Available online at www.sciencedirect.com
SCIENCE DIRECT®
Molecular Genetics and Metabolism 86 (2005) S86–S90
Molecular Genetics and Metabolism
www.elsevier.com/locate/ymgme

Long-term treatment with tetrahydrobiopterin increases phenylalanine tolerance in children with severe phenotype of phenylketonuria

Julia B. Hennermann [a,*], Christoph Bührer [a,b], Nenad Blau [c], Barbara Vetter [a], Eberhard Mönch [a]

[a] *Otto Heubner Center for Pediatric and Adolescent Medicine, Charité University Medical Center, Campus Virchow-Klinikum, Berlin, Germany*
[b] *University Children's Hospital, Basel, Switzerland*
[c] *Division of Clinical Chemistry and Biochemistry, University Children's Hospital, Zürich, Switzerland*

Received 30 April 2005; received in revised form 25 May 2005; accepted 26 May 2005
Available online 26 July 2005

Abstract

Hyperphenylalaninemia caused by phenylalanine hydroxylase (PAH) deficiency requires lifelong rigorous diet starting in early infancy to prevent severe neurodevelopmental handicap. In a considerable number of children with mild hyperphenylalaninemia, long-term tetrahydrobiopterin (BH4) treatment significantly improves phenylalanine (phe) tolerance, but it has never been investigated in classic phenylketonuria (PKU). We performed a BH4-loading test in 40 consecutive infants with phe serum concentrations exceeding 240 μM, who had been detected by newborn screening programs. Eighteen out of 40 infants were found to be BH4 responsive. Five of them, responding to the neonatal BH4-loading test, showed a phe tolerance of less than 20 mg/kg/day and a phe pretreatment level of >1000 μM. They were treated with BH4 (20 mg/kg/day) over a period of 24 months. All five children had a sustained response to BH4, allowing substantial easing of dietary restrictions. Before BH4 treatment daily phe tolerance was 18–19 mg/kg, increasing to 30–80 mg/kg on BH4 treatment and decreasing again to 12–17 mg/kg after termination of BH4 treatment. Mutation analysis revealed compound heterozygosity for a putative null and a variant *PAH* mutation in four patients and homozygosity for a variant *PAH* mutation in one patient. We conclude that BH4 sensitivitiy is not restricted to mild hyperphenylalaninemia and that long-term BH4 treatment may also improve phenylalanine tolerance in a considerable number of children with a more severe PKU phenotype.

Keywords: Tetrahydrobiopterin; BH4; BH4-responsive; Hyperphenylalaninemia; Phenylketonuria; Classic; PKU

Introduction

Hyperphenylalaninemia is caused either by phenylalanine hydroxylase (PAH)[1] deficiency (Online Mendelian Inheritance in Man number 261600) or by a defect in the synthesis or regeneration of its coenzyme tetrahydrobiopterin (BH4). While the latter requires supplementation of BH4 and various BH4-dependently synthesized neurotransmitters, normal psychomotor development can be achieved in PAH deficiency by early institution of phenylalanine (phe)-restricted diet. In 1999, Kure et al. reported for the first time on four infants with a novel subtype of BH4-responsive hyperphenylalaninemia. These infants showed a decrease of phe concentrations after oral administration of BH4 but displayed a normal BH4 metabolism and compound heterozygosity for

* Corresponding author. Fax: +49 30 450566918.
E-mail address: julia.hennermann@charite.de (J.B. Hennermann).

[1] *Abbreviations used:* BH4, tetrahydrobiopterin; bw, body weight; MHPA, mild hyperphenylalaninemia; phe, phenylalanine; PAH, phenylalanine hydroxylase; PKU, phenylketonuria.

1096-7192/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.ymgme.2005.05.013

different *PAH* mutations [1]. Since then, several infants with BH4-responsive PAH deficiency have been reported [2–9]. Different mechanisms had been discussed to cause BH4-responsive PAH deficiency [2]. All hypotheses put forward to date assume an association with specific, mainly milder *PAH* mutations. Of the almost 500 *PAH* mutations known, more than 35 have been associated with BH4 responsiveness [3–5,10,11].

Muntau et al. [4] showed that BH4 significantly lowered serum phe concentrations in the majority of patients with mild hyperphenylalaninemia (MHPA), while no patients with classic phenylketonuria (PKU) had a response. This is in line with previous reports, none of which found BH4 responsiveness in patients with classic PKU [1,6–8]. BH4 responsiveness in infants with classic PKU has been reported only twice, for the first time by our group [12] and recently by Matalon et al. [13]. Long-term BH4 treatment has been described several times in children with MHPA [6,8,14,15], but was never proved in children with classic PKU. We now report for the first time the effect of long-term BH4 treatment in children with a severe PKU phenotype.

Methods and patients

BH4 loading test

Over a period of 5 years, between October 1999 and October 2004, we performed the BH4 loading test in all consecutive infants with phe serum concentrations exceeding 240 μmol/L, as detected by newborn screening programs ($n = 40$). BH4 test was performed at the median age of 11 days of life. All infants received the fully active (6R)-BH4 (Schircks Laboratories, Jona, Switzerland; chemical purity 99.5%, application form: tablets solved in fluids) by mouth in one dose of 20 mg/kg body weight (bw) after a fasting period of 4 h. Phe serum concentrations were measured before, 4, 8, and in 32/40 children, 24 h after the application of BH4. During the BH4 test infants were still regularly breast- or bottle-fed. BH4 responsiveness was defined as a drop of serum phe levels 8–24 h after BH4 application by more than 30% from the value obtained before the administration of BH4 [4,9]. A defect of BH4 synthesis or regeneration was excluded in all 40 infants by measuring dihydropteridine reductase activity in dried blood filter card samples and by determination of pterins in urine samples collected before and during BH4 loading tests.

Classification

Adapted to previously established criteria, hyperphenylalaninemia was classified by using pretreatment phe levels [>1200 μM = classic PKU; 900–1200 μM = mild PKU; <900 μM = MHPA] and maximum daily phe tolerance [<20 mg/kg = classic PKU; 20–25 mg/kg = mild PKU; >25 mg/kg = MHPA] [16,17]. Pretreatment phe levels were established at a median age of 9.5 days (4–12 days of life) before starting any diet or medication. Classic PKU was diagnosed in 23 children, mild PKU in five children, and MHPA in 12 children. Two children (IDs 1 and 2) with the clinical phenotype of classic PKU but a milder genotype carrying the variant mutation Y414C were classified as "atypical" classic PKU. Two children (IDs 4 and 5) showed phe pretreatment levels between 1000 and 1200 μM and a low phe tolerance of <20 mg/kg/day. Due to their genotype they were classified as mild PKU, but—as their clinical course resembled that of children with classic PKU—they were included into the study (Tables 1 and 2).

Laboratory methods

Serum amino acids were analyzed by automated cation exchange chromatography (Biotronic/Eppendorf). Pterins in urine were analyzed by HPLC [18] and dihydropteridine reductase activity was measured in whole blood in filter cards as described previously [19]. Genomic DNA was amplified by polymerase chain reaction and sequenced as described previously [20]. Parental DNA analysis was performed to exclude monoallelic double heterozygosity.

Long-term BH4 treatment

Long-term BH4 treatment was started in all BH4-responsive PKU children with initial phe levels >1000 μM and a phe tolerance of less than 20 mg/kg (IDs 1–5). As there was no need for strong phe-restricted diet, the remaining children with mild PKU or MHPA (IDs 6–18) did not receive BH4 treatment. Long-term BH4 treatment was started at a median age of 9.25 months (from 2 weeks to 42 months) and continued over a median time of 24 months (5.5–29 months). BH4 was given in a dose of 10 mg/kg bw twice a day. Phe and tyrosine serum concentrations were measured weekly or fortnightly. Phe tolerance was evaluated by repeated 3 day dietary protocols. Side effects and positive effects of BH4 treatment were controlled regularly by interviews.

Results

The BH4 loading test revealed a significant decline of plasma phe concentrations in 18/40 infants with hyperphenylalaninemia: in 3/23 infants with classic/atypical classic PKU, in 4/5 infants with mild PKU, and in 11/12 infants with MHPA (Table 1). In all BH4-responsive children, a median decrease of phe serum concentrations of 46% (12.3–82.6%) was achieved 8 h after BH4 application, and of 46% (31.6–59.6%) 24 h after BH4