

Efficiency of long-term tetrahydrobiopterin monotherapy in phenylketonuria

R. STEINFELD, A. KOHLSCHÜTTER, K. ULLRICH and Z. LUKACS*

Klinik und Poliklinik für Kinder- und Jugendmedizin, University-Hospital Hamburg-Eppendorf, Hamburg, Germany

**Correspondence: Klinik und Poliklinik für Kinder- und Jugendmedizin, University-Hospital Hamburg-Eppendorf, Martinistr. 52, 20246 Hamburg, Germany. E-mail: lukacs@uke.uni-hamburg.de*

MS received 22.09.03 Accepted 06.03.04

Summary: Phenylketonuria, an inborn error of phenylalanine metabolism, occurs with a frequency of about 1 in 10 000 births and is treated with a strict dietary regimen. Recently, some patients with PKU have been found to show increased tolerance towards phenylalanine intake while receiving tetrahydrobiopterin (BH₄) supplementation. We have treated two infants with BH₄-responsive PKU with BH₄ for more than 2 years. No additional dietary control was required to maintain blood phenylalanine concentrations in the desired range. Both children have shown normal development. Generally, our results suggest that BH₄ treatment might be an option for some patients with mild PKU, as it frees them from dietary restrictions and thus improves their quality of life.

Phenylketonuria (PKU) is a frequent disorder of phenylalanine metabolism caused by functional deficiency of phenylalanine hydroxylase (PAH, EC 1.14.16.1). Although less common, a defect in the biosynthesis of (6*R*)-L-erythro-5,6,7,8-tetrahydrobiopterin (BH₄), the essential cofactor of PAH, also results in increased blood phenylalanine (Phe) concentrations. When blood Phe concentrations exceed 600 μmol/L, a phenylalanine-restricted diet is mandatory to allow normal development of the child (Schweitzer-Krantz and Burgard 2000; Walter et al 2002). Recent investigations have revealed that BH₄ supplementation has a beneficial effect on certain patients with mutations in the PAH gene, as it increases their phenylalanine tolerance (Kure et al 1999). However, to date, no long-term study has been published on the intake of BH₄ in patients with PKU.

PATIENTS AND METHODS

Patients: Two infants with BH₄-responsive PKU and known mutations in the PAH gene that usually cause mild classical PKU were selected for prolonged monotherapy

with BH₄. The parents were informed about the experimental approach chosen and were consulted throughout this study. No steps were carried out without their approval. Developmental progress and clinical conditions were regularly evaluated during visits to our outpatient ward.

Patient 1, the first child of unrelated parents was born spontaneously at the 38th week of gestation. The patient carries the Y414C mutation on both alleles and showed plasma phenylalanine concentrations above 600 µmol/L from the beginning. Treatment with BH₄ was initiated immediately after diagnosis.

Patient 2 is the second child of nonconsanguineous parents born spontaneously at the 42nd week of gestation. The postnatal and newborn periods passed without abnormalities. Initially, as plasma phenylalanine values were below 600 µmol/L, the patient was fed with normal infant formulas. However, at the age of 6 months plasma phenylalanine rose consistently above 600 µmol/L, so that BH₄ treatment was initiated. The patient is compound heterozygous for the A104D and the K320N mutations. Defects in BH₄ metabolism were excluded by analysis of urinary pterins in both patients.

Methods: Blood phenylalanine concentrations were measured regularly in dried blood samples over a period of ~2 years to evaluate the therapy. The phenylalanine assay by PerkinElmer Life Sciences (Turku, Finland) was used for all determinations.

RESULTS

Detailed studies of plasma Phe concentrations of both patients, measured during normal protein intake (~100 mg Phe per kg body weight (bw) per day), showed peak Phe concentrations approximately 4 h after food intake and an overall increase of Phe concentrations to up to 726 µmol/L or 1307 µmol/L in the evening. Phe concentrations dropped steeply during overnight fasting, when food intake was paused for more than 8 h, and reached a minimum in the morning (cf. Figure 1; Steinfeld *et al* 2002). These patients should therefore adhere to diet in order to keep their Phe concentrations below defined threshold levels (e.g. 360 µmol/L during the first 5 years of life according to British recommendations) (Schweitzer-Krantz and Burgard 2000). The dietary restrictions are far-reaching and difficult to follow, especially in early childhood and puberty when physical development is most demanding (Walter *et al* 2002). In contrast to the high Phe concentrations observed in our patients under normal dietary conditions, daily supplementation with 10 mg/kg bw of BH₄ led to significantly reduced Phe concentrations in blood, with values below 360 µmol/L, while the daily Phe intake was maintained at ~100 mg/kg bw (Figure 1).

Both infants with PKU were continued on oral BH₄ supplementation and were fed without protein restriction or special phenylalanine-free formula for at least 750 days. Blood Phe was determined regularly at 14-day intervals. Both patients were clinically evaluated every 4 weeks during the first 6 months and every 3 months thereafter. In addition, their individual development was assessed on the basis of the Denver development test at regular intervals, always with normal results.

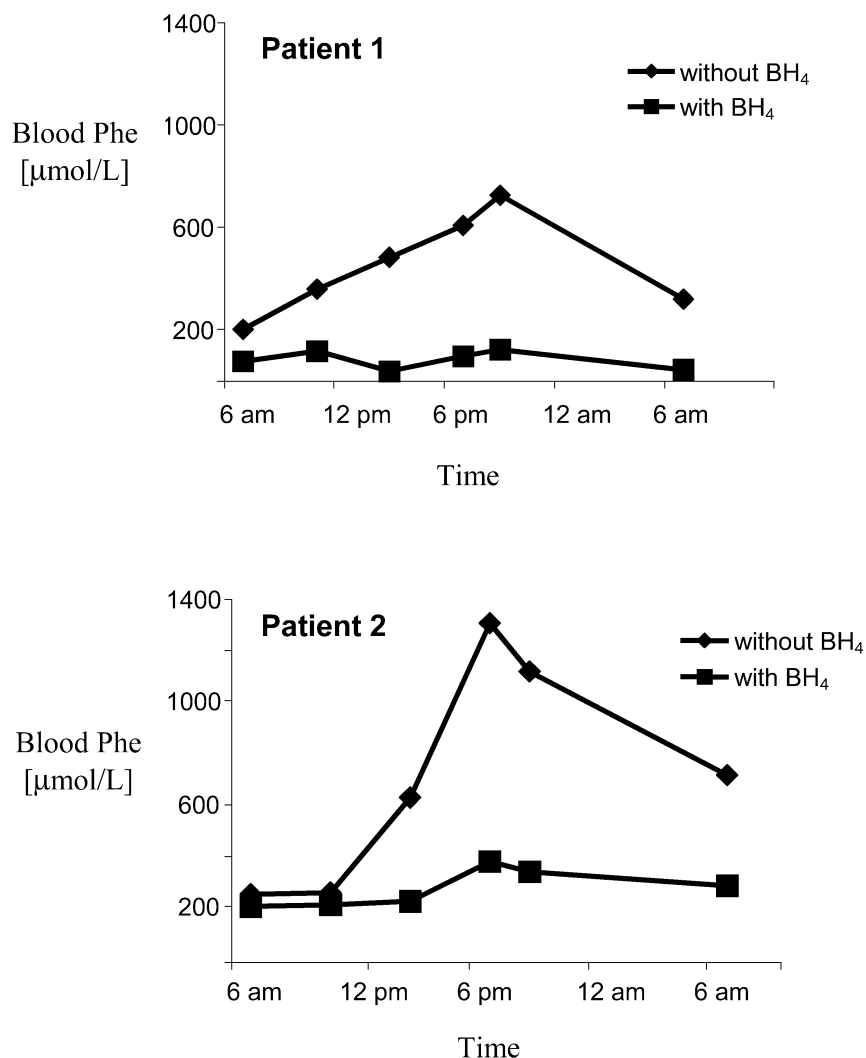


Figure 1 Blood Phe concentrations of patients 1 and 2 in the course of one day with and one day without additional BH₄ supplementation. Phe intake remained constant at all times. Residual activity of phenylalanine hydroxylase is responsible for metabolism of blood phenylalanine during overnight fasting periods. During the observation period of 24 h, blood phenylalanine concentrations remained largely constant under BH₄ therapy, and were generally lower than without BH₄ supplementation

Patient 1 was initially supplemented with 10 mg BH₄ per kg bw per day, but blood Phe concentrations occasionally increased beyond 400 µmol/L, in particular during febrile infections. After the daily BH₄ dose was increased to 20 mg/kg bw, blood Phe concentrations remained in the desired range (60–360 µmol/L). No abnormalities

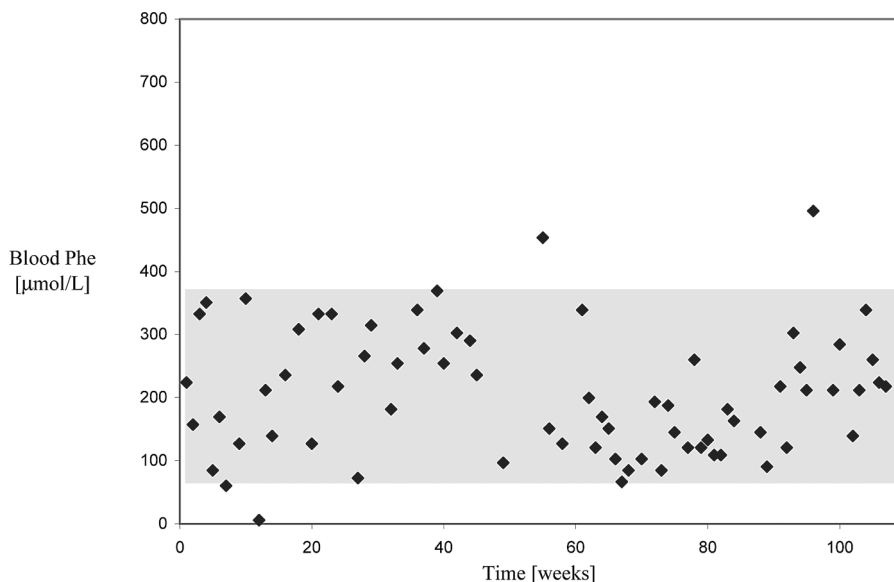


Figure 2 Blood Phe concentrations in patient 2 over a period of approximately 2 years. During that time the patient received daily BH_4 doses of 10 mg/kg bw without any additional dietary treatment. The shaded area indicates the therapeutic range for patients with PKU. Patient 2 maintained blood Phe concentrations below the upper therapeutic threshold except for two occasions during febrile infections. However, blood Phe concentrations returned to the desired values without dietary intervention. Phe concentrations of patient 1 were also within the desired range (data not shown).

were detected during BH_4 treatment. Psychomotor and speech development continued to correspond to peers.

Patient 2 was supplemented with a daily dose of 10 mg BH_4 per kg bw during the whole observation period. Somatic growth proceeded parallel to the 50th centile and developmental milestones were reached at the appropriate ages. Speech development was normal and equivalent to that of his older siblings. Plasma Phe concentrations remained in the desired range (60–360 $\mu\text{mol/L}$) throughout the 750-day study period and did not increase above 500 $\mu\text{mol/L}$ during periods of infections (Figure 2) (Schweitzer-Krantz and Burgard 2000).

DISCUSSION

Both patients maintained blood Phe concentrations within the therapeutic range when supplemented with daily doses of BH_4 . No additional dietary treatment has been necessary in the past 2 years. Hence, our observations suggest that long-term BH_4 therapy is feasible and can substitute for dietary treatment in selected cases of PKU. It freed our patients from strict dietary regimens and has thereby improved their quality of life. BH_4 is a natural cofactor for several enzymes, such as

hydroxylases and nitric oxide synthase. We have had no indication of any side-effects in our patients.

According to Muntau and colleagues, it is estimated that a large proportion of PKU patients with mutations causing a milder form of the disease can be expected to be BH₄ responsive (Muntau et al 2002). Here, it must be taken into consideration that in cases of PKU with some remaining enzyme activity, Phe concentrations will change spontaneously during the day and drop after overnight fasting periods. These variations in plasma Phe concentrations need to be heeded. Therefore, we strongly recommend that Phe values should be measured at constant intervals (e.g. every 4 h) during the day before and after administration of BH₄. The results for both tests should be compared and a significant difference between peak Phe concentrations should be apparent to classify the patient as BH₄ responsive. In addition, several mutations in the PAH gene, e.g. the Y414C allele, are associated with more than one PKU phenotype, so that only a prolonged BH₄ test (at least 24 h) under defined protein intake can disclose the individual PKU phenotype.

In general, BH₄ supplementation did not show any side-effects in our patients during the 2-year study period and has significantly increased their quality of life and that of their parents. Further studies involving a larger number of patients should be carried out to confirm our findings and to evaluate the feasibility of successful BH₄ treatment among PKU patients.

ACKNOWLEDGEMENTS

We express our gratitude to Dr J. Zschocke (Heidelberg, Germany) for genetic analysis of the PAH mutants and to Dr N. Blau (Zürich, Switzerland) for pterin analysis.

REFERENCES

- Kure S, Hou DC, Ohura T, et al (1999) Tetrahydrobiopterin-responsive phenylalanine hydroxylase deficiency. *J Pediatr* **135**: 375–378.
- Muntau AC, Roschinger W, Habich M, et al (2002) Tetrahydrobiopterin as an alternative treatment for mild phenylketonuria. *N Engl J Med* **347**: 2122–2132.
- Schweitzer-Krantz S, Burgard P (2000) Survey of national guidelines for the treatment of phenylketonuria. *Eur J Pediatr* **159**(supplement 2): S70–73.
- Steinfeld R, Kohlschütter A, Zschocke J, Lindner M, Ullrich K, Lukacs Z (2002) Tetrahydrobiopterin monotherapy for phenylketonuria patients with common mild mutations. *Eur J Pediatr* **161**: 403–405.
- Walter JH, White FJ, Hall SK, et al (2002) How practical are recommendations for dietary control in phenylketonuria? *Lancet* **360**: 55–57.