

Long-term treatment of patients with mild and classical phenylketonuria by tetrahydrobiopterin

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Abstract

Tetrahydrobiopterin (BH₄), the natural cofactor of phenylalanine hydroxylase (EC 1.14.16.1), can reduce blood phenylalanine (Phe) in BH₄ sensitive patients with hyperphenylalaninemia (McKusick 261600). We report on the long-term treatment of eight patients with mild and classical phenylketonuria (blood Phe levels maximum blood Phe levels between 771 and 1500 $\mu\text{mol/L}$) using BH₄ at a dosage of 8–12 mg/kg BW per day. In all patients reduction of blood Phe was >30% after BH₄ loading test. Three patients were treated from birth by BH₄ only, five after initial low Phe dietary treatment. Seven of them continue to be on BH₄ treatment only, one has a relaxed low protein diet. No side effects could be observed (longest observation time 5 years), somatic and psychomotor development were normal. The main problem of BH₄ treatment is finding an optimal dosage at different ages and an under special conditions like infectious diseases. There is evidence that in some patients BH₄ treatment may allow a more relaxed low protein diet showing positive effects on weight gain and quality of life. Further controlled studies are necessary not only to rule out any side effects but also for optimizing treatment strategies with BH₄ treatment in mild phenylketonuria.

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Introduction

Phenylketonuria (PKU) (McKusick 261600) is a heterogeneous inborn error of metabolism with a broad spectrum of phenotypes resulting in various elevations of phenylalanine in blood and tissues. About 500 different mutations of the phenylalanine hydroxylase (PAH) gene are described. Low phenylalanine (Phe) diet from birth proved to be effective to prevent mental retardation. In practice three groups are differentiated: mild hyperphenylalaninemia (HPA) with blood Phe levels less than 600 $\mu\text{mol/L}$, mild PKU showing Phe levels between 600 and 1200 $\mu\text{mol/L}$, and classical PKU with Phe levels above 1200 $\mu\text{mol/L}$. There is consensus in

Germany that treatment should only be performed in patients with mild and classical PKU (blood Phe >600 $\mu\text{mol/L}$). However, the low Phe diet is not easy to perform. Ability of the mother to understand their children's disease and social economic status are important parameters determining compliance of the diet and thus outcome of the children. For many years a better and more convenient treatment would be desirable. In the last years there were several reports showing that in some patients with PAH deficiency high doses of tetrahydrobiopterin (BH₄), the natural cofactor of PAH, can stimulate residual enzyme activity resulting in a reduction of blood Phe level. Certain mutations in the PAH gene were found to be BH₄ sensitive. A comprehensive review about the metabolic and molecular bases of BH₄ was published by Blau and Erlandsen [1] recently.

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So far many studies are published showing short-term effects of BH4 loading and the amount of reduction in Phe levels under BH4 which usually results in a reduction of about 60%. In addition many different BH4 sensitive mutations were identified [2–13]. Preliminary results of long-term treatment have been published in two patients [14,15]. In this communication, we will report more data on long-term treatment with BH4 in eight children with mild PKU covering a total of 18 treatment years.

Methods and study design

Decision for treatment with tetrahydrobiopterin was based on analysis of PAH gene and/or response on the BH4 loading test after excluding defects on the metabolism of BH4. All patients showed a more than 30% of blood Phe reduction after a standardized BH4 load (single dose of 20 mg/kg BW, blood drawings at 0, 4, 8, and 24 h post-load). In addition, only those children were treated where treatment was indicated (blood Phe levels >600 µmol/L). Informed consent was obtained after information about the so far experimental therapy using BH4 for treatment of PKU. Only those infants were selected for treatment with BH4 whose parents had problems keeping their children on a Phe restricted diet. Two parents were physicians and refused dietary treatment. Tetrahydrobiopterin was provided by Dr. Schircks, Swit-

zerland, and was given orally as a single dose in water or juice in the morning.

Similar to patients under a Phe restricted diet, regular clinical investigations, documentation of growth, weight gain, and measurement of routine blood chemistry was done. Blood phenylalanine control was performed using dried blood and measurement was done by tandem mass spectrometry. In the first year every week, from the second year two times per month blood drawings were recommended. Clinical visits in the first year were performed every 3–4 months, from the second year on every 6 months. Blood drawings were done at home by the parents, dried filter paper was sent to the laboratory by mail. Results were also provided to the parents on a next day service. Data were prospectively stored and analyzed using RAMEDIS database which was designed by our group (www.ramedis.de) [17].

Results

Summary of results is shown in Table 1. Patients 1–3 are treated from birth with BH4 as the only treatment, the oldest being on BH4 is now 62 months old. Blood Phe profile and BH4 dosages are shown in Figs. 1–3 and 5–7.

In patient ID 230 (Fig. 1) at day 168 of life treatment was stopped for 5 days which resulted in a rapid increase of blood phenylalanine up to 1180 µmol/L. At day 490, BH4

Table 1
Summary of patients data

No.	ID	Genotype of PAH gene	Ethnic origin	Phe max (µmol/L)	Start of treatment (days)	Latest age years	Range BH4 dose (mg/day) ^a	Time of treatment (months)
1	230	E390G/IVS10nt-11g > a	Italian	1180	10	5.2	50–150	62
2	837	E390G/R408W	German	1250	8	2.2	60–120	26
3	981	L48S/R261Q	German	771	10	0.4	30–50	5
4	494	Y414C/R408W	German	1500	1200	6.6	165–220	44
5	724	A300S/IVS12nt+1g > a	German	1200	120	2.4	60–100	24
6	445	L48S/L48S	Turkish	1400	1000	6.1	150	37
7	759	P281L/Y414C	German	1092	300	3.7	40–150	20
8	977	I65T/I95del	German	1293	320	1.4	75–150	8

“Phe max” is the highest blood Phe level observed during observation time.

^a Corresponds to 8–12 mg/kg BW per day.

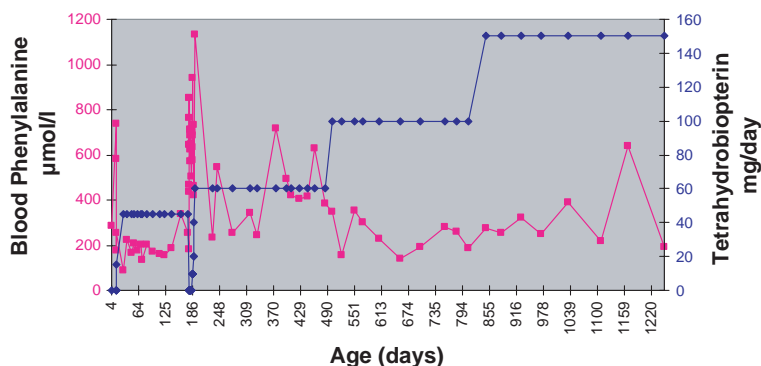


Fig. 1. Blood phenylalanine profile (red) and tetrahydrobiopterin dosage (blue) in Patient ID 230 from birth up 1300 days of life. At 180 days of life BH4 treatment was stopped for 5 days resulting in a rapid increase of blood phenylalanine. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)

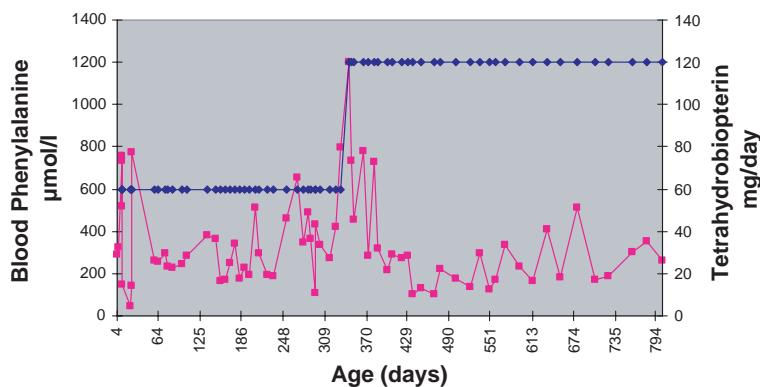


Fig. 2. Blood phenylalanine profile (red) and tetrahydrobiopterin dosage (blue) in Patient ID 494 from birth up to 800 days of life. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)

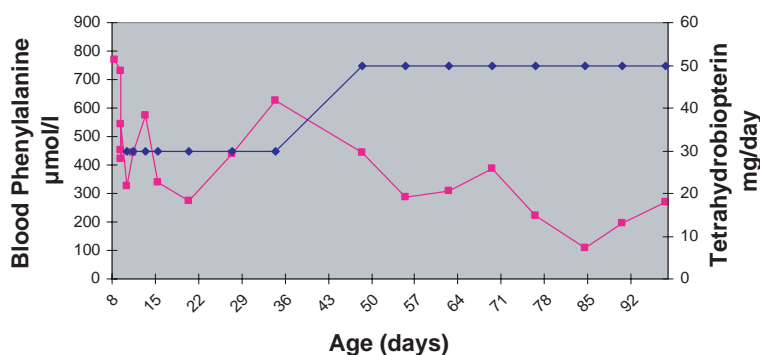


Fig. 3. Blood phenylalanine profile (red) and tetrahydrobiopterin dosage (blue) in Patient ID 981 from birth up to 100 days of life. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)

dosage was increased to 100 mg/day (corresponding to 10 mg/kg BW per day) to get the blood Phe down in the desired range of 60–240 µmol/L. In the following years, dosage was stepwise increased to 10 mg/kg BW per day. Even so there was a slow increase of blood Phe over time (range 200–450 µmol/L). The patient is now 5 years old and developing normally, no side effects were observed.

In patient 837 (Fig. 2) at the end of the first year blood Phe went up to 1250 µmol/L during infection. After increasing the BH4 dose to 120 mg/day blood Phe dropped down in the therapeutic range.

Recently, patient 981 (Fig. 3) was found in the newborn screening program with a blood Phe level of 771 µmol/L on a normal diet. BH4 treatment was started with a low dose of 30 mg/day (6–8 mg/kg BW per day), which proved to be too low and had to be increased to 50 mg/day. As can be seen good metabolic control was only achieved after adjusting the dosage to 50 mg/day corresponding to 8–10 mg/kg BW per day (see Fig. 4).

The other patients (No. 4–8 in Table 1) were later treated by tetrahydrobiopterin. A low phenylalanine diet plus a Phe-free amino acid formula (P-AM 1-2 from SHS or PKU 1-2 from Milupa) were given to two patients (see Figs. 5 and 6). All patients discontinued Phe restricted diet and BH4 is their only treatment with the exception of one patient (ID 759).

Patient 494 (Fig. 5) was found in the newborn screening program with elevated Phe level of 1020 µmol/L. BH4-loading in the newborn period showed no response on blood Phe level (for explanation see discussion in [1]), and dietary treatment was started in the second week of life. The patient's Phe tolerance was 220 mg/day. At 3 years of age after Phe intake was increased to 500 mg/day resulting in blood Phe levels above 600 µmol/L (see [16]), BH4 loading with 300 mg/day (20 mg/kg BW) over 5 days was repeated and showed a decrease from 827 to 199 µmol/L. In the following under BH4 supplementation of 165 mg/day, Phe intake could be increased as shown in Fig. 5. After another increase of BH4 dose to 220 mg/day, dietary treatment was stopped at 6 years of age.

In the same way as Phe intake was increased Phe free amino acid supplementation was decreased to a small amount of 15 g/day. Otherwise the patient has a normal diet. Psychomotoric development is excellent, no side effects were observed.

In patient ID 445 (Fig. 6), the Turkish parents refused dietary treatment because of mother child conflicts at 2 years of age. At that age BH4 loading test (20 mg/kg BW) over 5 days showed a good response from 830 µmol/L to the normal range. In the following BH4 treatment was continued (150 mg/day). Except for two episodes during infections blood Phe levels were in the therapeutic range.

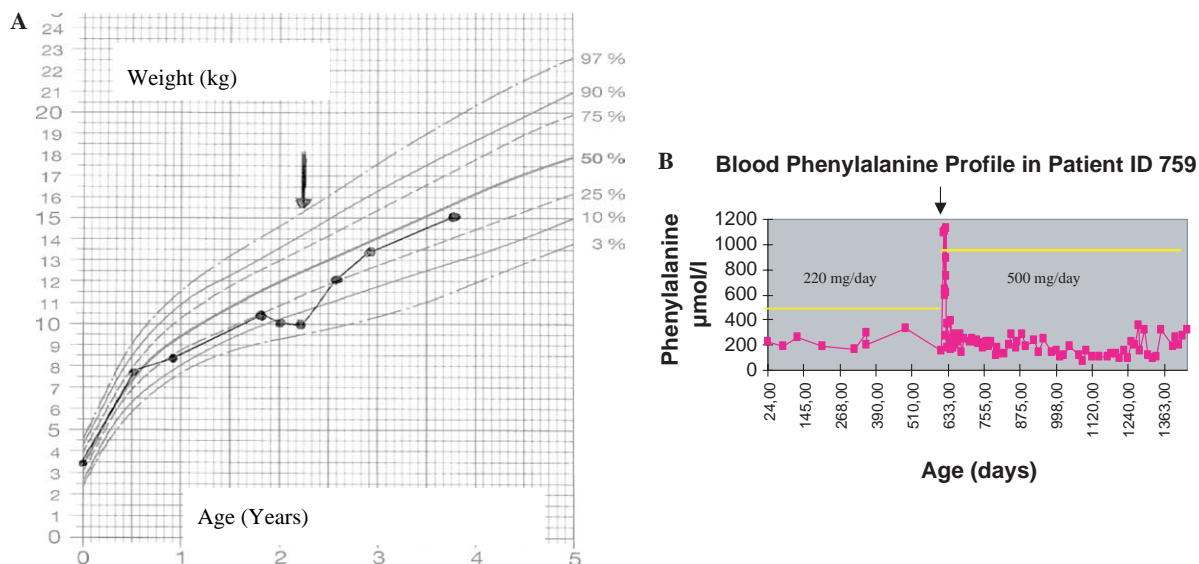


Fig. 4. Weight gain (A) in patient ID 759 before and after BH4 supplementation with 150 mg/day (arrow). And (B) blood Phe before and after introducing BH4 (10–15 mg/kg BW per day) (arrow) and increase of Phe intake per day (yellow line). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)

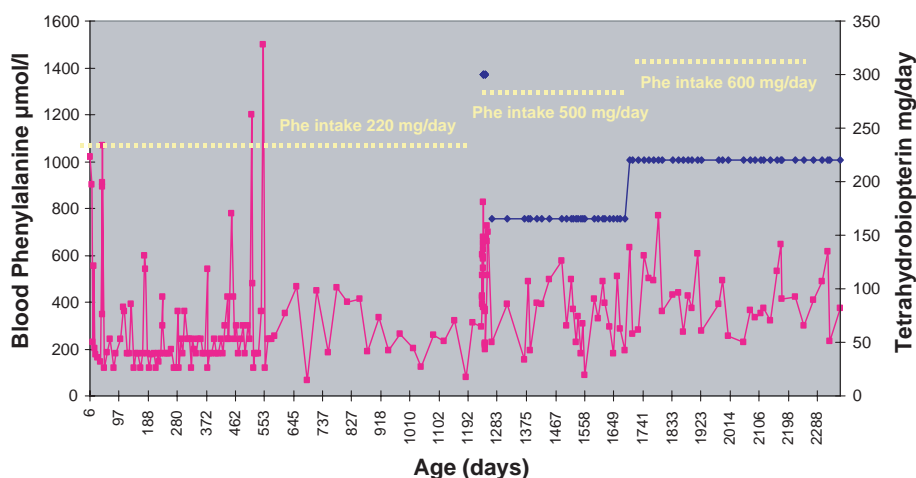


Fig. 5. Blood phenylalanine profile (red) and tetrahydrobiopterin dosage (blue) in Patient ID 494 from 1200 up to 2400 days of life. Phenylalanine intake (yellow) was stepwise increased (see text). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)

Patient ID 759 presented at 2 years of age as being on a Phe restricted diet since birth. Phe tolerance ranged from 220 to 250 mg/day. Blood Phe profile had been in the therapeutic range (Fig. 4B) but as can be seen from the weight gain in this patient (Fig. 4A) he suffered from failure to thrive because of diet refuse and problematic mother–child interaction. At this time blood Phe got out of control (up to 1100 µmol/L), so that we decided to start BH4 treatment with 150 mg/day (15 mg/kg BW per day). Under this treatment blood Phe dropped to 222 µmol/L. In the following, Phe tolerance could be increased to 500 mg/day and there was an excellent development and a better weight gain.

The most problematic patient on BH4 treatment is patient ID 977 (Fig. 7). The patient first was treated in another center. The parents refused a Phe restricted diet and tried a more or less low protein diet resulting in feeding

difficulties and insufficient weight gain. Since at the end of the first year blood Phe values were mostly above 600 µmol/L (Fig. 7). BH4 treatment was started with a dose of 70 mg/day (ca. 10 mg/kg BW per day). As can be seen there was a clear drop of blood Phe with mean Phe levels of ca. 350 µmol/L. At day 374–388 blood Phe values increased to 1000 µmol/L after the infant had developed high fever due to virus infection. The dose was increased to 150 mg/day (ca. 20 mg/kg BW per day) and blood Phe decreased to 150 µmol/L. However, after returning to 100 mg/day (ca. 12 mg/kg BW per day) blood Phe again increased and despite increase of BH4 to the high dose of 150 mg/day, blood Phe values remained very high for 10 days so that a low protein diet supplemented with 15 g of Phe-free amino acid mixture was started (day 425). Under this regimen there was a very rapid drop of blood Phe to a normal level

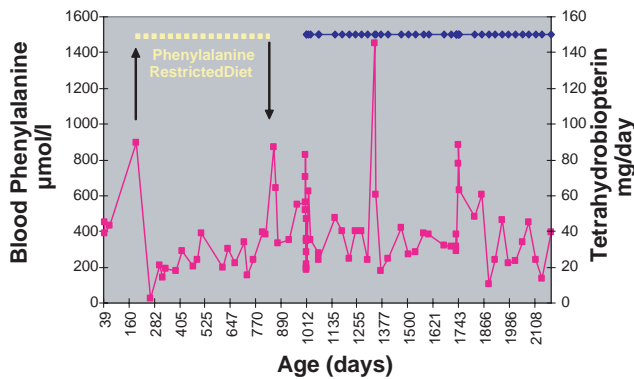


Fig. 6. Blood phenylalanine profile (red) and tetrahydrobiopterin dosage (blue) in Patient ID 445 from 1012 up to 2200 days of life. Phenylalanine restricted diet was stopped at about 800 days of life. At 1000 days of life BH4 loading (20 mg/kg BW per day) was performed for 5 days. At about 1300 days high increase of blood Phe due to virus infection occurred. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)

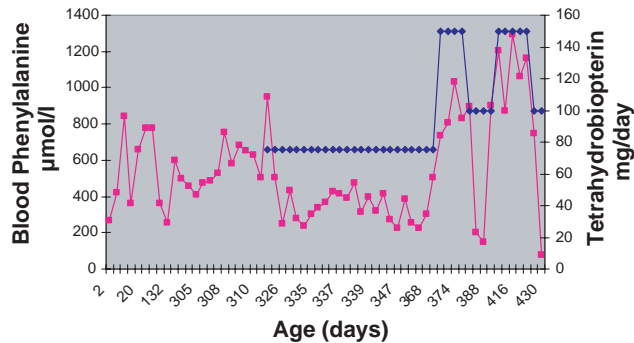


Fig. 7. Blood phenylalanine profile (red) and tetrahydrobiopterin dosage (blue) in Patient ID 977 from 300 up to 480 days of life. At days 380 and 415 high increase of blood phenylalanine although BH4 dosage was increased (see text). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)

so that BH4 was reduced to 100 mg/day again. No reason could be found for this second increase of blood Phe, clinical investigation, routine blood chemistry including infection parameters were perfectly normal, there was a normal weight gain.

Discussion

So far long-term experiences with BH4 treatment in patients with (Mild) PKU are very limited. BH4 dosage of ca. 10 mg/kg BW per day (in the first year 30–100 mg/day) in most cases seem to be sufficient. However, during febrile conditions (similar to the experiences in dietary treatment) blood Phe goes up, so that BH4 dosages may be increased. As in patients on dietary treatment it is very difficult always achieving therapeutic values between 60 and 240 µmol/L. To make BH4 regimen most practical, in the future self monitoring of blood Phe would be very desirable. In some situations, it may be necessary reducing protein intake for some days to stabilize Phe metabolism. One can speculate that supplementation of Phe free amino acid formula also

will contribute to a more stable metabolic condition. More data should be available also for BH4 serum concentrations to understand Phe tolerance in BH4 sensitive PKU patients.

The question of possible side effects cannot be answered by such a pilot study. From a theoretical point of view no side effects are reliable as pointed out by Blau and Erlandsen [1]. In our experience no side effects could be observed. However, further controlled studies are necessary not only to rule out any side effects but also for optimizing treatment strategies in mild and (also in rare cases) classical phenylketonuria. There is hope that from this new treatment at least these patients will profit who have a BH4 sensitive genotype. There is great evidence that this treatment will not only improve somatic but also intellectual development especially in patients with problems in staying on a Phe restricted semisynthetic diet.

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