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ORIGINAL ARTICLE

Pharmacokinetics of orally administered tetrahydrobiopterin in patients with phenylalanine hydroxylase deficiency

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Summary The oral loading test with tetrahydrobiopterin (BH_4) is used to discriminate between variants of hyperphenylalaninaemia and to detect BH_4-responsive patients. The outcome of the loading test depends on the genotype, dosage of BH_4, and BH_4 pharmacokinetics. A total of 71 patients with hyperphenylalaninaemia (mild to classic) were challenged with BH_4 (20 mg/kg) according to different protocols (1 × 20 mg or 2 × 20 mg) and blood BH_4 concentrations were measured in dried blood spots at different time points (T_0, T_2, T_4, T_8, T_{12}, T_{24}, T_{32} and $T_{48\,h}$). Maximal BH_4 concentrations (median 22.69 nmol/g Hb) were measured 4 h after BH_4 administration in 63 out of 71 patients. Eight patients presented with maximal BH_4 concentrations ~44% higher at 8 h than at 4 h. After 24 h, BH_4 blood concentrations dropped to 11% of maximal values. This profile was similar using different protocols. The following pharmacokinetic parameters were calculated for BH_4 in blood: t_{max} = 4 h, AUC ($T_{0–32}$) = 370 nmol × h/g Hb, and $t_{1/2}$ for absorption (1.1 h), distribution (2.5 h), and elimination (46.0 h) phases. Maximal BH_4 blood concentrations were not significantly lower in non-responders and there was no correlation between blood concentrations and responsiveness. Of mild PKU patients, 97% responded to BH_4 administration, while one was found to be a non-responder. Only 10/19 patients (53%) with Phe concentrations of 600–1200 μmol/L responded to BH_4 administration, and of the patients with the severe classical phenotype (blood Phe > 1200 μmol/L) only 4 out of 17 patient responded. An additional 36 patients with mild hyperphenylalaninaemia (HPA) who underwent the combined loading test with Phe+BH_4 were all responders. Slow responders and non-responders were found in all groups of HPA.

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Abbreviations

AUC	area under the curve
BH_4	tetrahydrobiopterin
HPA	hyperphenylalaninaemia
PAH	phenylalanine hydroxylase
PKU	phenylketonuria
$t_{1/2}$	half-life

Introduction

The loading test with tetrahydrobiopterin (BH_4) is an essential and integral part of the differential diagnosis for hyperphenylalaninaemia (HPA) (Blau et al 2001). This test discriminates between BH_4 responders and non-responders and is particularly important for detection of patients with BH_4-responsive phenylalanine hydroxylase (PAH; EC 1.14.16.1) deficiency (Blau and Erlandsen 2004). In BH_4 responders, blood phenylalanine (Phe) declines 4–24 h after BH_4 administration (20 mg/kg body weight), with almost complete

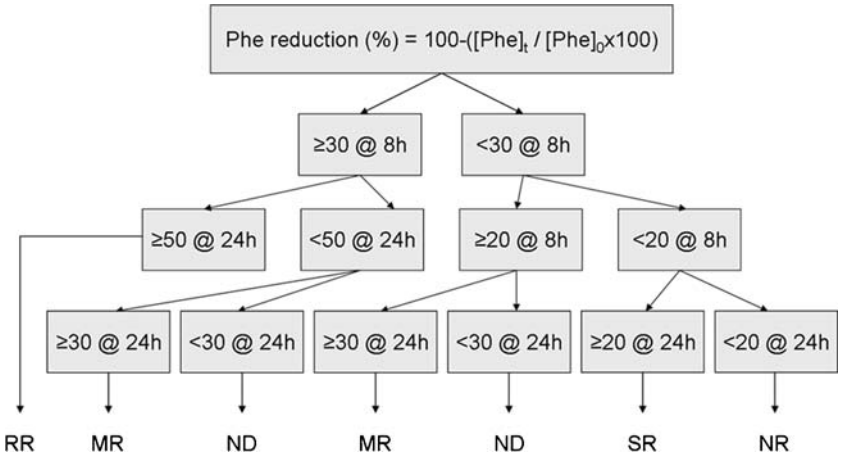

Fig. 1 Definition of the BH_4 responsiveness after oral administration of BH_4 (20 mg/kg). RR: rapid responder; MR: moderate responder; SR: slow responder; NR: non-responder; ND not defined

normalization after 4–8 h in patients with BH_4 deficiency. BH_4-responsive PAH patients were initially defined as showing a decrease of blood Phe concentrations of 30% after 8 h and 30–50% after 24 h (Bernegger and Blau 2002) and most of them belong to the group of mild HPA and mild and moderate phenylketonuria (PKU; OMIM 262600) (Desviat et al 2004; Fiori et al 2005; Kure et al 1999; Lässker et al 2002; Lindner et al 2003; Lücke et al 2003; Matalon et al 2004; Mitchell et al 2005; Muntau et al 2002; Perez-Duenas et al 2004; Spaapen et al 2001; Steinfeld et al 2003; Trefz and Blau 2003). Sensitivity of the test was further improved by multiple administrations of BH_4 and by longer observation time (Fiege et al 2005; Shintaku et al 2004). Efficiency and interpretation of the loading test depends on several factors such as amount of administered BH_4, severity of HPA, dietary management, and genotype. The pharmacokinetics of BH_4 was suggested as an additional factor affecting the loading test; however, this was never investigated in patients with PKU (Fiege et al 2004).

The aim of this study was to estimate basic kinetic parameters for BH_4 in blood after administration of BH_4 and following the combined Phe+BH_4 loading test, and to correlate the BH_4 concentrations in blood with the outcome of the test.

Materials and methods

Patients

A total of 71 HPA patients in whom BH_4 deficiency had been excluded were loaded with a single dose of BH_4 (20 mg/kg); 35 of them presented with basal blood Phe concentrations <600 μmol/L, 19 with Phe concentrations of 600–1200 μmol/L, and 17 with Phe concentrations >1200 μmol/L. In 11 patients with blood Phe concentrations of 278–1575 μmol/L the standard test was extended by the administration of another 20 mg/kg BH_4 after 24 h. An additional 36 patients with basal Phe concentrations of 384–739 μmol/L underwent a combined Phe (100 mg/kg) and BH_4 (20 mg/kg) loading test.

Mutation analysis was done in only a few patients and was not included in this study. The study was performed after a formal consensus of patients or their parents and in accordance with the Helsinki recommendations 1989.

Loading test

The single BH_4 test, the extended test, and the combined Phe+BH_4 loading test were performed as described previously using the 6R-BH_4 (Schircks Laboratories, Jona, Switzerland) (Blau and Erlandsen 2004; Fiege et al 2005). In patients loaded with a single dose of BH_4 (20 mg/kg), blood was collected at times T_0, T_4, T_8 and $T_{24\,h}$. In 45 of them additional blood collections were done at T_2, T_{12} and $T_{48\,h}$. In patients loaded with 2 × 20 mg/kg BH_4, blood was collected at T_0, T_4, T_8, T_{24}, T_{32} and $T_{48\,h}$, and in patients loaded with Phe+BH_4 the following blood collections were performed: T_{-3} (Phe administration), T_0 (BH_4 administration), T_4, T_8 and $T_{24\,h}$.

The following criteria were used to define BH_4-responsiveness over 24 h (Fiege et al 2005): 'rapid responder', reduction of blood Phe by ≥30% at T_8, and ≥50% at T_{24}; 'moderate responder', reduction of blood Phe by ≥20% at T_8, and ≥30% at T_{24}; 'slow responder', reduction of blood Phe by <20% at T_8, and ≥20% at T_{24} (Fig. 1).

BH_4 and Phe in blood

BH_4 was measured in dried blood spots according to the method of Zurflüh and colleagues (2005) and calculated as the sum of total biopterin and pterin (nmol BH_4/g Hb). The following protocol was used: For each measurement, four blood circles (Ø6 mm) were cut out and pterins were extracted with 250 μL of 20 mmol/L HCl and placed in an ultrasonic bath for 30 s. Pterins were extracted by mixing the filter

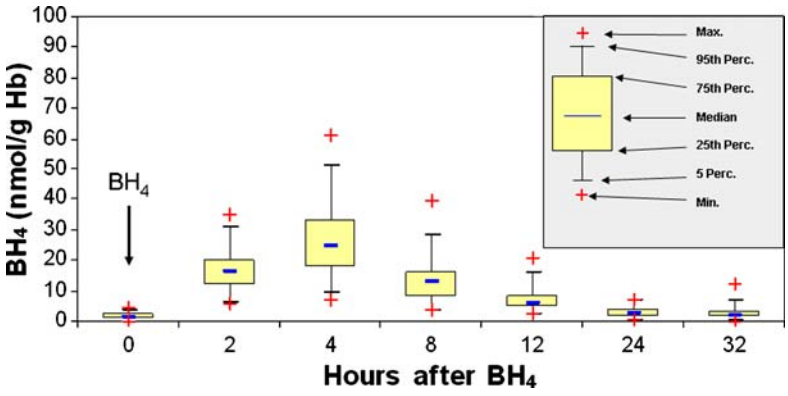

Fig. 2 BH_4 concentrations in blood after oral administration of BH_4 (20 mg/kg) in 47 patients with HPA. — Median, □ 25th–75th percentiles; ⊥ 5th percentile; ⊤ 95th percentile; + min/max

spot solution for 10 min at room temperature. The extract was centrifuged at 1800 × *g* for 5 min at room temperature. Clear supernatatant (60 μL) was used for analysis of haemoglobin on the haematology analyser Sysmex KX-21N (Sysmex Corporation, Japan). The remaining supernatant was ultrafiltered on Ultrafree (NMWL 10000; Millipore) at 5000 × *g* for 15 min. Pterins were analysed in clear filtrate by HPLC and fluorescence detection without prior oxidation (Zurflüh et al 2005).

Phe was measured using standard ion-exchange chromatography of amino acids or tandem-mass spectrometry.

Statistical analyses

WinSTAT for Excel (v. 2003.1) was used for descriptive statistics and for regression analysis. Pharmacokinetic parameters were calculated using the PK Solutions software, v. 2.0 (Summit Research Services, Montrose, CO).

Results

BH_4 kinetics in blood

In 63 out of 71 patients with HPA loaded with 20 mg/kg BH_4, blood BH_4 concentrations reached highest values after 4 h (median = 22.65 nmol/g Hb; 5th–95th percentiles = 10.07–49.97 nmol/g Hb). Four hours later (T_8) BH_4 concentrations decreased by 42% (median = 13.61 nmol/g Hb; 5th–95th percentiles = 5.25–31.63 nmol/g Hb) and after 24 h BH_4 concentrations were only 11% of maximal values (median = 2.29 nmol/g Hb; 5th–95th percentiles = 0.67–5.35 nmol/g Hb). In 8 out of 71 patients, blood BH_4 concentrations were ∼44 % higher at 8 h than values at 4 h after administration and the highest single BH_4 value was 96.39 nmol/g Hb at $T_{4\,h}$.

In 45 of the above patients the blood BH_4 profile was investigated over 32 h after BH_4 administration (20 mg/kg) with additional time points at T_2, T_{12} and $T_{32\,h}$ (Fig. 2). Two hours after BH_4 administration (median = 16.30 nmol/g Hb; 5th–95th percentiles = 6.84–28.44 nmol/g Hb) blood concentrations reached about 70% of the maximal concentrations found after 4 h. At 12 h (median = 6.15 nmol/g Hb; 5th–95th percentiles = 2.90–12.38 nmol/g Hb) and 32 h (median = 2.03 nmol/g Hb; 5th–95th percentiles = 0.51–5.47 nmol/g Hb) BH_4 blood concentrations were lower than those at 8 h (47% and 15%, respectively).

In 11 patients the protocol was extended to a second administration of BH_4 (20 mg/kg) after 24 h (Fig. 3). Blood was collected 8 (T_{32}) and 24 (T_{48}) hours after the second administration and compared with T_8 concentrations for BH_4 in blood (median = 15.37 nmol/g Hb; 5th–95th percentiles = 7.05–54.02 nmol/g Hb); T_{32} values were 17% lower (median = 12.80 nmol/g Hb; 5th–95th percentiles = 7.41–38.54 nmol/g Hb). There was no significant difference in BH_4 concentrations at T_{24} and $T_{48\,h}$.

Thirty-six patients with blood Phe concentrations of <336 μmol/L were loaded first with Phe (100 mg/kg) and three hours later with BH_4 (20 mg/kg), and blood samples were collected before Phe administration (T_{-3}), before BH_4 administration (i.e. 3 h after Phe loading; T_0), and 4, 8 and 24 h after BH_4 administration (T_{4-24}) (Fig. 4). BH_4 concentrations in blood increased in 26/36 patients after Phe administration by 111% (BH_4 at T_{-3}, median = 0.30 nmol/g Hb; 5th–95th percentiles = 0.12–2.77 nmol/g Hb; BH_4 at T_0, median = 0.52 nmol/g Hb; 5th–95th percentiles = 0.16–4.43 nmol/g Hb). The following profile of BH_4 kinetics was similar as the one described for a single BH_4 administration, with maximal blood concentrations at T_4 (median = 22.01 nmol/g Hb; 5th–95th percentiles = 10.40–46.58 nmol/g Hb), 25% lower concentrations at T_8 (median = 16.46 nmol/g Hb; 5th–95th percentiles = 4.66–33.49 nmol/g Hb), and 90% lower concentrations at T_{24} (median = 2.09 nmol/g Hb; 5th–95th percentiles = 0.55–6.84 nmol/g Hb). In 6 subjects maximal BH_4 concentrations were reached after 8 h.

Table 1 Summary of BH_4 loading tests (20 mg/kg) in 71 patients with PAH deficiency

		Initial blood Phe concentrations		
	n	<600 μmol/L ($n = 35$)	600–1200 μmol/L ($n = 19$)	>1200 μmol/L ($n = 17$)
Responder (total)	48	34	10	4
Rapid responder	34	26	6	2
Moderate responder	10	7	3	0
Slow responder	4	1	1	2
Non-responder	21	1	8	12
Not defined	2	0	1	1

Based on data obtained over 32 h after a single BH_4 administration (20 mg/kg) in 45 patients with HPA, basic pharmacokinetic parameters were calculated: t_{max} was 4 h, AUC (T_{0-32}) was 370 nmol × h/g Hb, and $t_{1/2}$ for absorption, distribution, and elimination phases was 1.1, 2.5, and 46.0 h, respectively.

Responsiveness to BH_4

Table 1 summarizes the results of the loading test with 20 mg/kg BH_4 in patients with HPA. Responsiveness was calculated according to criteria defined in Fig. 1. 34/35 patients (97%) with basal blood Phe concentrations <600 μmol/L responded to BH_4 administration, one patient was found to be a non-responder. Only 10/19 patients (53%) with basal blood Phe concentrations of 600–1200 μmol/L responded to BH_4 administration, and of the patients with the severe classical phenotype (blood Phe > 1200 μmol/L) only four patients responded (24%), and two of them were slow responders (Fig. 5). The lower the Phe at T_0 the higher the probability that a patient will respond to BH_4. Two patients could not be assigned to the above-mentioned criteria; one would have been positioned somewhere between moderate and slow responder and the other was at the level of a

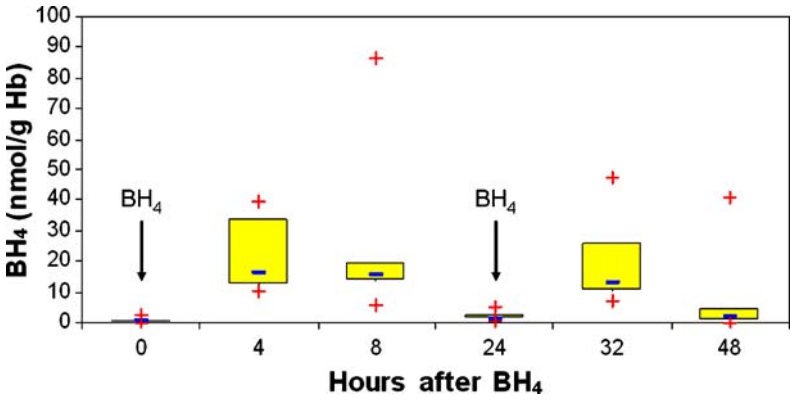

Fig. 3 BH_4 concentrations in blood after oral administration of BH_4 (2 × 20 mg/kg) in 11 patients with HPA. Second dosage of BH_4 was administered 24 h after the first application. — Median, □ 25th–75th percentiles; ⊥ 5th percentile; T 95th percentile; + min/max

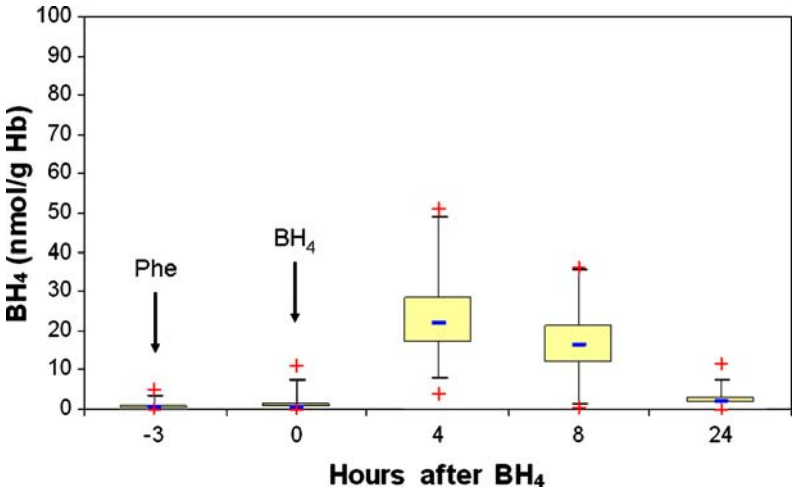

Fig. 4 BH_4 concentrations in blood after oral administration of Phe (100 mg/kg) and BH_4 (20 mg/kg) in 36 patients with mild HPA. BH_4 was administered 3 h after Phe application. — Median, □ 25th–75th percentiles; ⊥ 5th percentile; T 95th percentile; + min./max

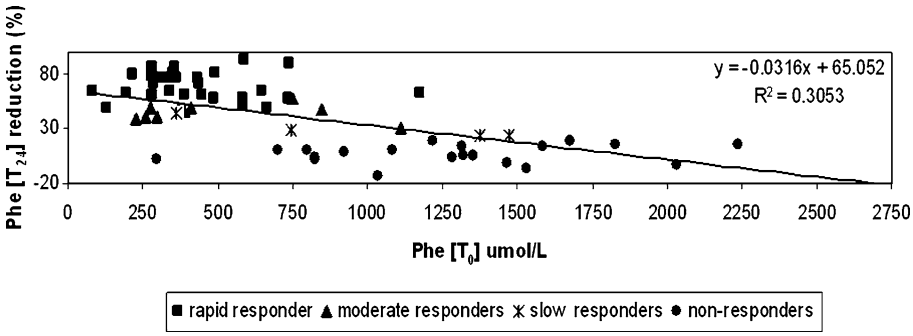

Fig. 5 Correlation between Phe decline in blood 24 h after administration of BH_4 (20 mg/kg) and basal Phe levels in 71 patients with HPA. ■ Rapid responder; ▲; moderate responder; * slow responder; · non-responder

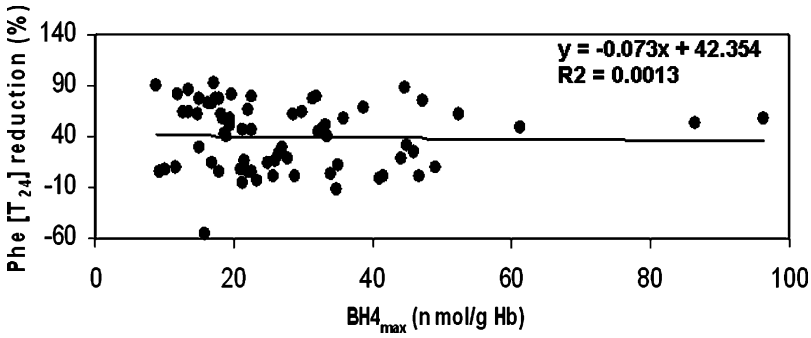

Fig. 6 Correlation between Phe decline in blood 24 h after administration of BH_4 (20 mg/kg) and maximal BH_4 levels in 71 patients with HPA

rapid responder after 8 h but dropped at 24 h below the level of a slow responder.

All 36 patients who underwent the combined loading test with Phe+BH_4 were classified as responders (data not shown).

In order to demonstrate whether absorption of BH_4 may affect the outcome of the loading test, maximal blood BH_4 concentrations were compared with responsiveness to BH_4, but no correlation was found (Fig. 6). Highest maximal blood BH_4 concentrations were found in two rapid responders.

Discussion

Extensive pharmacokinetic studies of BH_4 have been performed in animal models (Hayashi et al 1992), but only a few parameters are known from studies in humans (Fiege and Blau 2006). Some pharmacokinetic parameters are known from oral administration of BH_4 tablets to healthy adult human volunteers and might provide details on pharmacological response to BH_4 therapy (Fiege et al 2004). Plasma concentrations of BH_4 and total biopterin were assessed after oral administration of 6R-BH_4 at different doses to different healthy subjects and preliminary pharmacokinetic parameters have been determined (Fiege et al 2004). The plasma profile of total biopterin after oral administration exhibited first-order kinetics, showing a fast absorption phase (T_0–T_4), a rapid decline (T_4–T_{10}) corresponding to the absorption and distribution phase, followed by a slower decline in the final elimination phase (T_{10}–$T_{33\,h}$). Total biopterin concentrations in plasma have been studied after administration of different doses (10 and 20 mg/kg) to one healthy adult subject (Fiege et al 2004). Maximal plasma concentrations in this subject were reached 4 h after the 10 mg/kg dose and 3 h after the 20 mg/kg dose, at concentrations of 258.7 and 441.7 nmol/L, respectively. The AUC_{0-10} after administration of 20 mg/kg was 1.6 times higher than the AUC after the 10 mg/kg dosage (3046 vs 1958 nmol h/L). Based on these data, the elimination kinetics seem to be only slightly faster at higher plasma concentrations (t_{max} = 4.2 h vs 5.1 h) (Fiege et al 2004).

Very little is known about BH_4 pharmacokinetics in patients with HPA. Shintaku and colleagues (2005) reported plasma biopterin concentrations in two patients with HPA who underwent a single BH_4 loading test (10 mg/kg) at different ages. In both patients plasma biopterin concentrations were ~100% higher at an early age (<1 month) compared with concentratiuons measured at the age of 2 months. Also, biopterin concentrations peaked at 4 h at the age of 1 month, compared with maximal concentrations at 2 h at the age of 2 months. The authors suggested that BH_4 responsiveness in the same individual or the same genotype may correlate with biopterin concentration, but in different genotypes this might not be the case (Shintaku et al 2005).

We were not able to see any statistical difference between different age groups in our patients (data not shown). Similarly to what was described for healthy controls (Fiege et al 2004), blood BH_4 peaked at 4 h in 90% of patients with HPA. This profile was consistent regardless of whether patients were loaded with one or two BH_4 doses or after Phe administration (Figs. 2–4). Two hours after BH_4 administration, blood concentrations were about 70% of the maximal

BH_4 concentrations, indicating a very fast absorption phase ($t_{1/2} = 1.1$ h). In our patients, oral administration of BH_4 resulted in a fast distribution phase ($t_{1/2} = 2.5$ h), followed by a slow elimination phase ($t_{1/2} = 46.0$ h). Thus, although a single BH_4 administration may be sufficient for the interpretation of the loading test, additional dosages can potentate the effect and increase the sensitivity (Fiege et al 2005; Shintaku et al 2004).

Data from the combined Phe+BH_4 loading test show that administration of Phe (100 mg/kg) almost doubled blood BH_4 concentrations after 3 h in 72% of patients (Fig. 4). This is consistent with previous findings that biopterin concentrations in urine or plasma correlate with blood phenylalanine concentrations (Dhondt and Farriaux, 1982; Ponzone et al 1993) but have no consequences on the outcome of the loading test. Phenylalanine administration does not influence BH_4 concentrations upon oral BH_4 administration. As expected, all patients in this group were classified as responders and one should question how useful this test is. Factors such as spontaneous Phe elimination (Desviat et al 2004) or daily fluctuations (Leuzzi et al 2006) may influence the interpretation, and from our experience the combined loading test is not recommended. It can be only used in patients who are already on a strict low-phenylalanine diet with normalized blood phenylalanine concentrations.

One of the main goals of this study was to evaluate the effect of blood BH_4 concentrations on the outcome of the loading tests. Recently we described a single case with HPA (BH_4-responsive genotype) showing intra-individual variations in BH_4 absorption on two occasions, which resulted in different BH_4 blood concentrations and influenced the responsiveness (Zurflüh et al 2005). We were not able to repeat the loading test in non-responders in this study, but maximal BH_4 blood concentrations were not significantly lower in this group of patients. Indeed, maximal BH_4 blood concentrations were only 3% lower in non-responders than in all responders, and slow responders had 7% and 42% higher concentrations than moderate or rapid responders, respectively (data not shown).

With regard to the responsiveness to BH_4, our data confirm previous observations that rapid responders belong mainly to the groups of mild HPA and mild PKU, and that patients with classical PKU show either only a slow response or none at all (Fig. 5). Nevertheless, slow responders and non-responders were found in all groups of HPA.

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References

Bernegger C, Blau N (2002) High frequency of tetrahydrobiopterin-responsiveness among hyperphenylalaninemias: a study of 1919 patients observed from 1988 to 2002. *Mol Genet Metab* **77**: 304–313.

Blau N, Erlandsen H (2004) The metabolic and molecular bases of tetrahydrobiopterin-responsive phenylalanine hydroxylase deficiency. *Mol Genet Metab* **82**: 101–111.

Blau N, Thöny B, Cotton RGH, Hyland K (2001) Disorders of tetrahydrobiopterin and related biogenic amines. In: Scriver CR, Beaudet AL, Sly WS, Valle D, eds; Childs B, Kinzler KW, Vogelstein B, assoc, eds. *The Metabolic and Molecular Bases of Inherited Disease*, 8th edn. New York: McGraw-Hill, 1725–1776.

Desviat LR, Pérez B, Bèlanger-Quintana A, et al (2004) Tetrahydrobiopterin responsiveness: results of the BH_4 loading test in 31 Spanish PKU patients and correlation with their genotype. *Mol Genet Metab* **82**: 157–162.

Dhondt JL, Farriaux JP (1982) Relationships between phenylalanine and biopterin metabolisms. In: Wachter H, Curtius HC, Pfleiderer W, eds. *Biochemical and Clinical Aspects of Pteridines*. Berlin: Walter de Gruyter, 319–336.

Fiege B, Blau N (2006) Pharmacokinetic of tetrahydrobiopterin in humans and rats. *PKU and BH_4: Advances in Phenylketonuria and Tetrahydrobiopterin*. Heilbronn: SPS Verlagsgesellschaft, 638–651.

Fiege B, Ballhausen D, Kierat L, et al (2004) Plasma tetrahydrobiopterin and its pharmacokinetics following oral administration. *Mol Genet Metab* **81**: 45–51.

Fiege B, Bonafé L, Ballhausen D, et al (2005) Extended tetrahydrobiopterin loading test in the diagnosis of cofactor-responsive phenylketonuria: a pilot study. *Mol Genet Metab* **86** (Supplement 1): 91–95.

Fiori L, Fiege B, Riva E, Giovannini M (2005) Incidence of BH_4-responsiveness in phenylalanine-hydroxylase-deficient Italian patients. *Mol Genet Metab* **86**(Supplement 1): 67–74.

Hayashi T, Ogata A, Takehisha M, Komoridani K, Oonuma N (1992) Studies on metabolism and disposition of sapropterine hydrochloride (SUN-0588) L-erythro-tetrahydrobiopterin dichloride in rats. *Clin Report* **26**: 3471–3495.

Kure S, Hou DC, Ohura T, et al (1999) Tetrahydrobiopterin-responsive phenylalanine hydroxylase deficiency. *J Pediatr* **135**(3): 375–378.

Lässker U, Zschocke J, Blau N, Santer R (2002) Tetrahydrobiopterin responsiveness in phenylketonuria. Two new cases and a review of molecular genetic findings. *J Inherit Metab Dis* **25**: 65–70.

Leuzzi V, Carducci C, Carducci C, et al (2006) The spectrum of phenylalanine variations under tetrahydrobiopterin load in subjects affected by phenylalanine hydroxylase deficiency. *J Inherit Metab Dis* **29**(1): 38–46.

Lindner M, Steinfeld R, Burgard P, Schulze A, Mayatepek E, Zschocke J (2003) Tetrahydrobiopterin sensitivity in German patients with mild phenylalanine hydroxylase deficiency. *Hum Mutat* **21**(4):400.

Lücke T, Illsinger S, Aulehla-Scholz C, Sander J, Das AM (2003) BH_4-sensitive hyperphenylalaninemia: new case and review of literature. *Pediatr Neurol* **28**(3): 228–230.

Matalon R, Koch R, Michals-Matalon K, et al (2004) Biopterin responsive phenylalanine hydroxylase deficiency. *Genet Med* **6**(1): 27–32.

Mitchell JJ, Wilcken B, Alexander I, et al (2005) Tetrahydrobiopterin-responsive phenylketonuria: the New South Wales experience. *Mol Genet Metab* **86**(Supplement 1): S81–85.

Muntau AC, Roschinger W, Habich M, et al (2002) Tetrahydrobiopterin as an alternative treatment for mild phenylketonuria. *N Engl J Med* **347**: 2122–2132.

Perez-Duenas B, Vilaseca MA, Mas A, et al (2004) Tetrahydrobiopterin responsiveness in patients with phenylketonuria. *Clin Biochem* **37**(12): 1083–1090.

Ponzone A, Guardamagna O, Spada M, et al (1993) Hyperphenylalaninemia and pterin metabolism in serum and erythrocytes. *Clin Chim Acta* **216**: 63–71.

Shintaku H, Kure S, Ohura T, et al (2004) Long-term treatment and diagnosis of tetrahydrobiopterin-responsive hyperphenylalaninemia with a mutant phenylalanine hydroxylase gene. *Pediatr Res* **55**: 425–430.

Shintaku H, Fujioka H, Sawada Y, Asada M, Yamano T (2005) Plasma biopterin levels and tetrahydrobiopterin responsiveness. *Mol Genet Metab* **86**(Supplement 1): S104–S106.

Spaapen LJM, Bakker JA, Velter C, et al (2001) Tetrahydrobiopterin-responsive phenylalanine hydroxylase deficiency in Dutch neonates. *J Inherit Metab Dis* **24**: 325–358.

Steinfeld R, Kohlschutter A, Ullrich K, Lukacs Z (2003) A hypothesis on the biochemical mechanism of BH(4)-responsiveness in phenylalanine hydroxylase deficiency. *Amino Acids* **25**(1): 63–68.

Trefz FK, Blau N (2003) Potential role of tetrahydrobiopterin in the treatment of maternal phenylketonuria. *Pediatrics* **112**: 1566–1569.

Zurflüh MR, Fiori L, Fiege B, et al (2005) Screening for tetrahydrobiopterin deficiencies using dried blood spots on filter paper. *Mol Genet Metab* **86**(Supplement 1): 96–103.