

Pharmacokinetics of tetrahydrobiopterin following oral loadings with three single dosages in patients with phenylketonuria

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Summary

Background: Tetrahydrobiopterin (BH₄) loading has been performed for many years in patients detected by newborn screening for hyperphenylalaninaemia (HPA) to distinguish BH₄ cofactor synthesis or recycling defects from phenylalanine hydroxylase (PAH)-deficient HPA. Previous studies have shown that the pharmacokinetics of BH₄ shows high intra-individual and inter-individual variability.

Methods: Seventeen adult patients with PAH-deficient HPA were classified in one of three phenotypic groups (mild, moderate, classical PKU) according to their response to a standardized protein loading test. Genotype information was available for all participants. In a randomized controlled double-blind design, BH₄ loadings in single oral dosages of 10, 20 and 30 mg

BH₄/kg body weight (bw) were performed to assess BH₄ responsiveness. As part of this study, levels of BH₄ metabolites in dried blood spots were studied to provide information on the pharmacokinetics of BH₄ following oral administration.

Results: Levels of biopterin and pterin (B+P) increased significantly with increasing BH₄ dose ($p < 0.0001$). Maximum B+P levels were reached 4 hours after application of BH₄. There was no significant difference in BH₄ pharmacokinetics between the three phenotypic groups of PKU. Male and female patients showed different levels of BH₄ metabolites following 10 mg BH₄/kg bw, but not following 20 and 30 mg BH₄/kg bw. There was no relationship between age of patients and BH₄ pharmacokinetics. There was no correlation between B+P levels and decrease in Phe level ($p = 0.69$).
Conclusion: BH₄ pharmacokinetics are variable between patients regarding absolute levels of BH₄ metabolites reached after BH₄ loading, but are similar regarding the interval to individual maximum B+P levels. Levels of B+P increase significantly with increasing BH₄ doses. There is no correlation between B+P levels and decrease in Phe level.

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Abbreviations

B+P biopterin + pterin
BH₄ tetrahydrobiopterin
HPA hyperphenylalaninaemia
PAH phenylalanine hydroxylase
PKU phenylketonuria

Introduction

Phenylketonuria (PKU, OMIM #261600) was the first metabolic disorder diagnosed in population-based

newborn screening programmes. Tetrahydrobiopterin (BH₄) cofactor loading with consecutive analysis of phenylalanine (Phe) concentrations has been performed for many years in patients detected in newborn screening to distinguish BH₄ cofactor synthesis or recycling defects from PAH-deficient hyperphenylalaninaemia (HPA) (Blau 2006; Niederwieser et al 1985). In the United States, BH₄ has recently obtained marketing approval as an alternative treatment option (KUVAN, Biomarin Pharmaceutical Inc., USA) for BH₄-responsive HPA patients older than 4 years.

The pharmacokinetics of BH₄ has been reported to be highly variable between patients and also in individuals at different ages (Zurflüh et al 2005). BH₄ pharmacokinetics was evaluated in patients with mild, moderate and classical PKU as part of a study evaluating the response to three different dosages of BH₄. A correlation between BH₄ levels and decrease in Phe level was evaluated.

Study design

Patients

Seventeen patients with PKU (10 male, 7 female; mean age 27.8 years, range 19.3–36.8 years) were included in the study. All patients had been diagnosed in the newborn period and had been continuously treated during childhood and adolescence. Exclusion

criteria were pregnancy or breast-feeding in women, neuropsychiatric diseases and chronic diseases (e.g. seizures, asthma and diabetes mellitus).

All patients gave written informed consent to participation in the study. The study conformed to the tenets of the Declaration of Helsinki (Version Tokyo 2004) and was approved by the institutional review board of Heidelberg University (EC I, Number 272/2004).

Phenotype

According to the 72 h Phe level in a standardized loading test with 180 mg Phe/kg body weight (bw) performed during the German collaborative study of phenylketonuria between 1978 and 1984 (Lutz et al 1990) or as part of clinical routine evaluation, patients were assigned to three groups: group 1, mild PKU, 72 h Phe level 10–17 mg/dl (600–1020 µmol/L); group 2, moderate PKU, 72 h Phe level 17.1–26 mg/dl (1021–1560 µmol/L), group 3, classical PKU, 72 h Phe level >26 mg/dl (>1560 µmol/L) (Table 1).

Genotype

Genotypes in both alleles were available for all patients (Table 1). For genotypes R158Q/R408W and R408W/IVS12+1G>A, pairs with identical genotype were included; for genotype Y414C/IVS12+1G>A, three patients with identical genotype were included.

Table 1 Patients' genotype and phenotype. Maximum levels of biopterin and pterin following 10, 20, and 30 mg BH₄/kg bw were achieved 4 hours after intake in all 17 PKU patients

Patient	Phenotype	Genotype		Maximum biopterin (B)+pterin (P) (nmol/g Hb)		
		Allele 1	Allele 2	10 mg BH ₄ /kg bw	20 mg BH ₄ /kg bw	30 mg BH ₄ /kg bw
2	Mild	Y414C	IVS12+1G>A	4.68	6.63	12.65
9	Mild	Y414C	IVS12+1G>A	1.75	2.27	4.26
16	Mild	Y414C	R261Q	3.96	2.95	9.12
17	Mild	A395G	IVS10–11G>A	m	5.04	9.47
5	Mild	Y414C	IVS12+1G>A	1.43	4.65	7.16
11	Mild	R261Q	R261Q	1.96	4.33	3.88
7	Moderate	R261Q	Y166X	4.50	10.23	13.09
10	Moderate	R261Q	K274_Y277>Nfs	2.64	13.42	8.3
14	Classical	P281L	R408W	1.86	2.95	4.69
4	Classical	P281L	P281L	7.33	7.76	7.88
12	Classical	R158Q	R158Q	4.82	3.76	6.80
3	Classical	R158Q	R408W	12.46	17.40	22.64
8	Classical	R158Q	R408W	2.97	8.20	8.58
15	Classical	R408W	IVS12+1G>A	5.89	4.98	7.04
6	Classical	R408W	IVS12+1G>A	2.24	3.92	5.64
13	Classical	IVS12+1G>A	E221_D222>Efs	3.08	6.11	8.26
18	Classical	IVS12+1G>A	L48S	2.07	3.74	4.79

m=missing value.

BH₄ loadings

BH₄ (6-(R)-L-erythro-5,6,7,8-tetrahydrobiopterin) was obtained in 50 mg tablets from Schircks Laboratories (Jona, Switzerland). Following a double-blind randomized protocol, all patients received BH₄ in doses of 10, 20 and 30 mg/kg bw, respectively, in a single morning dose after an overnight fast at days 0, 7 and 14 of the study. BH₄ was dissolved in water or orange juice immediately before application. All patients were off diet.

Blood sampling

Thirty-six blood samples per patient were taken for analysis of Phe and tyrosine. BH₄ metabolites were analysed before as well as 4 h and 8 h after each BH₄ administration. Capillary blood samples were spotted on Whatman S&S 903 filter paper. Samples taken by the patients at home were stored at ambient temperature for a maximum of 7 days. All samples were stored at -18°C until analysis. Pterin concentrations were measured as described previously by Zurflüh and colleagues (2005). Because BH₄ is extremely unstable

in collected blood and about 30–40% is turned into pterin (Zurflüh et al 2005), total biopterin was calculated as the sum of biopterin and pterin (B+P; nmol/g Hb).

Phe and tyrosine levels were analysed at two single measuring days using the same tandem mass spectrometer (Micromass Ultima; metabolic laboratory, Heidelberg) to minimize inter-analysis and inter-device deviations using tandem mass spectrometry following standard methods as previously described (Schulze et al 2003).

Results

Levels of B+P were highly variable between patients after identical oral doses of BH₄. B+P levels varied between 1.43–12.46 nmol/g Hb after 10 mg BH₄/kg bw, 2.95–17.40 nmol/g Hb after 20 mg BH₄/kg bw, and 3.88–22.64 nmol/g Hb after 30 mg BH₄/kg bw (Table 1, Fig. 1). In all patients, maximum B+P levels were reached 4 h after BH₄ loading (Fig. 2).

In a linear mixed-effect model we evaluated the relationship between maximum B+P levels (dependent

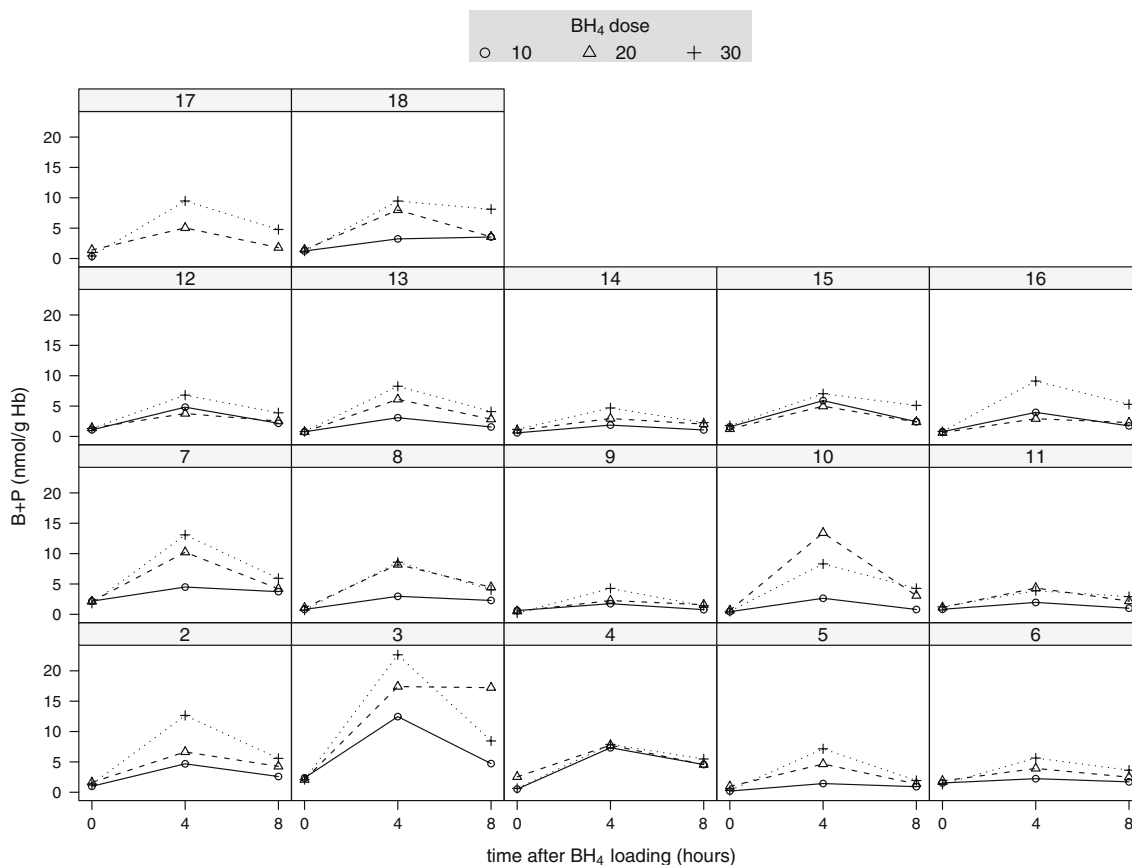


Fig. 1 Individual kinetics of biopterin and pterin (B+P) following BH₄ loading with 10, 20 and 30 mg BH₄/kg body weight in 17 PKU patients (patient ID 2–18)

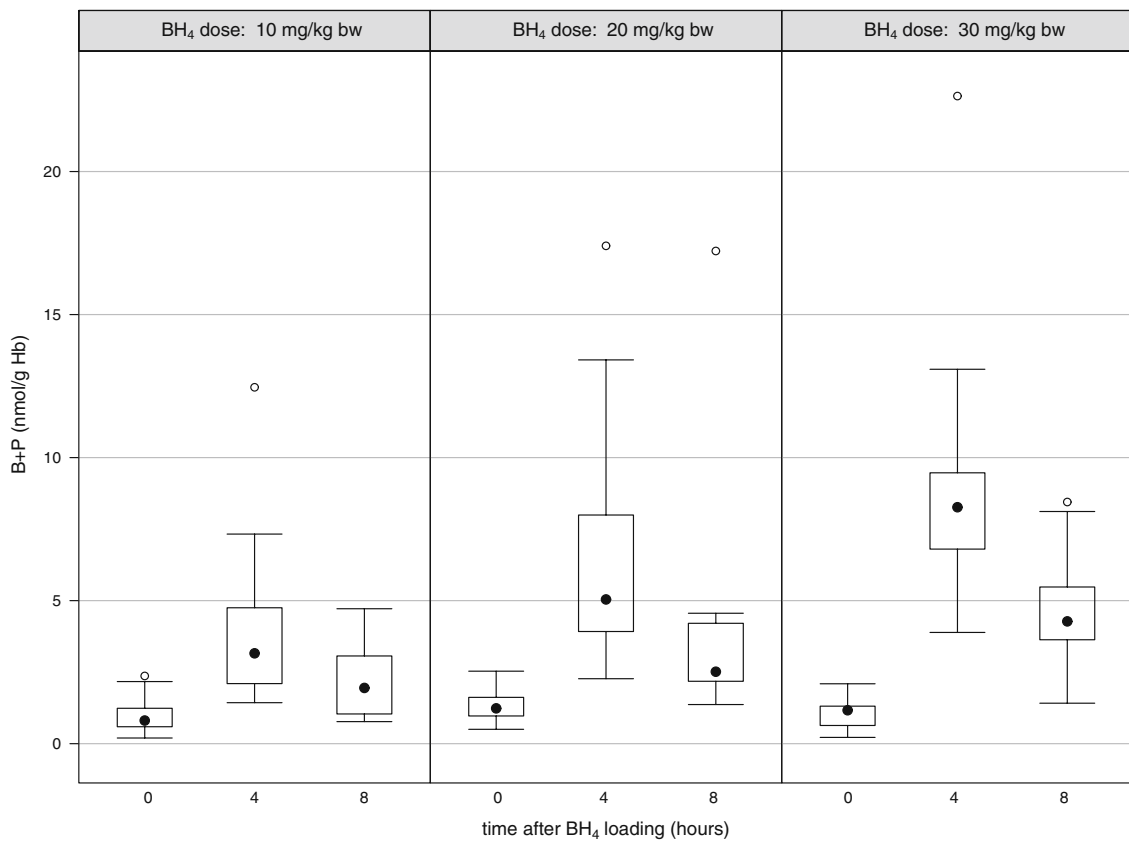


Fig. 2 Levels of biopterin and pterin (B+P) 0, 4 and 8 h after BH₄ loading with 10, 20 and 30 mg BH₄/kg body weight

variable), phenotype (mild, moderate and classical PKU) and BH₄ dose (10, 20, 30 mg BH₄/kg bw). Maximum levels of B+P increased with increasing dosage of BH₄ administered. The differences in maximum B+P after 10 vs 20 vs 30 mg BH₄/kg bw were statistically highly significant ($F(2,28)=30.86$; $p<0.001$) (Fig. 2).

There was no significant difference in maximum B+P levels between patients in the three different phenotype groups ($F(2,14)=1.27$; $p=0.311$) at any of the three BH₄ dosages.

In a linear mixed-effect model we evaluated the relationship between Phe level changes (dependent variable), phenotype, B+P levels (numerical) and BH₄ dose. Assessment of a possible relationship between BH₄ metabolites and BH₄ response in terms of decrease in Phe level showed that there was no interaction of maximal B+P level with Phe level decrease in any of the three phenotypic groups ($F(4,27)=1.36$; $p=0.273$). There was no significant relationship between maximal B+P levels and Phe level changes ($F(1,27)=0.16$, $p=0.69$).

To assess the relationship between age of the patients and maximum B+P levels, we computed three linear regressions, one for each BH₄ dose. There was

no relationship between age of the patients and maximum B+P levels ($[B+P]_{\max}$) at any of the BH₄ doses administered (10 mg BH₄: $p=0.7023$, 20 mg BH₄: $p=0.7698$, 30 mg BH₄: $p=0.7051$).

Differences between male and female patients in B+P levels following administration of 20 and 30 mg BH₄/kg bw were non-significant (Mann–Whitney *U*-Test, $p=0.669$ and $p=0.8868$). After loading with 10 mg BH₄/kg bw, B+P levels in men were significantly lower than in women (Mann–Whitney *U*-Test, $p=0.01357$).

Discussion

The pharmacokinetics of BH₄ in PKU patients following different dosages of BH₄ was evaluated by analysis of biopterin and pterin from dried blood spots. This method of measuring BH₄ metabolites shows a good correlation of results for biopterin and pterin levels in plasma and urine (Zurflüh et al 2005).

In accordance with previous studies (Fiege et al 2004; Zurflüh et al 2005) we found that the pharmacokinetics of BH₄ is highly variable between subjects.

After all three dosages of BH₄, levels of B+P in dried blood spots differed substantially between patients.

Zurflüh and colleagues (2005) reported that, in addition to inter-individual differences, the level of dried blood B+P was highly variable in one individual who underwent BH₄ loading twice. On the first test this patient showed low B+P levels and only a minor decrease in blood phenylalanine; several weeks later the same patient showed markedly higher levels of B+P and a good response to BH₄ loading in terms of Phe level decrease (Zurflüh et al 2005).

The reasons for these inter- and intra-individual differences in BH₄ metabolism are not well understood to date. Shintaku and colleagues (2005) reported two patients who underwent repeated BH₄ loadings in their first months of life and showing a decrease in plasma pterin levels following oral loads with 10 mg BH₄/kg bw with increasing age. In one patient this was correlated with a less marked lowering of phenylalanine levels from 54% at age 30 days to 16% at age 55 days and only 4% at 19 months. In this patient the maximum phenylalanine decline was in proportion to the square of each peak biopterin value. This correlation was not found in the second patient. The authors postulate that BH₄ absorption from the intestine might decrease with age. A study on BH₄ pharmacokinetics in rats (Fiege and Blau 2006) found higher absorption of BH₄ in younger animals than in older ones and attributed this to physiologically incomplete gut closure in newborn animals.

Our patients aged 19–36 years showed a high inter-individual variability of B+P levels in dried blood spots. This variability did not show any association with age or phenotype group. Although the levels of B+P were highly variable between patients, the time course of BH₄ kinetics was almost identical in all patients with a maximum B+P level 4 hours after BH₄ loading confirming the findings of Fiege and colleagues in healthy individuals (Fiege et al 2004) and Zurflüh and colleagues in HPA patients (Zurflüh et al 2006).

Levels of [B+P]_{max} increased significantly for all patients following BH₄ loading with 10, 20 and 30 mg BH₄/kg bw. Maximum B+P levels did not show a significant association with decrease in Phe level in any of the phenotypic groups, as documented by Zurflüh and colleagues in 71 patients (Zurflüh et al 2006).

This lack of correlation between B+P levels and Phe level decrease is not surprising in the group of patients with classical PKU, because patients without residual enzyme activity of PAH are not likely to gain from higher BH₄ doses and therefore higher B+P levels. In the mild PKU group the lack of correlation

between B+P levels and phenylalanine decrease might be due to the fact that some patients already show a BH₄ response to 10 mg BH₄/kg bw that does not increase further on higher BH₄ levels. Numerous studies indicate that 10 mg BH₄/kg bw should be a sufficient dose in patients with mild PKU (Bélanger-Quintana et al 2005; Levy et al 2007; Matalon et al 2005; Trefz et al 2005). In moderate PKU with intermediate residual enzyme activity, adequate dosage of BH₄ might need to be determined individually and larger series of patients would have to be studied to find a possible correlation between B+P levels and phenylalanine decrease.

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