

Tetrahydrobiopterin-responsive phenylketonuria: The New South Wales experience

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Abstract

Recent studies have shown that a subgroup of phenylketonuric patients respond to high doses of BH₄ (20 mg/kg) by a decrease of plasma phenylalanine. A clinically significant response has been defined as a decrease in phenylalanine by more than 30% within 24 h, after a BH₄ challenge. We report our experience with 37 patients diagnosed with hyperphenylalaninemia, mild, moderate, or classical Phenylketonuria (PKU) using a seven day combined BH₄ and phenylalanine load. Nine of the 37 patients responded with a 30% decrease in their phenylalanine levels in the first 8 h of treatment. A total of 17 patients (46%) had a decrease of at least 30% during the study period. This study confirms that a significant number of patients with mild to moderate PKU will respond to a BH₄ load. Furthermore, it confirms that the seven-day phenylalanine test is more sensitive in detecting BH₄ responsive patients.

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Introduction

Early treatment with a diet restricted in phenylalanine has been the core treatment for phenylketonuria (PKU) for almost 40 years and has succeeded in preventing intellectual disability in affected patients. While the diet is effective, it is difficult for children and adolescents to follow because of its restrictive nature, poor palatability, and social pressures. An exciting alternative to phenylalanine restriction was proposed by Kure et al. [1] when they reported that tetrahydrobiopterin (BH₄) given to four patients with phenylalanine hydroxylase deficiency reduced the blood phenylalanine concentration over an

8 h period. Other reports of BH₄ responsive phenylalanine hydroxylase deficiency patients have since been reported and up to 70% of hyperphenylalaninemic patients and mild PKU patients are responsive [2–5]. The frequency of responsiveness in classical PKU patients is much lower [6,7].

The optimal way to detect patients with BH₄ responsiveness remains to be determined and several protocols have been described for detecting BH₄ responsive patients. The loading dose of BH₄ has ranged from 10 to 20 mg/kg and the duration of the trial has varied from 8 h to 1 week. [2–4,8]. A one-week trial of BH₄ has proven the most sensitive test to determine BH₄ responsiveness as it may detect slow responders who would otherwise be missed [8]. Additionally, some protocols use a combination of BH₄ and either phenylalanine load or

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relaxation of the diet to enhance the BH₄ effect in mildly affected individuals [2,9,10].

We describe a one week combined trial of BH₄ and phenylalanine to evaluate BH₄ responsiveness in our patient population. Our aim was to determine which of our patients on a phenylalanine restricted diet were responsive to BH₄.

Methods

Patients

Patients were ascertained through the New South Wales Newborn Screening database that includes all individuals screened in New South Wales for hyperphenylalaninemia. Urinary pterin levels and DHPR activity had previously been measured in all patients to exclude the possibility of BH₄ deficiency. Patients were invited to take part if they were between 2 and 18 years, were currently on a phenylalanine restricted diet, and had an elevated phenylalanine level (>300 μmol/L) at the first clinic visit after a positive newborn screening result. The patients' families were approached by one of the investigators in clinic or by telephone to discuss the research protocol. The patients were classified based on pretreatment phenylalanine levels into mild hyperphenylalaninemia (phenylalanine 300–599 μmol/L), mild PKU (phenylalanine 600–900 μmol/L), moderate PKU (phenylalanine 900–1199 μmol/L), and classical PKU (phenylalanine >1200 μmol/L).

Combined phenylalanine and BH₄ load

Subjects were given oral phenylalanine at a dose of 25 mg/kg/day divided into three daily doses for a lead-in period of two days to bring the phenylalanine levels above the target therapeutic range while continuing their regular phenylalanine-restricted diet. The patients started oral 6R-BH₄ (Schirck's Laboratory) on the third day of the study at a dose of 20 mg/kg/day divided into two doses. In an attempt to increase phenylalanine levels without compromising the diet, the oral phenylalanine supplement was continued throughout the study period.

Sample collection and amino acid analysis

All blood was collected by finger prick onto filter paper at the patient's home. On the first day of BH₄ administration, blood was collected at initiation of treatment and then 8 h after the first BH₄ dose. Samples were then obtained daily for the duration of the study. Parents were asked to collect samples at the same time each day to avoid daily variation in phenylalanine levels. At the end of the study period, all samples were sent to our newborn screening laboratory for analysis.

Phenylalanine measurement

Phenylalanine levels were determined by electrospray ionization-tandem mass spectrometry. Each subject had day 1 pre-BH₄ phenylalanine levels compared with 8 h, 36 h, and 7th day levels. A decrease of phenylalanine of 30% was deemed to be clinically significant [5].

Results

Sixty-three patients were approached and 37 (59%) agreed to participate. There were 6 mild hyperphenylalaninemia patients (MHP), 9 mild, 7 moderate, and 15 classical PKU patients in this cohort.

Milder phenotypes were more likely to respond to the BH₄ challenge. Five out of six HPA patients, 8/9 mild, 3/7 moderate, and 1/15 classical PKU patients showed a positive response. The average phenylalanine level at time 0 in the positive responders was 498 (range 180–890) and in the non-responders the value was 632 (range 200–1200). In the BH₄ responders, the mean decrease at 8 h, 32 h, and 7 days was 31, 57, and 59%, respectively. The values for the same time periods in the non-responders were an increase of 8, 11, and 35%, respectively.

Of the 37 patients studied, nine showed a positive response to BH₄ in the first 8 h (Table 1). At the end of seven days, there were 17 positive responders with the majority showing a positive response within the first 36 h. Of the 17 patients who had a decrease in phenylalanine by 30%, 12 remained in the therapeutic treatment range (<360 μmol/L) for the duration of the trial period. Patients 3, 4, and 13 were not above the target level of 360 μmol/L at time 0 but they both showed significant decreases of 79, 40, and 39%, respectively. Patients 3, 6, 9, 10, 12, 13, 15, and 16 did not show a significant decrease of phenylalanine levels in the first 8 h and can be classified as slow responders. Patient 10 showed a positive response at 36 h and maintained this response until the fifth day (data not shown) but returned to a value greater than baseline in the last two days of the study.

A number of patients classified as non-responders approached a 30% decrease at one point in the study. Patient 19 became ill on the fourth day with gastroenteritis and missed two doses of BH₄. Two non-responders (19 and 20) did not increase above 360 μmol/L despite receiving phenylalanine.

Discussion

Since a BH₄ response was first demonstrated in patients with phenylalanine hydroxylase deficiency, a number of studies have attempted to document this effect. Results have varied with some investigators showing infrequent response to BH₄ [11], while other studies

Table 1
Phenylalanine response following 7 days with BH₄

	Initial phenylalanine at diagnosis (μmol/L)	Phenotype	Phenylalanine level in μmol/L (percent change)			
			0 h	8 h	36 h	7 days
<i>Responders</i>						
1	400	HPA	470	330 (–30)	90 (–81)	110 (–77)
2	500	HPA	410	220 (–46)	100 (–76)	200 (–51)
3	410	HPA	290	270 (–7)	180 (–38)	60 (–79)
4	290	HPA	200	120 (–40)	160 (–20)	120 (–40)
5	290	HPA	520	220 (–58)	140 (–73)	220 (–58)
6	700	Mild	890	770 (–13)	210 (–76)	180 (–80)
7	750	Mild	550	340 (–38)	150 (–73)	100 (–82)
8	700	Mild	570	340 (–40)	300 (–47)	240 (–58)
9	830	Mild	400	400 (0)	140 (–65)	220 (–45)
10	840	Mild	470	460 (–2)	260 (–45)	410 (–13)
11	750	Mild	630	320 (–49)	200 (–68)	320 (–49)
12	660	Mild	710	730 (3)	430 (–39)	580 (–18)
13	750	Mild	180	150 (–17)	140 (–22)	110 (–39)
14	1080	Moderate	700	190 (–73)	190 (–73)	70 (–90)
15	1100	Moderate	450	390 (–13)	250 (–44)	120 (–73)
16	1010	Moderate	520	440 (–15)	280 (–46)	190 (–63)
17	1200	Classical	510	50 (–90)	90 (–82)	100 (–80)
Average			498	338 (–31)	195 (–57)	197 (–59)
SD			180	192 (27)	88 (20)	135 (23)
<i>Non-responders</i>						
18	570	HPA	360	260 (–28)	460 (28)	410 (14)
19	860	Mild	250	260 (4)	180 (–28)	380 (52)
20	900	Moderate	200	360 (80)	180 (–10)	230 (15)
21	1130	Moderate	740	750 (1)	1050 (42)	1000 (35)
22	1000	Moderate	230	290 (26)	300 (30)	460 (100)
23	1670	Moderate	510	400 (–22)	640 (25)	620 (22)
24	1250	Classical	680	970 (43)	550 (–19)	710 (4)
25	1530	Classical	600	620 (3)	610 (2)	480 (–20)
26	1970	Classical	700	630 (–10)	700 (0)	620 (–11)
27	1560	Classical	740	940 (27)	790 (7)	610 (–18)
28	1300	Classical	800	630 (–21)	1000 (25)	650 (–19)
29	1300	Classical	690	670 (–3)	680 (–1)	780 (13)
30	1700	Classical	700	840 (20)	710 (1)	730 (4)
31	1400	Classical	580	470 (–19)	450 (–22)	570 (–2)
32	>2000	Classical	1200	1100 (–8)	1050 (–13)	1390 (16)
33	1340	Classical	1030	1070 (4)	1120 (9)	1320 (28)
34	1500	Classical	530	530 (0)	680 (28)	860 (62)
35	1300	Classical	810	850 (5)	690 (–15)	1940 (140)
36	>2000	Classical	970	1000 (3)	1620 (67)	1870 (93)
37	2300	Classical	310	460 (48)	510 (65)	870 (181)
Average			632	655 (8)	699 (11)	825 (35)
SD			271	275 (27)	345 (27)	468 (55)

have found a majority of patients with mild PKU and virtually all MHP patients respond [6,7,12,13]. The variation may be due in part to different doses of BH₄, different formulations of BH₄, and the inclusion of classical patients that have a lower response rate [7,12]. Our results support these studies in that 16 out of 22 patients (73%) who did not have classical PKU (as defined by a phenylalanine level of <1200 μmol/L at first clinic visit) responded to BH₄ with a decrease of phenylalanine of at least 30%. Furthermore, only one of the 15 classical PKU patients responded to BH₄ loading, which is also in agreement with the literature. Although we did not have

mutation analysis available on our subjects available for genotype/phenotype correlation, our study shows a high rate of responders could be determined based on phenotype alone.

A combination of phenylalanine and BH₄ loads may accentuate BH₄ responsiveness. It is recommended that a dose of phenylalanine of 100 mg/kg is used in the standard loading test for diagnosis of BH₄ deficiencies in patients with phenylalanine levels less than 400 μmol/L [7]. However, some of the decrease in phenylalanine attributed to the BH₄ load may be due to spontaneous elimination of phenylalanine [12]. We chose to challenge

the patients continually with phenylalanine throughout the trial period. We were wary of relaxing the diet. We were unable to continue BH₄ treatment after the close of the trial and we felt this was unfair to our patients as dietary relaxation may have been difficult to rectify after the study concluded, so we gave a modest supplement of phenylalanine in a divided daily dose. We thought this was an accurate representation of what would happen if we relaxed the diet in responsive individuals. Despite the load of phenylalanine, we had four patients who remained below 360 μmol/L throughout the study (patients 3, 4, 13, and 20). Patient 13 did not show a significant decrease until day 5. The responsiveness in this patient has been confirmed with a 100 mg/kg/phenylalanine load followed by 20 mg/kg BH₄. This test revealed a phenylalanine drop from 950 to 120 μmol/L at 24 h. Therefore, the seven-day test seems capable of detecting BH₄ responsiveness (albeit at a slower rate) even at phenylalanine levels that are lower than the minimal level of 400 μmol/L that is proposed in the literature [7]. The other patients (3, 4, and 20) have not yet been tested to confirm this finding.

With respect to the best testing procedure for detecting BH₄ responsiveness, 8/16 patients who ultimately responded did not respond in the first 8 h of the study. Slow responders have been described in 20–35% of cases in the literature [6,8]. The one-week BH₄ load test has been found to be the most sensitive for determining responsive patients and some of these patients would be missed by the 24 h test alone. A number of reasons may explain why we had a larger number of slow responders. The optimal dose of BH₄ for the slow responders is 20 mg/kg [7]. The estimated half life of BH₄ is 8 h; therefore, the medication should be given twice a day to maintain a constant blood BH₄ concentration [7]. Our protocol divided the 20 mg/kg dose of BH₄ into two doses. Some of the children in our study may not have responded in the first 8 h as they had only received a 10 mg/kg dose. Another possible reason for the higher number of slow responders may stem from the relatively low values of some of the patients at initiation of BH₄ and the fact that it may take longer to demonstrate responsiveness at the lower range of phenylalanine levels. This was indeed documented in Patient 13 as mentioned in the previous paragraph. Of our slow responders, patients 6 and 12 clearly did not fit this pattern and it is possible that BH₄ is exerting its effects through molecular mechanisms which may not be immediate. It has been hypothesized that slow responders may have a time-dependent increase of phenylalanine hydroxylase transcription [6,10]. However, *in vitro* studies of PAH and BH₄ have shown that increased activity is not due to activation of transcription or mRNA stabilization [15]. Therefore, if there is a molecular mechanism that can explain why some patients are slow responders, it remains to be elucidated.

The protocol for this study was done on an outpatient basis. This is advantageous for the patients as they can continue their normal routine and they could arrange to do the study when it was convenient for them. The seven-day trial allowed identification of several patients who would not be detected by the 24 h load (patients 3, 6, 9, 10, 12, 13, 15, and 16). Most of these patients were detected by 36 h. These results confirm that the seven-day protocol is more sensitive in detecting BH₄ responsive patients. However, as most of our patients showed a positive response in the first 36 h, perhaps the optimal protocol would be to test patients for 48 h. At this time, clear responders could terminate the trial and the protocol could be prolonged for a full seven day period in only those showing a partial response. One disadvantage to our protocol was that we did not have results of initial phenylalanine levels until after all samples were sent in. A modification of the protocol to include a phenylalanine result prior to starting the BH₄ would ensure that adequate phenylalanine levels were obtained to document BH₄ effect.

The clinical implication for our patients is that BH₄ could reduce the stringency of phenylalanine restriction in our cohort. The effects have been reported to be long lasting [2,8,14]. Long-term follow-up with phenylalanine tolerance followed by BH₄ titration will need to be performed on an individual basis in our patients but this offers some hope in improving the quality of life amongst responders. Currently, two of our patients have been on long-term therapy with BH₄ for over one year. Patient 3 was taking 405 mg phenylalanine per day prior to BH₄ therapy (20 mg/kg/day) and has now increased her phenylalanine intake to 1125 mg/day. Patient 15 is now taking 10 mg/kg/day of BH₄ and has increased phenylalanine tolerance from 375 to 1125 mg/day. Both patients have maintained their blood phenylalanine levels in the target range. The results of this pilot study support the need for a longer term systematic trial of BH₄.

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References

- [1] S. Kure, D.C. Hou, T. Ohura, H. Iwamoto, S. Suzuki, N. Sugiyama, O. Sakamoto, K. Fujii, Y. Matsubara, K. Narisawa, Tetrahydrobiopterin-responsive phenylalanine hydroxylase deficiency, *J. Pediatr.* 135 (1999) 375–378.
- [2] A.C. Muntau, W. Roschinger, M. Habich, H. Demmelmair, B. Hoffmann, C.P. Sommerhoff, A.A. Roscher, Tetrahydrobiopterin as an alternative treatment for mild phenylketonuria, *N. Engl. J. Med.* 347 (2002) 2122–2132.

- [3] L.J. Spaapen, J.A. Bakker, C. Velter, W. Loots, M.E. Rubio-Gozalbo, P.P. Forget, L. Dorland, T.J. De Koning, B.T. Poll-The, H.K. Ploos van Amstel, J. Bekhof, N. Blau, M. Duran, M.E. Rubio-Gonzalbo, Tetrahydrobiopterin-responsive phenylalanine hydroxylase deficiency in Dutch neonates, *J. Inherit. Metab. Dis.* 24 (2001) 352–358.
- [4] U. Lassker, J. Zschocke, N. Blau, R. Santer, Tetrahydrobiopterin responsiveness in phenylketonuria. Two new cases and a review of molecular genetic findings, *J. Inherit. Metab. Dis.* 25 (2002) 65–70.
- [5] R. Steinfeld, A. Kohlschutter, J. Zschocke, M. Lindner, K. Ullrich, Z. Lukacs, Tetrahydrobiopterin monotherapy for phenylketonuria patients with common mild mutations, *Eur. J. Pediatr.* 161 (2002) 403–405.
- [6] L.R. Desviat, B. Perez, A. Belanger-Quintana, M. Castro, C. Aguado, A. Sanchez, M.J. Garcia, M. Martinez-Pardo, M. Ugarte, Tetrahydrobiopterin responsiveness: results of the BH₄ loading test in 31 Spanish PKU patients and correlation with their genotype, *Mol. Genet. Metab.* 83 (2004) 157–162.
- [7] C. Bernegger, N. Blau, High frequency of tetrahydrobiopterin-responsiveness among hyperphenylalaninemias: a study of 1919 patients observed from 1988 to 2002, *Mol. Genet. Metab.* 77 (2002) 304–313.
- [8] H. Shintaku, S. Kure, T. Ohura, Y. Okano, M. Ohwada, N. Sugiyama, N. Sakura, I. Yoshida, M. Yoshino, Y. Matsubara, K. Suzuki, K. Aoki, T. Kitagawa, Long-term treatment and diagnosis of tetrahydrobiopterin-responsive hyperphenylalaninemia with a mutant phenylalanine hydroxylase gene, *Pediatr. Res.* 55 (2004) 425–430.
- [9] T. Bardelli, M.A. Donati, S. Gasperini, F. Ciani, F. Belli, N. Blau, A. Morrone, E. Zammarchi, Two novel genetic lesions and a common BH₄-responsive mutation of the PAH gene in Italian patients with hyperphenylalaninemia, *Mol. Genet. Metab.* 77 (2002) 260–266.
- [10] N. Blau, H. Erlandsen, The metabolic and molecular bases of tetrahydrobiopterin-responsive phenylalanine hydroxylase deficiency, *Mol. Genet. Metab.* 82 (2004) 101–111.
- [11] J. Weglage, M. Grenzebach, A. Teeffelen-Heithoff, T. Marquardt, R. Feldmann, J. Denecke, D. Godde, H.G. Koch, Tetrahydrobiopterin responsiveness in a large series of phenylketonuria patients, *J. Inherit. Metab. Dis.* 25 (2002) 321–322.
- [12] L.J. Spaapen, M.E. Rubio-Gozalbo, Tetrahydrobiopterin-responsive phenylalanine hydroxylase deficiency, state of the art, *Mol. Genet. Metab.* 78 (2003) 93–99.
- [13] B. Perez-Duenas, M.A. Vilaseca, A. Mas, N. Lambruschini, R. Artuch, L. Gomez, J. Pineda, A. Gutierrez, M. Mila, J. Campistol, Tetrahydrobiopterin responsiveness in patients with phenylketonuria, *Clin. Biochem.* 37 (1983) 1083–1090.
- [14] R. Steinfeld, A. Kohlschutter, K. Ullrich, Z. Lukacs, Efficiency of long-term tetrahydrobiopterin monotherapy in phenylketonuria, *J. Inherit. Metab. Dis.* 27 (2004) 449–453.
- [15] B. Thony, Z. Ding, A. Martinez, Tetrahydrobiopterin protects phenylalanine hydroxylase activity in vivo: implications for tetrahydrobiopterin-responsive hyperphenylalaninemia, *FEBS Lett.* 577 (2004) 507–511.