPA-PAH = hyperphenylalaninemia due to phenylalanine hydroxylase deficiency; Ind = indefinite; ND = not determined; Phe = phenylalanine; PKU = phenylketonuria.
* Siblings: 1 and 20; 4, 16 and 21; 7 and 8; 14 and 17. Patients 7, 10 and 23 were responsive according to criterion 1, and patients 2, 7, 10, 20 and 23, according to criterion 2.

4, 16 and 21) included in the study, only one of the pairs (patients 14 and 17) and the trio had equal results for presence or absence of responsiveness.

Discussion

To our knowledge, this was the first study with Brazilian patients with HPA-PAH to detect responsiveness to the oral administration of BH_4. The purpose of the inclusion criteria was to select the most collaborative patients, which explains the 7 years as a cut-off point for age, and the least adherent to treatment, which explains the 6 mg/dL cutoff point for previous Phe levels. Therefore, most patients included in the study belonged to the classical PKU group. In addition, in patients with normal Phe levels (< 6 mg/dL), studies to evaluate BH_4 responsiveness should be conducted using the combined administration of BH_4 and Phe loading test,[15] which was not included in our protocol.

Phe plasma levels on the day before the BH4 loading test did not change at the different collection time points. However, the comparison of Phe levels at the time points of the days before and after BH_4 administration revealed significant differences, which adds support to the hypothesis that changes in Phe levels after BH_4 administration may be an effect of BH_4.[16] The molecular mechanisms responsible for responsiveness to BH_4 in patients with HPA-PAH have not been fully elucidated. Several hypotheses have been raised, such as: a) BH_4 chaperone effect; b) induction of PAH expression by BH_4; and c) stabilization of PAH mRNA.[9,17]

Although the number of siblings included was small (n = 4/18), genotype data and intrafamilial variability were in agreement with the multifactorial character of responsiveness to BH_4. This finding suggests that, although genotyping is useful in predicting BH_4 responsiveness, further studies should be conducted before it can be used as a standard test.[8]

The rate of BH_4 responsiveness varied according to the criterion used to define responsiveness, and was greater when criterion 2 was used (reduction ≥ 30% 24 h after drug administration). The comparisons between the 8 h and the 24 h protocol also revealed that, the longer the test time, the greater the chance of detecting "slower responders."[8,13]

Table 2 - BH_4 responsiveness: genotype-phenotype association in a sample of Brazilian patients and comparison with findings in the literature

Patients*	Genotype†		Phenotype	BH4 responsiveness (this study)‡	BH4 responsiveness according to genotype in other studies[14] (number of patients described)
	Allele 1	Allele 2			
1	p.R158Q	p.R408W	Ind PKU	No	No (1 patient), Yes (1 patient)
2	p.I65T	p.R408W	Cla PKU	Yes	Yes (1 patient)
3	p.L249F	p.V388M	Mild PKU	No	ND
6	p.V388M	IVS71G>A	Cla PKU	No	ND
7	p.L348V	p.R408W	Mild PKU	Yes	ND
8	p.L348V	p.R408W	Ind PKU	No	ND
12	p.I65T	IVS2+5G>C	Cla PKU	No	ND
14	p.R261Q	IVS12+1G>A	Cla PKU	No	ND
17	p.R261Q	IVS12+1G>A	Cla PKU	No	ND
20	p.R158Q	p.R408W	Ind PKU	Yes	No (1 patient), Yes (1 patient)
22	p.I65T	p.R261X	Cla PKU	No	No (1 patient), Yes (1 patient)
23	p.I65T	p.R176X	Cla PKU	Yes	ND

BH_4 = tetrahydrobiopterin; Cla = classical; Ind = indefinite; ND = no data available; PKU = phenylketonuria.
* Siblings 1 and 20; 7 and 8; 14 and 17.
† The genotype of patient 10 was unknown; therefore, table shows only four patients responsive to BH_4.
‡ Patients met same responsiveness criteria, that is, responsive patients had a Phe reduction ≥ 30% at 8 h and/or 24 h after the oral administration of BH_4, and nonresponsive patients did not have reductions at both time points.

Moreover, studies in the literature suggest that the cut-off point for the definition of BH_4 responsiveness should vary according to the patient's clinical phenotype, and should be lower for those with classical PKU (reduction > 20% in Phe level) and greater for those with mild PKU (reduction ≥ 30% in Phe level.[18] If this criterion had been adopted in this study, the responsiveness rate for our patients with classical PKU would increase from 2/18 (11.12%) to 5/18 (27.78%).

The comparison of what type of PKU responded to BH_4 and the two responsiveness criteria adopted revealed that patients with classical PKU had a substantial percentage increase along time, from one to two patients. However, the BH_4 responsiveness rate of patients with mild PKU remained the same (two patients in both criteria).

Our findings are in agreement with results reported in the literature. The significant reduction in Phe levels in response to the oral administration of BH_4 is usually seen in about 50% of patients with mild PKU,[8,19] and the reduction of Phe levels is greater in patients with milder PKU phenotypes than in patients with more severe phenotypes because PAH residual activity is greater in the less severe forms of the disease.[20,21] Our results showed that patients with classical PKU may also be responsive to BH_4 loading. In the literature, less than 10% of the patients that are responsive to BH_4 belong to the classical PKU group.[8] This is explained by the fact that these patients have a low or even inexistent PAH residual activity. However, we cannot rule out the possibility that patients classified as nonresponsive according to the protocol used in this study may still be responsive if tested for a longer period of time. According to clinical studies, long protocols, such as the 48 h ones, are essential to detect slower responders.[4,22,23] Fiege & Blau[18] reported that a high number of patients with classical PKU were responsive to BH_4 loading test, particularly when the cutoff point was lowered from 30 to 25% (6.8 for 26%).

There is no consensus about a standard protocol for the diagnosis of BH4 responsiveness.[24] Several protocols have been used in different studies both with the unregistered formulation of BH_4 (produced by Schirks Laboratory, Switzerland) and the new BH_4 formulation [(6R)-5,6,7,8-tetrahydro-L-biopterin dihydrochloride, Sapropterin dihydrochloride, the synthetic form of (6R)-5,6,7,8-tetrahydro-L-biopterin dihydrochloride, Sapropterin dihydrochloride used in our study]. The latter has been approved by the FDA for patients 4 years and older and by the EMEA for patients 5 years and older to treat HPA-PAH in the United States and Europe.[17,25,26] These studies also included a normal or Phe-restricted diet, different BH_4 doses and different time points to evaluate Phe levels, for example.[13,27]

Results are difficult to compare, but the reduction of at least 30% in Phe levels is frequently classified as a clinically significant response to treatment. However, this threshold is arbitrary.[10] A recent study was conducted in Europe to better understand the HPA-PAH diagnostic and treatment practices. Blau et al.[28] developed a questionnaire with 33 questions that was sent to 243 healthcare workers in 165 PKU treatment centers in 23 European countries. One hundred and one questionnaires from 93/165 PKU treatment centers (56%) of 19/23 European countries (83%) were returned. According to the study data, the BH_4 loading test was performed routinely in 54% of the PKU treatment centers, and in 61%, the test was a single dose of BH_4 (20 mg/kg). When asked about how they defined responsiveness to BH_4, 34% answered that it is defined by a reduction ≥ 30% in Phe levels after 24 h, and 17% and 12%, by a reduction ≥ 30% in Phe levels at any time point and 8 h after drug administration. For the classification of HPA-PAH type, more than 70% of the interviewees classified classical PKU as Phe level > 20 µmol/L at diagnosis (no treatment). However, the definitions of mild PKU and non-PKU HPA varied substantially, not only between countries, but also between different centers in the same country. These findings demonstrate the difficulty in defining the severity of PAH deficiency and the need to standardize the classification of HPA-PAH types.

We believe that a single, simple and universal test should become the criterion standard to facilitate the identification of patients responsive to BH_4. It should be easy to apply and should define the adequate amount of BH_4 to be administered, Phe intake, and the standard time points to measure Phe levels, thus limiting the number of Phe measurements that have to be made and defining a criterion standard for the interpretation of responsiveness to this drug.

The potential benefits of treatment with BH_4 for patients with PKU responsive to BH_4 include the reduction of Phe levels in blood and less strict dietary restrictions, as a result of the higher tolerance to natural proteins, which may, consequently, result in a greater adherence to treatment by the patient.[17] However, as this is a new drug, better evidence is not currently available about its efficacy and effectiveness in the long term treatment of milder cases of PKU, as a single treatment, or of classical PKU, as a complementary treatment, associated with Phe-restricted diets.

Although limited by its sample size, our study suggests that, in the near future, the treatment with BH_4 may be beneficial to a considerable number of the Brazilian patients with HPA-PAH that poorly tolerate dietary restrictions. This may improve their quality of life and be an important contribution for their treatment because it may generate better adherence. Additional studies should be conducted in an attempt to confirm this hypothesis.

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Correspondence:
Roberto Giugliani
Serviço de Genética Médica, Hospital de Clínicas de Porto Alegre
Rua Ramiro Barcelos, 2350
CEP 90035-903 – Porto Alegre, RS – Brazil
Tel.: +55 (51) 3359.8011
Fax: +55 (51) 3359.8010
E-mail: rgiugliani@hcpa.ufrgs.br