Early Screening for Tetrahydrobiopterin Responsiveness in Phenylketonuria

Francesco Porta, MD, Marco Spada, MD, Alberto Ponzone, MD

abstract

Since 2007, synthetic tetrahydrobiopterin (BH4) has been approved as a therapeutic option in BH4-responsive phenylketonuria (PKU) and since 2015 extended to infants younger than 4 years in Europe. The current definition of BH4 responsiveness relies on the observation of a 20% to 30% blood phenylalanine (Phe) decrease after BH4 administration, under nonstandardized conditions. By this definition, however, patients with the same genotype or even the same patients were alternatively reported as responsive or nonresponsive to the cofactor. These inconsistencies are troubling, as frustrating patient expectations and impairing cost-effectiveness of BH4-therapy. Here we tried a quantitative procedure through the comparison of the outcome of a simple Phe and a combined Phe plus BH4 loading in a series of infants with PKU, most of them harboring genotypes already reported as BH4 responsive. Under these ideal conditions, blood Phe clearance did not significantly differ after the 2 types of loading, and a 20% to 30% decrease of blood Phe occurred irrespective of BH4 administration in milder forms of PKU. Such early screening for BH4 responsiveness, based on a quantitative assay, is essential for warranting an evidence-based and cost-effective therapy in those patients with PKU eventually but definitely diagnosed as responsive to the cofactor.

Phenylketonuria (PKU) is an autosomal recessive disorder of phenylalanine (Phe) metabolism due to more than 800 mutations in the gene encoding Phe-hydroxylase (PAH). Their different severity results in variable PAH impairment from mild hyperphenylalaninemia (HPA) to classic PKU, with residual or absent enzyme activity and high or reduced Phe dietary tolerance, respectively. As Phe itself is a toxic substrate for brain development and function, a calibrated dietary restriction of Phe ensured remarkable results since the second half of the past century.

In past years, many authors reported that a consistent rate of patients with PKU may be responsive to tetrahydrobiopterin (BH4), the natural cofactor of PAH.[1,2] In 2007, a pharmaceutical formulation of BH4 (sapropterin dihydrochloride) was approved for treatment of PKU in addition to a Phe-restricted diet in the United States, and in 2008 in Europe. In 2015, its approval was extended to infants younger than 4 years in Europe. The cost of BH4 therapy ranges from $10 000 to more than $80 000 per patient per year, depending on dose and bodyweight. Such an expensive treatment should be offered only to patients with PKU assessed as BH4 responsive. The current definition of BH4 responsiveness is based on the decrease of blood Phe >20% to 30% after BH4 loading or prolonged administration. With these criteria, however, patients with an identical genotype have been alternatively classified as BH4 responders or nonresponders, and ~60% of

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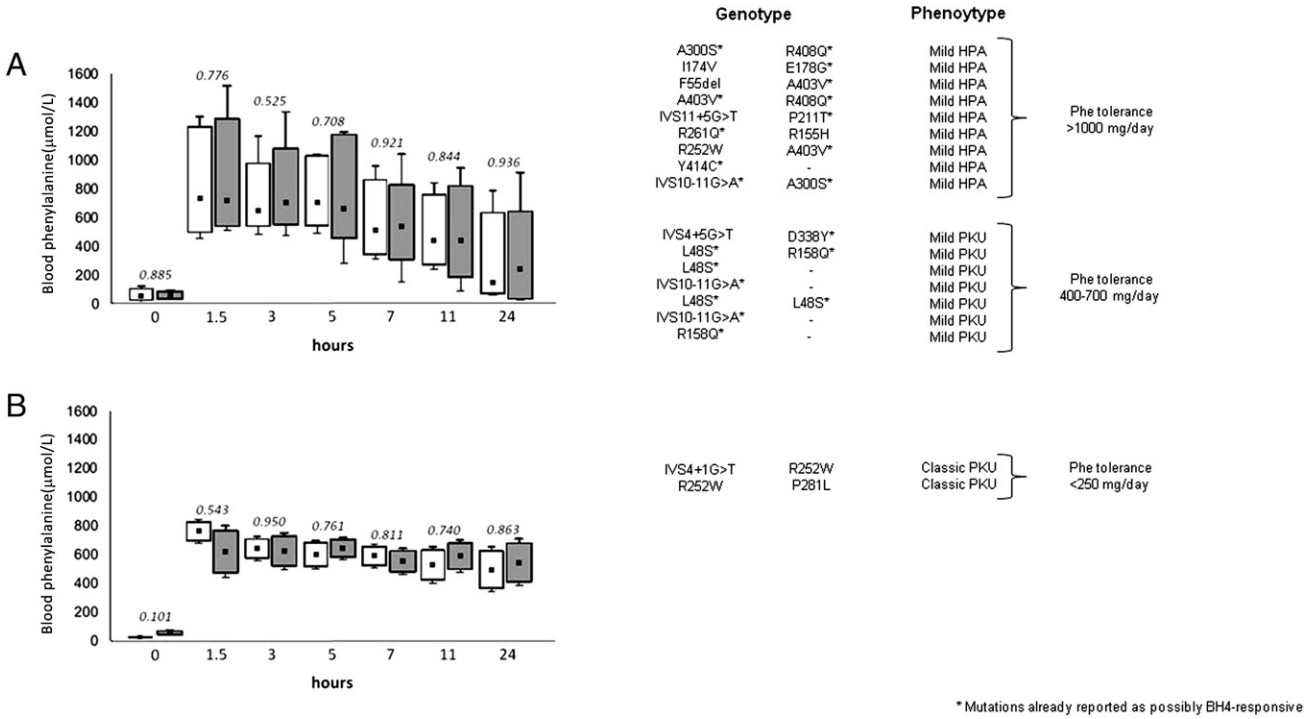

FIGURE 1

Comparative outcome of a simple Phe (150 mg/kg) oral loading test (white boxes) and a combined Phe plus BH4 (20 mg/kg 3 hours after Phe administration) (gray boxes) oral loading test in 18 infants with PAH deficiency due to mutations previously associated with BH4-responsiveness* (A) or not (B). *P* values are indicated for each comparison at each time. Boxes represent 10th to 90th percentile ranges with median value (point), I bars indicate 1st and 99th percentiles. Differences between groups were established by using the Student's *t* test, with statistical significance achieved with $P < .05$.

"responders" to BH4 loading showed nonsignificant Phe reduction after 6 weeks of cofactor treatment.[2] These erratic results are likely consequent to variable blood Phe concentration at the time of BH4 loading, variable Phe intake before and during the challenge with cofactor, inconsistent assignation of a patient's phenotype, and diurnal fluctuations of blood Phe concentration, also occurring on steady dietary treatment.[3–5] Furthermore, the largely accepted simple BH4 loading is not comparative.

We already argued that the assessment of BH4 responsiveness should rely on a reproducible, quantitative, and controlled procedure, taking into account all the determinants of blood Phe level.[6,7] According to other experts in the field, "under ideal conditions, one would need to perform both the combined Phe+BH4 as well as a single Phe loading test" to definitely prove the effectiveness of BH4 in enhancing Phe hydroxylation in patients with PKU.[8] This procedure was previously applied by us in a small series of patients with PKU of different age, phenotype, and genotype, all resulting definitely unresponsive to the administration of synthetic cofactor.[3] With the recent approval of BH4 therapy in children and infants, this procedure has been adopted and tested in a larger cohort infants with PKU, homogeneous for weight and age, to early screen their definite responsiveness to BH4 to anticipate treatment optimization.

METHODS

The procedure was applied to 18 infants with PKU consecutively detected at newborn mass screening. Sixteen of 18 patients harbored mutations in the *PAH* gene already reported as BH4 responsive and 2 harbored severe nonresponsive mutations (Fig 1).

Their age ranged between 3 and 6 months and weight between 5 and 7 kg. Patients were classified according to the Phe tolerance into 3 classes: mild HPA, mild PKU, and classic PKU (Fig 1). The outcome of a simple Phe oral loading (150 mg/kg, an amount corresponding to 3 g/kg natural protein) was compared with that of a combined oral Phe (150 mg/kg) followed by oral BH4 loading (sapropterin dihydrochloride 20 mg/kg, 3 hours after Phe administration). Blood Phe concentration was measured at 0, 1.5, 3, 5, 7, 11, and 24 hours. Prerequisite before the procedure was the normalization of blood Phe by diet (≤120 µmol/L at time 0) as different basal Phe concentration does alter the outcome of the loading. To avoid any additional Phe intake, during the test (24 hours), a Phe-free, normocaloric diet was administered. Written

informed consent for inclusion in the study was obtained from the parents.

RESULTS

Blood Phe clearance was not significantly different after the 2 types of loading, only with Phe or with Phe plus BH4. Moreover, a >20% to 30% decrease with respect to peak levels (3–5 hours after Phe loading) of blood Phe was usual in patients with mild PKU and mild HPA, not in classic PKU, within 24 hours after Phe loading, irrespective of BH4 administration (Fig 1).

DISCUSSION

None of the 18 consecutive infants with PKU, loaded with equimolar Phe amounts, showed significant variation of the spontaneous Phe hydroxylation rates if challenged with equimolar amounts of synthetic BH4, independently from their genotypes and phenotypes.

Three main conclusions can be drawn from this study. In the first instance, previous and present observations clearly show that a 20% to 30% decrease of blood Phe after BH4 administration to patients with PKU with spontaneous or induced HPA is a deceptive criterion of responsiveness to cofactor. Actually, such blood Phe fluctuations do occur in patients on a steady Phe-restricted diet,[5] and blood Phe decrease can attain even 100% within 24 hours after patients with milder PKU are loaded with 150 mg/kg Phe, a dose exceeding the daily Phe requirement. This behavior is in line with the high Phe tolerance of these patients.[4]

Second, no evident effect of BH4 on Phe hydroxylation was observed even in those patients with mutations already reported as BH4 responsive, with Phe hydroxylation rates not significantly differing either after a simple Phe or a combined Phe+BH4 loading. Based on these results, on our experience in handling the use of BH4 in HPAs,[9,10] and on the nonreproducible clinical results in front of its high cost, we encourage early screening for BH4 responsiveness with suitable procedures in all patients with PKU. Actually, as Phe is the physiologic enhancer of PAH activity, any eventual effect of BH4 administration on Phe hydroxylation must be validated by a quantitative comparison with the outcome of spontaneous or induced HPA. This crucial assay, however, has been omitted from the first[11] to the last[12] study concerning this topic, and these same authors using nonquantitative procedures do suggest the investigation of other alternatives. The same conclusion came from the article by Langenbeck.[13] For clinical purposes, the procedure we here described and tested can be applied soon in PKU neonates. As based on an internal control, patients' genotype and phenotype are unnecessary. Reaching a stronger definition of BH4-responsive PKU is essential for warranting an evidence-based and cost-effective therapy in selected patients definitely responsive to the cofactor.

ABBREVIATIONS

BH4: tetrahydrobiopterin
HPA: hyperphenylalaninemia
PAH: phenylalanine hydroxylase
Phe: phenylalanine
PKU: phenylketonuria

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