

Plasma biopterin levels and tetrahydrobiopterin responsiveness

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Received 29 April 2005; received in revised form 16 June 2005; accepted 24 June 2005

Available online 23 September 2005

Abstract

Tetrahydrobiopterin (BH₄) responsive hyperphenylalaninemia (HPA) with a mutant phenylalanine hydroxylase (PAH) gene was found during neonatal screening for PKU. This study determined blood BH₄ and phenylalanine in two patients with hyperphenylalaninemia following oral load with BH₄ 10 mg/kg. Our patients underwent neonatal screening for PKU, had normal biopterin metabolism and their PAH mutations were determined. Peak plasma biopterin levels in Case 1, which were reached at between 2 and 4 h after loading, were 612, 297, and 178 nmol/L at age 30 days, 55 days, and 19 months, respectively, and the maximum phenylalanine decreasing rates, which were found at 24 h, were 54, 16, and 4%, respectively. In Case 2, peak plasma biopterin levels were 747 and 327 nmol/L at age 20 and 55 days, respectively, and the maximum phenylalanine decreasing rates were 39 and 32%, respectively. In the BH₄ loading test, the peaks of BH₄ in both patients lowered (~50%), on the same dose schedule of BH₄, as patients got older.

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Keywords: Tetrahydrobiopterin; BH₄; Mild PKU; Mild hyperphenylalaninemia; Biopterin; Phenylalanine hydroxylase; Gene mutation; BH₄ loading test

Introduction

Since Kure et al. [1] reported on patients with phenylalanine hydroxylase (PAH) deficiency, who showed a decrease in blood phenylalanine after tetrahydrobiopterin (BH₄) loading, there has been an explosion of interest in patients with mild phenylketonuria (MPKU) who are responsive to BH₄. Over the past 5 years the consistent prediction of BH₄ responsiveness has been studied based on the genotype and may, as yet, remain incomplete. Moreover, Lindner et al. [2] found that three patients with the same genotype (Y414C/R408W) showed differing responses in a single-dose BH₄ loading test (20 mg/kg). We found that a patient had differing responses in a single-dose BH₄ loading test (10 mg/kg). To identify the precise

biochemical mechanisms involved in BH₄ responsiveness, we examined the plasma biopterin levels during a BH₄ loading test, and found a correlation between plasma biopterin and phenylalanine levels.

Patients and methods

Case 1 and Case 2 were detected by neonatal PKU screening, and initial phenylalanine levels were 600 and 480–600 μmol/L, respectively. Both infants had normal biopterin metabolism and their PAH mutations were P407S/R158W and R241C/R111X, respectively [3]. The BH₄ loading test was performed at 30 days, 55 days, and 19 months in Case 1, and at 20 days and 55 days in Case 2. An oral BH₄ (Suntory, Tokyo, Japan) loading test was performed upon instituting a normal diet, which was maintained during loading tests. In the BH₄ loading test, BH₄ (10 mg/kg) was administered before breakfast;

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blood samples were collected at 0, 4, 8, and 24 h after loading [3].

Serum phenylalanine concentrations were determined using an automated amino acid analyzer (L-8800; Hitachi, Tokyo, Japan). Serum pteridine was measured by high performance liquid chromatography (LC-10; Shimadzu, Kyoto, Japan) after iodine oxidation. Dihydropteridine reductase (DHPR) activity was measured in Guthrie card specimens as described previously [4].

Results and discussion

In the BH₄ loading test, plasma biopterin levels are shown in Table 1. Peak plasma biopterin levels were

reached at between 2 and 4 h after loading. Plasma phenylalanine and tyrosine levels are shown in Table 2. Decreases in plasma phenylalanine were maximum at 24 h except at 19 months in Case 1, which was 4 h after loading, while plasma tyrosine levels did not increase very much during the BH₄ loading test. Plasma biopterin levels after BH₄ loading decreased with age in both Case 1 (Fig. 1) and Case 2 (Fig. 2). Peak plasma biopterin levels in Case 1 were 612, 297, and 178 nmol/L at age 30 days, 55 days, and 19 months, respectively, and decreased by half with age while the maximum phenylalanine decreasing rates were 54, 16, and 4%, respectively (Table 2). Therefore, the maximum phenylalanine decreasing rate was in proportion to the square of each peak biopterin value. These results indicated that BH₄ responsiveness in

Table 1
Plasma biopterin (nM) levels after BH₄ loading tests by age

Time after BH ₄ loading	Case 1			Case 2	
	30 days	55 days	19 months	20 days	55 days
0	75	111	8	197	133
2	249	297	33	598	272
4	612	275	178	747	327
8	259	117	60	389	180
24	61	73	21	150	88

Table 2
Plasma phenylalanine and tyrosine levels (μmol/L) after BH₄ loading tests by age

Time after BH ₄ loading	Case 1						Case 2			
	30 days		55 days		19 months		20 days		55 days	
	Phe	Tyr	Phe	Tyr	Phe	Tyr	Phe	Tyr	Phe	Tyr
0	638	100	593	72	263	50	1180	91	848	81
2	632	119	608	119	292	45	978	76	809	73
4	505	62	499	71	254	42	932	91	672	73
8	452	107	536	97	317	44	935	122	599	76
24	297	111	525	65	284	32	719	97	581	82
Phe decline	54%		16%		4%		39%		32%	

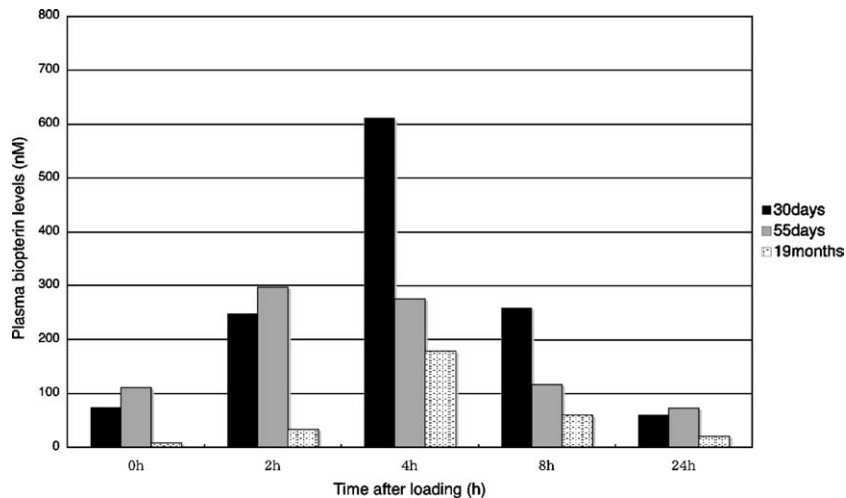


Fig. 1. Plasma biopterin levels after BH₄ loading test at different ages in Case 1.

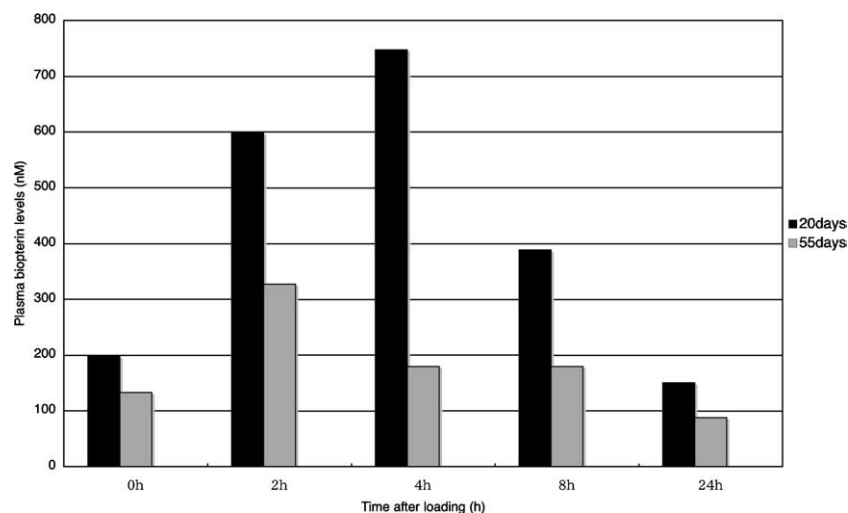


Fig. 2. Plasma bipterin levels after BH₄ loading test at different ages in Case 2.

the same individual depends on the total BH₄ amount absorbed from the intestine, and BH₄ absorption probably decreases with age. In Case 2, peak plasma bipterin levels were 747 and 327 nmol/L at age 20 days and 55 days, respectively, and also decreased by half with age. However, the maximum phenylalanine decreasing rates were 39 and 32%, respectively (Table 2). In the BH₄ loading test, the peaks of BH₄ in both patients lowered, (~50%), on the same dose schedule of BH₄, as patients got older. It seems that BH₄ absorption from the intestine might decrease with age. However, the maximum decreasing rate of plasma phenylalanine in Case 2 did not correlate with plasma bipterin levels as it did in Case 1. These results suggest that BH₄ responsiveness in the same individual or the same genotype correlates with bipterin levels, but among different genotypes it might not correlate very well and further examination is necessary.

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