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Cognitive functions in patients with phenylketonuria in long-term treatment with tetrahydrobiopterin ☆

R. Gassió [a,*], M.A. Vilaseca [b], N. Lambruschini [c], C. Boix [a], M.E. Fusté [d], J. Campistol [a,e]

[a] Neuropediatrics Department, Hospital Sant Joan de Déu, Universitat de Barcelona, Spain
[b] Biochemistry Department, Hospital Sant Joan de Déu, Universitat de Barcelona, Spain
[c] Nutrition Department, Hospital Sant Joan de Déu, Universitat de Barcelona, Spain
[d] Psychology Department, Hospital Sant Joan de Déu, Universitat de Barcelona, Spain
[e] CIBERER–ISC III, Pº Sant Joan de Déu 2, 08950 Esplugues, Barcelona, Spain

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ABSTRACT

Cognitive functions were assessed in 9 patients with mild to moderate phenylketonuria (PKU) ranging from 6 to 18 years of age, who were in long-term treatment (>5 years) with 5–9 mg/kg/day tetrahydrobiopterin (BH4) on compassionate use, provided by Schircks Inc. An extensive study of cognitive functions (intelligence quotient (IQ), visuospatial, visual memory, fine motor, executive and attentional functions) was conducted, and behavior was assessed using the ADHD Rating Scale and the Behavior Rating Inventory of Executive Function (BRIEF). All patients had normal IQ (M = 107, SD = 10). The most notable area of impairment was fine motor function, but no significant difference was found between the PKU patients in BH4 treatment who participated in the current study and PKU patients in dietary treatment who participated in a previous study. These results, however, should be interpreted with caution. It is necessary to conduct further studies with a larger number of patients, using more sensitive tests of motor function and using the formulation of BH4 that is currently available.

Introduction

Phenylketonuria (PKU; OMIM 261600 and 261630)[1] is a metabolic disease caused by mutations in the phenylalanine hydroxylase (PAH; EC 1.14.16.1) gene. Mutations partially or totally reduce the PAH enzyme activity that catalyzes the hydroxylation of phenylalanine (Phe) to tyrosine (Tyr), using tetrahydrobiopterin (BH4) as the cofactor. Biochemical consequences are the accumulation of Phe and a relative deficiency in Tyr in tissues and biological fluids [1].

PKU is the metabolic disease that most often causes mental retardation, although the introduction of a Phe-restricted diet in the first weeks of life has changed the evolution of the disease, allowing physical and general intellectual development within the normal range. Nonetheless, mild cognitive impairments have been described in patients with PKU who undergo early dietary treatment. Although their intellectual capacity is normal, it is less than that of control groups. In addition, patients with PKU have deficits in executive functions such as attention, strategic planning, inhibitory control, working memory, and cognitive flexibility [2–15].

Deficits in cognitive functions in treated patients with PKU are fundamentally associated with dysfunction of the prefrontal cortex of the brain. Diamond et al. [8] claimed that these deficits are caused by reduced dopamine synthesis due to reduced Tyr. Dyer [16], on the other hand, related the deficits to impairment in the maintenance and production of myelin, which decreases dopamine production and affects axonal conduction velocity.

Until recently, the predominant treatment for PKU was a Phe-restricted diet. However, in 1999, when Kure et al. [17] described four patients who responded to the administration of BH4 with a decrease in blood Phe levels, a door was opened to a new treatment for PKU. The aim of treatment in PKU patients, whether dietary treatment or treatment with BH4, is to maintain blood Phe levels within 120–360 μmol/L, although these values are still 3–5 times higher than normal. Blood Phe levels >360 μmol/L are associated with the mild cognitive deficits described earlier [8].

Various studies have reported the efficacy of long-term treatment with BH4 as an alternative to dietary treatment, and in some cases patients are fully or partially free from dietary restrictions [18–26]. To date, however, there have been no published studies that assess cognitive functions in PKU patients in long-term

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* Corresponding authors. Address: Neuropediatrics Department, Hospital Sant Joan de Déu, Passeig Sant Joan de Déu 2, 08950 Esplugues, Barcelona, Spain. Fax: +34 932803626.
E-mail addresses: rgassio@hsjdbcn.org (R. Gassió), campistol@hsjdbcn.org (J. Campistol).

[1] *Abbreviations used:* PKU, phenylketonuria; PAH, phenylalanine hydroxylase; Phe, phenylalanine; Tyr, tyrosine; BH4, tetrahydrobiopterin; IQ, intelligence quotient.

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treatment with BH4. Due to the cognitive deficits shown by PKU patients in dietary treatment, we conducted a preliminary study to assess cognitive functions in patients who have been treated with BH4 for at least 5 years.

Materials and methods

Patients

From 15 patients with PKU in BH4 treatment (5–9 mg/kg/day) at our hospital (a reference center for PKU in Catalonia), we selected 9 patients who met the following inclusion criteria: (1) $\geqslant$6 years of age, and (2) in treatment with BH4 and following an unrestricted diet with controlled protein intake for at least 5 years. The BH4 treatment was provided by Schircks Inc. on the basis of compassionate use. A defect in synthesis or recycling of BH4 was ruled out in all patients. The sample consisted of 7 girls and 2 boys from 6 to 18 years of age (M = 11, SD = 4). Consistent with the Guttler classification [27], 5 had the mild variant of PKU (tolerance: 400–600 mg Phe/day) and 4 had the moderate variant (tolerance: 350–400 mg Phe/day). Prior to beginning treatment with BH4, the mean (SD) Phe level of the BH4 treatment group was 304 (126) μmol/L [22]. Previous studies from our group [22,28] described the treatment protocol with BH4, assessment of Phe tolerance, metabolic controls, and anthropometric and nutritional controls. Tolerance to BH4 in our 9 patients was good throughout treatment. The ethics committee of the Hospital Sant Joan de Déu approved the study, and informed consent was obtained from patients and their parents.

Procedures

The intelligence quotient (IQ) was measured using the Wechsler Intelligence Scale for Children-IV (WISC-IV) [29] for patients 6–16 years of age and the Wechsler Adult Intelligence Scale-III (WAIS-III) [30] for patients $\geqslant$17 years of age. Visuospatial functions and visual memory were examined using the Rey-Osterrieth Complex Figure Test (RCFT) [31]. Fine motor functions were studied with the Assembly Subtest of the Purdue Pegboard Test [32], which measures finger and hand dexterity. Several tests were used to assess executive functions. Sustained attention was assessed with the Perception of Differences Test (FACES) [33] in patients 6–10 years of age and with the Toulouse Piéron Test [34] in patients >10 years of age. Alternating attention and cognitive flexibility was measured using the Trail Making Test [35] in patients >8 years of age. Inhibitory control was assessed with the Stroop Color and Word Test [36] in patients >7 years of age. Cognitive flexibility and planning were measured using phonetic word fluency (FAS) [37]. Working memory was assessed using WISC-IV and WAIS-III subtests. In terms of behavior, the ADHD Rating Scale-IV [38] and the Behavior Rating Inventory of Executive Function (BRIEF) [39] were administered to parents and teachers to assess ADHD symptoms and executive function in daily life.

Data analysis

We used ANOVA with Bonferroni's correction for multiple comparisons to compare the means between BH4 and dietary treatment groups. Statistical significance was accepted at $p < 0.05$. Statistical analyses were performed using the package SPSS (version 17.0).

Results

Table 1 shows biochemical and genetic findings. All patients had a normal physical and neurological examination, with the exception of one patient who presented with tremor before the start of BH4 treatment. No patient experienced side effects attributable to BH4.

With regard to cognitive functions, mean (SD) IQ was 107 (10), with a range from 84 to 118. Of the functions assessed, the results for fine motor function were most noteworthy, with deficits in 7 of 9 patients (78%). Only one patient additionally showed an attention deficit, and two others additionally showed a deficit in cognitive flexibility (impairment on the Trail Making Test and/or phonetic word fluency). On the behavioral surveys, impulsivity and attentional difficulties were reported in two patients. School performance was adequate in all patients, with the exception of one patient who had learning difficulties prior to starting BH4 treatment.

Mean results from the Assembly Subtest of the Purdue Pegboard Test were compared for PKU patients in BH4 treatment and 3 groups from a previous study [14]: (1) PKU patients in dietary treatment (n = 28), (2) patients with mild hyperphenylalaninemia without dietary treatment (n = 28), and (3) a healthy control group (n = 23). There were no statistically significant differences between PKU patients treated with BH4 and those in dietary treatment. In contrast, there were statistically significant differences between these 2 groups and both the mild hyperphenylalaninemia and control groups (see Table 2).

Discussions

There are very few references to cognitive functions in studies conducted with PKU patients in BH4 treatment. Studies have only described psychomotor function and intellectual capacity within

Table 1
Biochemical and genetic data of PKU patients in BH4 treatment.

Patients	Sex	Age (years)	Genotype		Age at start of BH4 treatment (years)	Duration of BH4 treatment (years)	BH4 dose (mg/kg/day)	% BH_4 response	IDC (μmol/L) Phe Mean ± SD	
			Allele 1	Allele 2					With dietary treatment	With BH4 therapy
1	F	6.6	IVS10-11G>A	D415N	0.9	5.7	5.0	94.0	193 ± 93	272 ± 82
2	F	6.5	R241Q	IVS2+19T>C	0.8	5.7	9.0	72.3	291 ± 68	341 ± 100
3	F	11.3	IVS10-11G>A	E178G	5.5	5.8	5.0	70.4	255 ± 93	265 ± 51
4	F	13.3	R408W	P275S	8.0	5.3	6.4	49.6	254 ± 108	380 ± 65
5	F	18.2	S349P	E390G	12.2	6.0	5.0	66.1	412 ± 84	343 ± 100
6	F	7.6	R408W	E390G	2.0	5.6	6.0	83.9	434 ± 133	284 ± 65
7	M	16.1	V388M	R241H	10.5	5.6	5.5	72.0	295 ± 47	321 ± 79
8	M	11.9	Y168H	V388M	6 .2	5.8	5.0	83.8	252 ± 76	205 ± 59
9	F	7.5	Y414C	IVS2+19T>C	1.5	6.0	8.0	78.0	290 ± 94	329 ± 100

F, female; M, male; BH4, tetrahydrobiopterin; IDC, the index of dietary control was calculated as the mean of the median Phe values for 6-month periods before and during the BH4 treatment.

Table 2
Comparison of means on the Assembly Test of the Purdue Pegboard Test.

	N	Mean (SD) age (years)	Mean (SD) Phe (μmol/L)	Mean (SD) Purdue[a]
1. PKU with BH4	9	11 (4)	304 (53)[b]	38 (6)
2. PKU with diet	28	11.8 (4)	478 (185)[c]	41 (9)
3. mHPA	28	8.7 (3)	229 (75)[c]	49 (9)
4. CG	23	11 (4)	–	48 (8)

Statistical significance (ANOVA Bonferroni correction) of the Assembly Subtest of the Purdue Pegboard Test: NS (1 versus 2), $p = 0.006$ (1 versus 3), $p = 0.023$ (1 versus 4).

PKU, phenylketonuric patients; BH4, tetrahydrobiopterin; mHPA, mild hyperphenylalaninemia; CG, control group; *N*, number of cases; NS, non-significant.

[a] Results expressed as *T*-scores ($M = 50$, SD = 10).

[b] Index of dietary control (IDC) throughout the treatment period.

[c] IDC of the last 6 months.

the normal range [20,21,24,26]. In a previous study [22] we assessed 4 patients after 1 year of BH4 treatment. We found that their intellectual capacity was normal and we did not identify impairments in attention or executive functions. In the current study, we increased the sample size ($n = 9$) and the length of treatment (>5 years), which has enabled us to better assess cognitive functions. As expected, IQ was within normal limits in all patients.

Interestingly, the most affected area was fine motor function, as assessed by the Assembly Subtest of the Purdue Pegboard Test. Motor function impairments have been reported in PKU patients treated early with diet, although less often than deficits in executive functions. In an earlier study performed by our group [14], we found poorer performance on the Assembly Subtest of the Purdue Pegboard Test in a sample of 28 PKU patients treated early with diet compared with either a group with mild hyperphenylalaninemia without dietary treatment or a control group. Motor function was also related to most recent Phe level, with poorer motor performance at higher Phe levels.

Other researchers have reported similar findings in PKU patients treated early with diet. For example, in a study of 20 such patients (mean age of 10 years), Weglage et al. [40] described significant deficits in fine motor function measured by the Motorische Leistungsserie. Arnold et al. [41] also described poorer motor performance in a group of 18 children with an average age of 47 months on the Peabody Developmental Motor Scales. As in our study, poorer performance was correlated with concurrent Phe levels. Huijbregts et al. [42] also found poorer performance in 61 PKU patients ranging from 7 to 14 years of age when assessing motor functions using complex demands (pursuit task of the Amsterdam Neuropsychological Test). According to the authors, this was caused by slowed processing speed, which in turn could be explained either by prefrontal cortex dysfunction or a myelination deficit. Arnold et al. [41] asserted that poorer motor performance in children with high Phe levels may also be associated with a dopamine deficit that affects the motor cortex, as motor tracts in the brain are dopamine dependent.

Due to the small size of our sample, our results should be interpreted with caution. Further studies are needed with larger samples of patients who are assessed using more sensitive tests of motor function. In addition, findings of no statistically significant differences between the PKU groups in dietary versus BH4 treatment suggests that the BH4 group fared at least as well as the dietary treatment group. It should be noted, however, that the BH4 formulation we used was different from that which is currently used for clinical purposes. The dosage we used (5–9 mg/kg/day) was also less than half of that which is currently recommended (20 mg/kg/day). Thus, it is possible that treatment with the formulation and dosage now available may provide benefits that are greater than those of dietary treatment. Further research is clearly needed to reach definitive conclusions.

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