Pteridines
Vol. 3, pp. 13–15

Short Communication

Monitoring Treatment in Tetrahydrobiopterin Deficiency

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(Received March 1992)

Introduction

Tetrahydrobiopterin (BH4) deficiency leads to an impaired function of phenylalanine (Phe), tyrosine (Tyr) and tryptophan hydroxylases. Consequent hyperphenylalaninemia and dopamine and serotonin deficiency are the main metabolic disturbances on which depend the patient's symptoms and poor prognosis, irrespective of the enzyme defect causing impaired BH4 synthesis or regeneration. Three inborn errors of pterin metabolism are so far known: guanosine triphosphate cyclohydrolase (GTP CH I, EC 3.5.4.16) and 6-pyruvoyl tetrahydropterin synthase (6-PPH4S, Mc Kusick 26164) deficiency inhibit BH4 biosynthesis, and dihydropteridine reductase (DHPR, EC 1.66.99.7) deficiency prevents BH4 from being recycled after oxidation in the hydroxylating reaction (1).

The goals of the treatment are to control the hyperphenylalaninemia and to restore the homeostasis of neurotransmitters. In synthesis defects, hyperphenylalaninemia can be easily corrected by BH4 administration, while in DHPR deficiency Phe dietary restriction is generally applied. With the exception of some milder cases, where BH4 monotherapy or just dietary treatment is affordable, most patients need hydroxylated neurotransmitter precursor administration in addition to an inhibitor of peripheral aromatic amino acid decarboxylases to avoid clinical symptoms and to increase brain neurotransmitter levels (2).

The comparison of the biochemical picture in BH4 deficient patients at diagnosis with that observed at the time of optimal clinical response to therapy can be helpful in cases whose clinical monitoring is not sufficient to evaluate the adequacy of treatment.

Case Report and Methods

Three patients suffering from 6-PPH4S deficiency and three patients suffering from DHPR deficiency have been examined in two opposite conditions: at diagnosis, when hyperphenylalaninemia and biogenic amine deficiency symptoms were present, and during the follow up, when the hyperphenylalaninemia was controlled and symptoms disappeared (Table 1). As for the treatment, the 6-PPH4S deficient patients were on free diet but received a single daily dose of 5 mg/kg synthetic BH4, while the DHPR deficient patients were given a Phe-restricted diet adjusted to the individual tolerance. Both types of patients needed neurotransmitter substitutive therapy in three daily divided doses (L-dopa: 5–6 mg/kg; 5-OH-tryptophan: 4–5 mg/kg; Carbidopa: 1 mg/kg).

Serum, urine, and cerebrospinal fluid (CSF) samples were taken in the morning after midnight semifasting at diagnosis, and, when patients were on therapy, half way between two successive drug administrations. Serum Phe and Tyr were measured chromatographically with a Kromakon 500 automatic analyzer. Serum, urine and CSF neopterin (N) and biopterin (B) were measured by HPLC after oxidation with manganese dioxide and subsequent deproteinization with trichloroacetic acid; 5-hydroxyindolacetic acid

Enzymes: dihydropteridine reductase (EC 1.66.99.7)
guanosine triphosphate cyclohydrolase (EC 3.5.4.16)
6-pyruvoyl tetrahydropterin synthase (Mc Kusick 26164)

Table 1. Biochemical findings in 6 tetrahydrobiopterin (BH_4) deficient patients, 3 suffering from 6-pyruvoyl tetrahydropterin synthase (6-PPH_4S) and 3 from dihydropteridine reductase (DHPR) deficiency. Each patient was evaluated at diagnosis, prior to treatment, and when a good clinical response to the therapy had been obtained.

Patients	Age	Treatment			Serum				Urine		CSF			
		BH4	diet	NT	Phe (μmol/L)	Tyr	N (nmol/L)	B	N (mmol/mol C)	B	N (nmol/l)	B	HVA	5-HIAA
1. 6-PPH_4S	7 d	–	–	–	1589	34	43	0	28	0	449	0	34	13
	4.5 y	+	–	+	70	46	1.3	12.6	1.4	2.4	115	2.1	288	162
2. 6-PPH_4S	5 m	–	–	–	743	52	61	0	27	0	206	6	70	21
	4 y	+	–	+	32	68	4.5	19	2.3	1.7	124	5.5	283	202
3. 6-PPH_4S	11 m	–	–	–	1282	45	229	0	13.4	0	215	9.7	91	15
	8 y	+	–	+	161	66	4.6	9.2	1.7	1.3	77	13.7	349	120
I. DHPR	14 m	–	–	–	1458	66	17	98	2.8	13.8	17	61	71	14
	6.5 y	–	+	+	57	72	17	28	1.1	3.1	18	41	123	65
II. DHPR	14 m	–	–	–	553	41	24	100	2	6.5	11	57	81	23
	2 y	–	+	+	55	51	18	32	1	2.8	20	42	233	164
III. DHPR	1 m	–	–	–	1993	42	17	35	12.6	9.3	47	117	59	4
	3 y	–	+	+	61	69	35	28	0.8	1.2	16	37	168	125
				Controls	31	30	12	5	1.1	0.5	9	10	250	110
					91	147	24	13	4	3	20	34	880	360

NT = neurotransmitter precursors; N = neopterin; B = biopterin; C = creatinine; HVA = homovanillic acid; 5-HIAA = 5-hydroxyindolacetic acid

(5-HIAA) and homovanillic acid (HVA) were measured by HPLC with an ESA Coulochem 5100 A electrochemical detector (3).

Results and Discussion

Results are summarized in Table 1. As previously reported (4), high serum Phe levels are a limiting factor in the response to neurotransmitter precursor therapy, by interfering with their membrane transport or metabolism, and so altering the dose-effect relationships. Normal serum Phe levels are easily attained in synthesis defects by small doses of synthetic cofactor, while DHPR deficient patients need a strict dietary control. This strategy allows to reduce the dose of neurotransmitter precursors, as well as to avoid most of the clinical fluctuations in symptomatology. In practice, the patient's individual optimal dosage is judged on a clinical basis, with only the monitoring of serum Phe concentration. Unfortunately, dopamine and serotonin can produce agonist and antagonist effects, and side effects, which can also mimic the symptoms of deficiency (4). As a consequence, the direct analysis of neurotransmitter metabolites in CSF might be necessary in some cases to evaluate the adequacy of treatment.

Since reference parameters of pterin and neurotransmitter metabolism in treated BH4 deficient patients are very scarce in literature, the present investigation offers new insights into the biochemical expression of a favourable outcome. The administration of BH4 and the restriction of Phe dietary intake, respectively applied in 6-PPH4S and in DHPR deficiency, are both able to correct significantly the pattern of pterin metabolism at the peripheral level, with only minor modifications of B and N concentrations in CSF (Table 1). Particularly, the urinary pattern of pterin excretion appears to be almost normalized, and this is the reason why the analysis of urinary pterins can miss the diagnosis of DHPR deficiency when serum Phe is low (5).

The concentration of CSF neurotransmitter metabolites, extremely low prior to hydroxylated precursors administration, increased only up to the lowest normal values on therapy. These findings show that in BH4 deficient patients a good clinical result is achieved at CSF biogenic amine levels below those of age-matched controls. In addition to the low dosage of neurotransmitter precursors required, as compared to other conditions such as Parkinson's disease (2), these data suggest that synaptic receptors are very sensitive in these patients, and that the attempt to reach therapeutically higher values of CSF neurotransmitter metabolites may result in overdose symptoms.

Acknowledgements

This work was supported in part by the Swiss National Science Foundation (to N. B.), project No. 31-28797.90.

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