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Clinical and nutritional evaluation of phenylketonuric patients on tetrahydrobiopterin monotherapy

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Abstract

The clinical, nutritional, and neuropsychological data of 11 mild/moderate PKU patients after one year of treatment with BH4 are evaluated. BH4 monotherapy was introduced at 5 mg/kg/day in 14 PKU patients. In 11/14 patients, Phe tolerance increased significantly from 356 ± 172 to 1546 ± 192 mg/day ($p = 0.004$), and special PKU formula was gradually reduced until complete removal. In them, mean plasma Phe concentrations remained below 360 μmol/L at 5 mg BH4/kg/day (7 mg/kg/day in one patient). BH4 therapy was stopped in three patients (V388M/P362T and R243Q/IVS10-11G > A genotypes) because it was not possible to improve Phe tolerance and to remove formula intake. Serum micronutrients were not significantly different at the start of treatment and at one year follow-up, except for selenium, which increased significantly after one year of therapy ($p = 0.017$). Anthropometric, and nutritional measurements were within the age- and sex-specific percentiles for a healthy population after one year therapy. Neuropsychological follow-up indicated that intelligence scores persisted within normal limits. In terms of patients' genotype, we confirmed that the P275S mutation combined with R408W was associated with long-term BH4 responsiveness, while the combination of P362T/V388M, and R243Q/IVS10-11G > A resulted in poor metabolic control in long-term BH4 therapy. In summary, our data confirm that BH4 is a safe, and effective therapy in a selected group of mild, and moderate PKU patients who respond to the BH4 loading test. Low doses of BH4 in monotherapy permit withdrawal of the special formula and guarantee a good clinical and nutritional outcome with no adverse side effects in PKU patients.

Keywords: Tetrahydrobiopterin; BH4 responsiveness; Hyperphenylalaninemia; Phenylketonuria; Nutritional status; PKU

Introduction

Phenylketonuria (PKU; OMIM 261600) is an autosomal recessive metabolic disease caused by a deficiency of phenylalanine hydroxylase (PAH, EC 1.14.16.1), a hepatic enzyme which catalyses the conversion of phenylalanine (Phe) to tyrosine, using tetrahydrobiopterin (BH_4) as coenzyme [1]. The mainstay of dietary treatment is restriction of the Phe intake which in practice means restriction of nearly all protein-rich foods, and supplementation with Phe-free amino acid mixtures [2]. The description by Kure et al. [3] of four phenylketonuric patients with known mutations on the *PAH* gene who responded to BH4 supplementation by lowering plasma Phe concentrations

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opened a wide field of research into this novel therapeutic strategy in PKU [4]. Since then, many mutations of the *PAH* gene have been reported to be BH_4 responsive [5–9], and several investigation groups are currently studying the reasons for this responsiveness [10–12]. Furthermore, BH_4 treatment of some responsive patients resulted in successful control of blood Phe levels with a progressive relief or withdrawal from the Phe restricted diet [13–19]. However, in spite of a growing experience in BH4 therapy, few data concerning the clinical and nutritional evolution have been reported.

Working at a reference centre for PKU in Catalonia (Spain), we systematically investigated BH4 responsiveness in patients at diagnosis from the neonatal screening, as well as in PKU patients on a Phe restricted diet [20], so as to be able to offer this alternative therapy to the responsive patients. After the selection of the responsive patients, the initiation of BH4 treatment would bring about an increase in Phe tolerance as well as removal of the special PKU formula.

Here, we present the clinical, nutritional, and neuropsychological evaluation after one year of treatment with BH4 of 11 mild/moderate PKU patients.

Materials and methods

Patient selection

We investigated BH4 responsiveness in a group of 73 PKU patients. The differential diagnosis of hyperphenylalaninemia was performed in all patients and a defect in BH4 synthesis or recycling was excluded. In seven patients, the BH4 loading test was applied at diagnosis from the neonatal screening before starting the Phe restricted diet, following the protocol described by Blau et al. [4], while in 66 patients the combined Phe/BH4 loading test was applied as previously described [20]. Fourteen patients were initially selected owing to good response to the BH4 loading test (a decrease of 45–94% in plasma Phe). Nine patients were mild PKU (tolerance: 400–600 mg Phe/day), four patients moderate PKU (tolerance: 350–400 mg Phe/day), and one classic PKU (tolerance: <350 mg Phe/day). All of them were on a Phe-restricted diet supplemented with special PKU formula at the start of BH4 treatment.

All children or their guardians in this study signed an informed consent agreement in accord with the Helsinki Declaration of 1964, revised in Edinburgh in 2000. Our hospital Ethics Committee approved the study. Compassionate use authorization for the BH_4 loading test and treatment was obtained from the health authorities.

BH4 therapy protocol

BH_4 was obtained in 50 mg tablets from Schircks Laboratories (Jona, Switzerland). An initial dose of 5 mg/kg/day was applied to the selected PKU patients. Since the elimination half-life time of orally administered BH4 is about 4 h [21], the BH4 was administered in three daily doses. Phe restricted diet was progressively liberalised by adding 200 mg Phe/day every week for two months, while formula was gradually reduced (from a mean ± SD of 51 ± 40 g/day) until complete removal was achieved. BH4 therapy was discontinued when tolerance could not be increased more than 400 mg Phe/day and formula could not be completely removed. Patients were clinically and nutritionally evaluated monthly throughout the BH4 treatment.

Anthropometric and nutritional examination

Anthropometric evaluation was performed by the measurement of weight (kg) and height (cm). Assessment of nutritional status was performed on the basis of brachial areas of fat and muscle. Brachial area was calculated by measuring arm circumference. Brachial muscular area (mm^2) was calculated as the ratio of triceps skinfold thickness and arm circumference, while brachial adipose area (mm^2) was expressed as the difference between the brachial area and the brachial muscular area. Values obtained for all these measurements were compared to previously established age- and sex-specific percentiles for healthy population [22].

Tolerance evaluation

Tolerance is defined as the highest Phe intake tolerated while keeping blood Phe levels within the safe range (120–360 μmol/L). Tolerance was assessed before the start of BH4 therapy and whenever an increase in daily Phe-intake was introduced. A three-day questionnaire was used to calculate the Phe intake with the DietSource 2.0 Sanutrin Program (Novartis Consumer Health).

Biochemical procedures

Metabolic control: Plasma phenylalanine and tyrosine were analysed by ion exchange chromatography with ninhydrin detection (Biochrom 20, Pharmacia Biotech, Cambridge, England) [23]. Controls were performed weekly until complete introduction of natural proteins and removal from formula, every fortnight for the first three months and monthly for the last nine months. The index of dietary control for the last year before the start of BH4 therapy and for the one year BH4 treatment was calculated as the mean of the median of all Phe values for one year.

Nutritional control: Serum albumin was analysed by standard procedures with a Cobas Integra 700 Analyser (Roche Diagnostics). Serum vitamins E and A were determined by HPLC with UV detection [24], and B vitamins (folate, B_{12}), and ferritin by a competitive