Long-term follow-up of a patient with mild tetrahydrobiopterin-responsive phenylketonuria

R. Cerone, a, * M.C. Schiaffino, a A.R. Fantasia, a M. Perfumo, a L. Birk Moller, b and N. Blau c, *

a Department of Pediatrics, G. Gaslini Institute, University of Genoa, Genoa, Italy
b The John F. Kennedy Institute, Glostrup, Denmark
c Division of Clinical Chemistry and Biochemistry, University Children's Hospital, Zurich, Switzerland

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Abstract

We report on the long-term follow-up of the first Italian patient with the tetrahydrobiopterin (BH4)-responsive type of phenylalanine hydroxylase deficiency (R243X/Y414C genotype). The patient was diagnosed by the newborn screening for phenylketonuria (PKU) and with a positive BH4 loading test. Introduction of BH4 (initially 10 and later 20 mg/kg/day) in addition to reduced low-phenylalanine diet resulted in therapeutic plasma phenylalanine concentrations (< 340 μmol/L). Very good compliance and no side effects in this patient demonstrate the great potential of BH4 in the treatment of some patients with mild PKU.

Introduction

Since the first report of a BH4-responsive type of phenylalanine hydroxylase (PAH) deficiency [1], an increasing number of new cases has been reported (for review see Spaapen and Rubio-Gonzalgo [2]). It has been further documented that almost 70% of all patients with mild hyperphenylalaninemia (HPA) and mild PKU respond to BH4 by lowering plasma phenylalanine levels 8 h after administration [3]. Although a number of patients with mild HPA and PKU are now on BH4 treatment worldwide, reports on long-term follow-up are scarce. Here, we report the first Italian patient with BH4-responsive PAH deficiency (mild PKU) and discuss the therapeutic efficacy of BH4 supplementation in this patient during the last two years.

Case report and methods

A girl, born December 2000, after normal pregnancy and delivery was found in the newborn screening program with blood phenylalanine levels of 696 μmol/L and at 13 days of age with 1005 μmol/L. BH4 loading (20 mg/kg body weight) resulted in a decrease of blood phenylalanine to 725 and 528 μmol/L, 4 and 8 h post loading, respectively. Pterin analysis in urine and dihydropteridine reductase activity in red blood cells were both normal (for methodology see Blau et al. [4]). The BH4 used was from Schircks Laboratories (Jona, Switzerland).

Mutation analysis was performed using denaturing gradient gel electrophoresis (DGGE) and direct sequencing of exons 7 and 12.

Results and discussion

Fig. 1 shows plasma phenylalanine concentrations in relation to different treatment protocols. From 14 days of age, the patient was put on a low-phenylalanine diet (160 mg/day). Because of rather poor compliance phenylalanine concentrations ranged between 350 and 520 μmol/L. While still on low-phenylalanine diet, supplementation with 10 mg/kg body weight of BH4 per day, divided into two doses, was started. Under this treatment phenylalanine levels persisted between 380 and 470 μmol/L. Subsequently, the BH4 dose was increased to 20 mg/kg
body weight per day, resulting in phenylalanine concentrations mostly below 300 \( \mu \text{mol/L} \). At the same time, the low-phenylalanine diet was adjusted to 180 mg/day. In contrast to some patients who benefit from the BH₄ alone, our patient need a combination of BH₄ and a low-phenylalanine diet. To achieve optimal BH₄ dosage, each single patient need to be titrated. In some patients BH₄ dosage can be reduced from the initial 10 mg/kg and in some need to be increased. The preliminary pharmacokinetic data show the elimination half-life time for BH₄ between 3.3 and 5.1 h [5]. Thus, for the optimal activation of phenylalanine hydroxylase, BH₄ should be given in at least two doses.

DNA analysis of the PAH gene revealed two mutations: R243X (727C>T) and Y414C (1241A>G). The Y414C mutation is the most frequent one found in patients with BH₄-responsive HPA/PKU. According to the database of BH₄-responsive HPA/PKU (BIOPKU; www.bh4.org) this mutation found in 19 out of 77 alleles is potentially associated with BH₄-responsiveness. Y414C has been reported as inconsistently associated with BH₄-responsiveness [6]; however, this was probably due to the use of a different BH₄ product in the past [7]. When expressed recombinantly in the eukaryotic cell system the Y414C mutant protein expresses 28% of the wild-type activity.

Between 1984 and 2002 53 HPA patients were investigated at the G. Gaslini Institute. In all of them BH₄ deficiency was excluded, but four out of the 53 patients showed a significant decrease of phenylalanine levels 8 h after BH₄ loading (20 mg/kg). However, one should consider that some older patient, tested before October 1999, used an old formulation of BH₄ containing about 33% of inactive 6S-BH₄. These patients may have respond to the new fully active BH₄ product (6R-BH₄) and thus, the actual number of BH₄-responsive patients may be higher. In three of these four patients without protein restriction or BH₄ supplementation phenylalanine concentrations never exceeded 300 \( \mu \text{mol/L} \); the patients are developing normally. In one patient, the genotype is A403V/W187X, while in the other two the DNA genotyping is still in progress.

Our data confirm the therapeutic potential of BH₄ in some patients with mild BH₄-responsive PKU; however, further long-term studies are necessary to evaluate the optimal dosage of BH₄ in this group of patients.

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References


