Molecular Genetics and Metabolism 104 (2011) S80–S85

Contents lists available at SciVerse ScienceDirect

Molecular Genetics and Metabolism

journal homepage: www.elsevier.com/locate/ymgme

Optimized loading test to evaluate responsiveness to tetrahydrobiopterin (BH_4) in Brazilian patients with phenylalanine hydroxylase deficiency

Tatiéle Nalin [a], Ingrid Dalira Schweigert Perry [b], Angela Sitta [c], Carmen Regla Vargas [c,d,e], Maria Luiza Saraiva-Pereira [d,f], Roberto Giugliani [a,d,g,h], Nenad Blau [i,j], Ida Vanessa Doederlein Schwartz [a,d,g,*]

[a] Post-Graduation Program in Medicine: Medical Sciences, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil
[b] Department of Internal Medicine, Universidade Federal do Rio Grande do Sul. Food and Nutrition Research Center of Hospital de Clínicas de Porto Alegre, Porto Alegre, Brazil
[c] Post-Graduation Program in Biochemistry, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil
[d] Medical Genetics Service, Hospital de Clínicas de Porto Alegre, Porto Alegre, Brazil
[e] School of Pharmacy, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil
[f] Department of Biochemistry, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil
[g] Department of Genetics, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil
[h] INAGEMP — Instituto Nacional de Genética Médica Populacional, Porto Alegre, Brazil
[i] Division of Clinical Chemistry, University Children's Hospital, Zürich, Switzerland
[j] Zürich Center for Integrative Human Physiology (ZIHP), Zürich, Switzerland

ARTICLE INFO

Article history:
Received 27 July 2011
Received in revised form 13 September 2011
Accepted 13 September 2011
Available online 20 September 2011

Keywords:
Inborn errors of metabolism
Hyperphenylalaninemia
Phenylketonuria
Phenylalanine
Tetrahydrobiopterin

ABSTRACT

Introduction: Recent studies showed that phenylalanine (Phe) plasma concentrations may decrease in some patients with hyperphenylalaninemia (HPA) due to phenylalanine hydroxylase (PAH) deficiency, after the administration of tetrahydrobiopterin (BH_4).
Objective: To determine responsiveness to a single dose of BH_4 administered according to an innovative protocol using a combined Phe and BH_4 loading test in Brazilian phenylketonuria (PKU) patients.
Methods: Patient age should be ≥4 years, and median Phe plasma concentration ≤600 μmol/L when following dietary restrictions. Participants received a simple Phe loading test using 100 mg/kg L-Phe (Test 1) and a combined Phe + BH_4 loading test using 100 mg/kg L-Phe and 20 mg/kg/BH_4 (Test 2). Blood samples were collected at baseline and 3, 11 and 27 h after Phe ingestion (T0, T1, T2 and T3). Responsiveness was defined as: criterion A: plasma Phe reduction of ≥30% at T1 and T2 for Tests 1 and 2; criterion B: plasma Phe reduction of ≥30% at T1 and T3 for Tests 1 and 2; and criterion C: at least 30% difference of the areas under the Phe curve for Tests 1 and 2.
Results: Eighteen patients (median age 12 yrs; 8 classical PKU; 10 mild PKU) participated in the study. Six patients (2 classical PKU; 4 mild PKU) were classified as responsive according to at least one of the criteria. Responsiveness was concordant when criteria A + B we compared with criterion C (kappa = 0.557; p = 0.017). Of the patients whose genotype was available (n = 16), six had data about BH_4-responsiveness genotypes described in the literature, which were in agreement with our findings.
Conclusion: The comparison of simple Phe loading and combined Phe + BH_4 loading seems to be an optimal method to evaluate responsiveness to BH_4 in patients with good metabolic control.

1. Introduction

Phenylketonuria (PKU) or hyperphenylalaninemia due to phenylalanine hydroxylase (PAH) deficiency is an inborn error of amino acid metabolism characterized by the persistent increase of phenylalanine (Phe) plasma concentration. PAH converts Phe into tyrosine (Tyr), in the presence of its cofactor tetrahydrobiopterin (BH_4) [1].

The standard PKU treatment is based on a Phe-restricted diet and ingestion of a Phe-free amino acid-rich metabolic formula that supplies the daily protein requirements of a patient [1,2]. Because of the toxic effects of high Phe levels, this condition, if left untreated, may lead to neurological impairment, mental retardation and behavioral disorders [3,4].

The necessary dietary restriction and the associated difficulty to adhere to the treatment [4–6], have motivated the search for new PKU management strategies [7]. Since the publication of the study by Kure et al. [8], who described the first case of patients with PKU whose Phe levels decreased after BH_4 administration, several studies

* Corresponding author at: Serviço de Genética Médica, Hospital de Clínicas de Porto Alegre, Rua Ramiro Barcelos 2350, 90035-903 Porto Alegre, RS, Brazil.
E-mail address: ischwartz@hcpa.ufrgs.br (I.V.D. Schwartz).

1096-7192/$ – see front matter
doi:10.1016/j.ymgme.2011.09.019

have been conducted to confirm the efficacy and safety of this medication, which has already been approved by the FDA and the EMEA. Patients are usually evaluated to check their responsiveness and, in case results suggest that they are responsive, that is, that Phe levels will decrease after BH_4 administration, BH_4 supplementation is initiated. Studies, however, have used different protocols to evaluate responsiveness: BH_4 doses are different and may range from 10 to 20 mg/kg/day in a single dose or distributed along the day; test evaluation times range from some hours to weeks or even months; the cut-off point of Phe variation defined to determine responsiveness also varies, and the criterion most frequently adopted is a decrease of 30% in Phe levels 24 h after BH_4 administration. In addition, different diets are used during tests: normal diets, Phe-restricted diet, or even a Phe-loading diet using, for example, powder milk or L-Phe [7,9–22].

This study describes responsiveness to a single dose of BH_4 in a sample of Brazilian patients with PKU and good metabolic control. For that purpose, an innovative protocol with a single Phe plus a combined Phe and BH_4 loading tests was used.

2. Material and methods

This study included patients with PKU seen in the Outpatient Metabolic Disorder Treatment Clinic of the Medical Genetics Service of Hospital de Clínicas de Porto Alegre (ATDM-SGM/HCPA), Porto Alegre, Brazil. At the time this study was conducted, 68 patients with different phenotypes were followed up in the ATDM-SGM/HCPA and 64 of them underwent dietary treatment.

This study was approved by the Ethics in Research Committee of HCPA, and all patients or their guardians signed a written informed consent term.

2.1. Patients

Patients included in the study should be aged ≥4 years and should be under dietary treatment; median Phe plasma levels should be ≤600 μmol/L in the 12 months before the start of the study. The Phe cut-off point adopted ensured that patients with the mild form of the disease, good metabolic control, or both were also included in the test. A previous trial conducted by our study team [23] adopted a different protocol and different inclusion criteria to evaluate responsiveness to BH_4, and most patients had the classical form of the disease and inadequate metabolic control, as their Phe plasma levels had to be ≥360 μmol/L in all measurements during the previous 12 months.

Exclusion criteria were: pregnancy, clinical signs suggestive of liver disease; use of levodopa; allergy to any component of BH_4; median Phe level>600 μmol/L in the measurements made in the 12 months before inclusion in the study; irregular follow-up in the ATDM-SGM/HCPA in the same 12 months; and probable non-compliance with study procedures according to evaluations made by the authors.

PKU types were defined according to Nalin et al. [6], and the patients were classified as having classical PKU or mild PKU.

2.2. Single Phe and combined Phe + BH_4 loading tests

The patients were asked to come to two visits in HCPA and to stay under evaluation for 27 h each time; the two visits were made at a one-week interval.

2.2.1. Simple Phe loading (Test 1)

In the first week, after overnight fasting, blood was collected to measure Phe and Tyr plasma concentrations (T0). After that, patients ingested 100 mg/kg of L-Phe and resumed their usual diet (Phe-restricted diet and supplementation with Phe-free metabolic formula). Blood for Phe and Tyr was then collected at 3 (T1), 11 (T2) and 27 h (T3) after Phe loading.

2.2.2. Combined Phe and BH_4 loading (Test 2)

In the second week, evaluation was conducted using the protocol described by Blau et al. [24], with a modification, as Phe and Tyr levels were not analyzed 7 h after Phe loading. The initial phases of Test 2 (collection at T0, Phe loading, food ingestion, and blood collection at T1) were similar to those described for Test 1. In addition, immediately after collection at T1 bloods sample, a single dose of 20 mg/kg BH_4 (sapropterin dihydrochloride, KUVAN®, Merck Serono) was administered orally and samples were collected at 8 h (T2) and 24 h (T3) after BH_4 ingestion. Time points T0 and T1 of Tests 1 and 2 were, therefore, equivalent to each other, whereas T2 and T3 were differed in that BH_4 administration was included in Test 2.

L-Phe and BH_4 were dissolved in orange juice before administration. Patients were told to fast for at least 1 h before all blood collections.

Phe and Tyr plasma concentrations were measured using tandem mass spectrometry (MS/MS) in the Laboratory of Inborn Errors of Metabolism of SGM/HCPA, as described by Rashed et al. [25]. All measurements were made in duplicate, and the mean of the two measurements was calculated. In Test 2 samples, the levels of BH_4 (total biopterins) were also measured, according to the method of Opladen et al. [26].

2.3. Responsiveness to BH_4

Patients were defined as responsive to BH_4 if they met at least one of the criteria listed below:

Criterion A: this criterion used Phe values at T1 [3 h after Phe loading in Tests 1 (1T1) and 2 (2T1)] and at T2 [11 h after Phe loading in Tests 1 (1T2) and 2 (2T2) and 8 h after BH_4 administration in Test 2]. The following equation was used for calculations: $[((2T2-2T1)/2T1)\times 100]-[((1T2-1T1)/1T1)\times 100]$.
Individuals were responsive if the values found corresponded to a reduction of ≥30% in Phe levels in Test 2.
Criterion B: this criterion used Phe values at T1 and T3 [27 h after Phe loading in Tests 1 (1T3) and 2 (2T3) and 24 h after BH_4 administration in Test 2]. The following equation was used for calculations: $[((2T3-2T1)/2T1)\times 100]-[((1T3-1T1)/1T1)\times 100]$.
Individuals were responsive if the values found corresponded to a reduction of ≥30% in Phe levels in Test 2.
Criterion C: this criterion used the percentage difference of the value found for the area under the Phe curve in Tests 1 (AUC1) and 2 (AUC2). The following equation was used for calculations: $[((AUC2-AUC1)/AUC1)\times 100]$. Individuals were responsive if the difference was ≥30%, as long as the Test 1 area was greater than the Test 2 area.

To compare the classification of responsiveness, four additional criteria were used, as described below:

Criterion D: only the Phe values at T1 and T2 of Test 2 were used. The following equation was used for calculations: $[((2T2-2T1)/2T1)\times 100]$. Individuals were responsive if the values found corresponded to a reduction of ≥30% in Phe levels in time point 2.
Criterion E: only the Phe values at T1 and 3 of Test 2 were used. The following equation was used for calculations: $[((2T3-2T1)/2T1)\times 100]$. Individuals were responsive if the values found corresponded to a reduction of ≥30% in Phe levels in time point 3.
Criterion F: this criterion was used by the authors in a previous study [23] to evaluate the responsiveness of 5 patients also included in this study, and was defined as a reduction of ≥30% in Phe levels 8 h after simple BH_4 loading (single-dose of BH_4 at 20 mg/kg, without a concomitant load of Phe or L-Phe).