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Hyperphenylalaninemia Due to Impaired Dihydrobiopterin Biosynthesis

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Abstract. A fourteen month-old boy with atypical phenylketonuria was treated with 5-hydroxytryptophan, L-dopa and peripheral aromatic amino acid decarboxylase inhibitor (Ro 4-4602:benserazide). Despite the good control of plasma phenylalanine on a low phenylalanine diet, he had shown no improvement in his development but progressive neurological symptoms, such as irritability, convulsions and decrease voluntary movement. After beginning neurotransmitter therapy, his irritability disappeared promptly and the other symptoms diminished. He gradually reached his developmental milestones. At two and a half years of age, he had recovered sufficiently to be able to walk freely on treatment with 13 mg/kg/day of 5-hydroxytryptophan, 11 mg/kg/day of L-dopa and 2.7 mg/kg/day of benserazide in combination with slight restriction of phenylalanine intake (100 mg/kg/day).

Levels of serotonin and 5-hydroxyindoleacetic acid were low in the patient's CSF. His urinary biopterin (*Crithidia* factor) excretion was low. An increase in serum biopterin following L-phenylalanine loading was not found. Dihydropteridine reductase activity in his skin fibroblasts was normal. He excreated large amounts of *erythro-* and *threo*-neopterins (but only a trace of biopterin) in his urine. After loading with phenylalanine the urinary excretion of neopterins was even more enhanced, but biopterin remained at low levels. These findings indicated that the patient has a dihydrobiopterin synthetase deficiency.

Key words: Atypical PKU – Deficiency of dihydrobiopterin – Neurotransmitter treatment

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Introduction

A variant of phenylketonuria—atypical PKU—, which differs from classic PKU in progressive neurologic damage despite adequate dietary control of plasma phenylalanine (Phe) levels has been described [31]. It is now clear that these patients have a deficiency of tetrahydrobiopterin (BH_4). BH_4 deficiency can be caused by defective re-cycling of BH_4 due to deficient activity of dihydropteridine reductase [17], or to defective biosynthesis of BH_4 [16]; from the known cases, a frequency of 1-3% among all PKU patients can be presumed [9, 12]. Since BH_4 is not only the co-factor of phenylalanine hydroxylase, but also of tyrosine hydroxylase [30] and tryptophan hydroxylase [13] (Fig. 1), a deficiency of BH_4 causes impaired biosynthesis of the neurotransmitters dopamine and serotonin, which accounts for the progressive neurological damage in atypical PKU [13]. Supplementation with 5-hydroxytryptophan (5-HTP) and L-dopa—precursors of the deficient neurotransmitters—has been recommended [2, 9, 17]. Improvement has been reported in some cases, but not in all [3, 4, 9, 12].

The present paper describes the clinical and the biochemical findings in a patient with impaired biopterin biosynthesis.

Case Report

A 3.26 kg male infant (M.S.) was born to non-consanguinous Japanese parents after an uncomplicated pregnancy and delivery. There was no family history of any central nervous system disease. Screening tests for metabolic diseases were not performed in the neonatal period. He was healthy until two months

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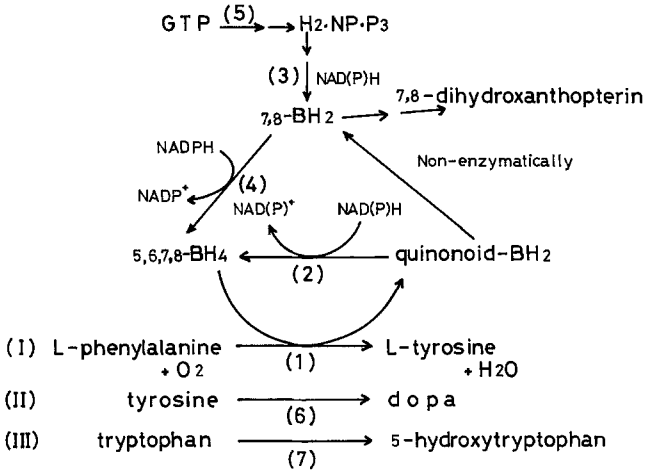

Fig. 1. Enzymic hydroxylation of phenylalanine to tyrosine, and the biosynthesis of biopterin and its function. BH_2 = dihydrobiopterin, BH_4 = tetrahydrobiopterin, $H_2 \cdot NP \cdot P_3$ = 7,8-dihydroneopterin triphosphate, GTP = guanosine triphosphate. (*1*) Phenylalanine hydroxylase, (*2*) dihydropteridine reductase, (*3*) dihydrobiopterin synthetase, (*4*) dihydrofolate reductase, (*5*) GTP cyclohydrolase, (*6*) tyrosine hydroxylase, (*7*) tryptophan hydroxylase

of age and then gradually developed feeding difficulties, stridor and irritability. At four months of age he began to have daily episodes of stiffening of the extremities and arching of his back. At six months of age he could not control his head. Hyperphenylalaninemia above 20 mg/100 ml was demonstrated by the Guthrie test for PKU, but the urinary ferric chloride test was negative.

At seven months of age, he was admitted to our hospital for dietary control. His mental development was estimated to be one to two months of age by the Denver Developmental Screening Test. Skin pigmentation and hair color were normal. Stiffening of his extremities and trunk hypotonia were noted, but the deep tendon reflexes were normal and no pathological reflexes were observed. Plasma Phe was 15.9 mg/100 ml and the results of the Phe loading test (L-Phe 100 mg/kg) were similar to those of classic PKU. Only during this test was the urinary ferric chloride test positive. The EEG and computerized tomogram of the brain were normal.

A low Phe diet was begun with an initial amount of 30 mg/kg/day. The plasma Phe level fell quickly to below 1 mg/100 ml after two days of treatment. A relatively high intake of Phe (50 to 80 mg/kg/day) was required to maintain the plasma levels between 1 and 4 mg/100 ml. Despite the good control of plasma Phe on this diet, there was no clinical improvement. He suffered frequent stiffening of his extremities with a sharp cry and rolling up of the eyes which was poorly controlled with various anticonvulsants. Irritability and stridor were persistent and progressive. Voluntary movements of his extremities gradually decreased. Abnormalities in the EEG (occipital focal spikes) and computerized tomogram of the brain (frontal lobe atrophy) were noted at 12 and 14 months of age, respectively. The low Phe diet brought neither relief of symptoms nor improvement in his development.

Materials and Methods

The drugs 5-HTP, L-dopa and a peripheral aromatic amino acid decarboxylase inhibitor (Ro 4-4602; benserazide) [1] were kindly supplied by Kyowa Hakko Co., Sankyo Co. and Daiichi Seiyaku Co. (Japan). The levels of serotonin and its metabolite (5-hydroxyindoleacetic acid) in the lumbar cerebro-spinal fluid (CSF) were determined by the method of Curzon and Green [8]. Biopterin in serum and urine was determined by an assay using *Crithida fasciculata* [15].Serum biopterin and urinary pterins after Phe loading (L-Phe 100 mg/kg per os) were analysed at 22 months of age. The pterin compounds in urine were measured using reversed-phase high-performance liquid chromatography (HPLC) as described previously [20]. Simultaneously, biopterin levels in serum were determined in a 5-year-old girl with classic PKU who was not under PKU diet. The activity of dihydropteridine reductase in skin fibroblasts was examined at two years of age according to the method of Milstien et al. [24].

Informed parental consent was obtained before each test.

Results

Treatment with 5-HTP, L-dopa and benserazide: At 14 months of age, a trial administration of 5-HTP (25 mg), L-dopa (50 mg) and benserazide (12.5 mg) was started before determination of the defective metabolic site. After a few days of treatment, stridor and irritability disappeared. When the therapy was discontinued to estimate its effectiveness, the symptoms reappeared promptly. After one month of therapy, he could raise his head and grasp a small object. The seizures of stiffening of the extremities and rolling up of the eyes gradually decreased. There was improvement in the EEG with decreased spike discharges. At 16 months of age, he could control his head and laugh aloud. His disposition improved. He could sit alone at 18 months of age. The atrophy of the frontal lobe was no longer apparent in the computerized tomogram. He has been free of convulsions since 20 month of age. He could stand at two years of age, and could walk with minimum support at 2.5 years and without support at 3 years. As shown in Fig. 2, he gradually reached his developmental milestones during therapy with these neurotransmitters.

The serotonin levels in the lumbar CSF of this patient are shown in Table 1. Before therapy, serotonin and 5-hydroxyindoleacetic acid were very low. Samples were drawn about four hours after the morning doses of 5-HTP, L-dopa and benserazide. The serotonin levels became higher than the normal level for 1- to 4-year-old children. However, the level of 5-hydroxyindoleacetic acid was lower than normal. To date there have been no side effects with this therapy. The CSF level of norepinephrine was determined simultaneously by the method of Chang [6], and was normal during the neurotransmitter therapy (data not shown).

At two and a half years of age, his Phe intake was slightly restricted (100 mg/kg/day) and he received 150 mg of 5-HTP, 125 mg of L-dopa and 31.25 mg of benserazide daily in three equal doses. His physical development has been within normal limits.

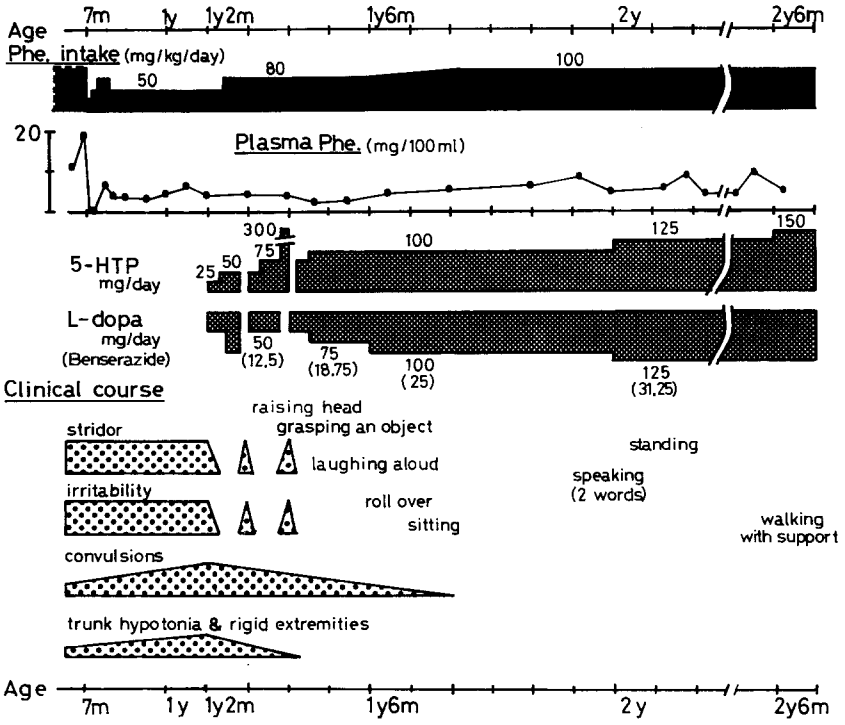

Fig. 2. Clinical course of a patient with biopterin deficiency following neurotransmitter therapy

Table 1. The changes in CSF serotonin and 5-HIAA levels following neurotransmitter therapy

Age of the patient		1 year 2 months	1 year 2 months	1 year 4 months	2 years	Control[b] mean (range)
Dose[a]	5-HTP	(−)	50	100	125	
(mg/day)	L-dopa	(−)	100	75	125	
	Benserazide	(−)	25	18.75	31.25	
Serotonin (ng/ml)		24	139	77	158	45 (40–53)
5-HIAA (ng/ml)		5.6	19.9	21.9	ND	73 (60–90)

[a] The dose was divided into three equal doses during the day
[b] Samples were obtained from 1- to 4-year-old children without central nervous disease ($n = 5$)
5-HIAA, 5-hydroxyindoleacetic acid; ND, not determined

Diagnostic Studies

(i) Biopterin Levels in Serum and Urine: The serum biopterin (Crithidia factor) level of the patient (0.34 ng/ml) did not differ from that of the control (0.37 ng/ml), but the urinary excretion (0.2 μg/ml) measured in a random sample of urine was lower than the controls (1.0 - 1.5 μg/ml, n=4). The serum biopterin level of a patient with classic PKU was 1.67 ng/ml when she was not under treatment. This finding agrees with the observation of Leeming et al. [22] that patients with classic PKU have higher than normal serum levels of biopterin-related compounds.

(ii) Serum Biopterin Levels After Oral Loading of L-Phe: We examined the fluctuation of serum biopterin levels after oral loading with L-Phe (100 mg/kg), as was reported by Kaufman et al. [16] as a diagnostic method in a patient with biopterin deficiency. As shown in Fig 3 and in Table 2, the serum biopterin level in this patient did not increase after the Phe loading, although the Phe levels rose to 30 mg/100 ml. On the other hand, in a patient with classic PKU the basal biopterin level was higher than in our atypical PKU patient or the control. After loading, the patient with classic PKU showed a 2.3-fold increase of biopterin which persisted for 24 hours. The maximum biopterin increase in a normal control was 4.7 times the basal level two hours after the loading. Thus, this patient with atypical PKU was similar to the case of impaired biopterin synthesis reported by Kaufman et al. [16].

(iii) Excretion of Urinary Pterin Compounds: The results of urinary pterins measured by HPLC are shown in Table 3. The patient excreted large amount of *erythro-* and *threo*-neopterins, but small amount of biopterin. After loading with L-Phe (100 mg/kg, per os), excretion of neopterins was even more enhanced. However, biopterin and isoxanthopterin excretion were not. These findings suggest that dihydrobiopterin (BH_2) synthetase was deficient or repressed in this patient. The amount of biopterin measured by HPLC differed from that measured as a *Crithidia* factor. The biopterin-related compounds have a variety of the biological activity for *Crithidia fasciculata*. The former finding was quite compatible with the latter when the amounts were converted into the biological activity with the coefficients for each of them [27].

(iv) Dihydropteridine Reductase Activity in Cultured Skin Fibroblasts: The activity of dihydropteridine reductase in the patient's fibroblasts was 19.2 n mol/mg protein/minute, which was in the normal range (15-58 n mol/mg protein/minute). In patients with dihydropteridine reductase deficiency, decreased folate levels in serum and CSF have been reported [5, 12, 17, 32]. The serum folate level of this patient was normal.

Hepatic phenylalanine hydroxylase activity has not yet been determined.

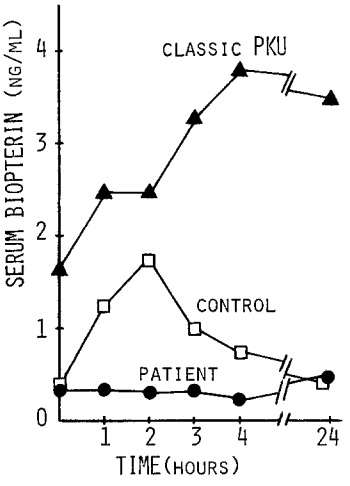

Fig. 3. Serum biopterin (Crithidia factor) levels after an oral phenylalanine load (L-phenylalanine, 100 mg/kg)

Discussion

After Kaufman had elucidated the details of the phenylalanine hydroxylating system he predicted and demonstrated the specific deficiencies—such as dihydropteridine reductase and biopterin biosynthesis—in patients with atypical PKU [16, 17]. In our patient hyperphenylalaninemia was due to an impairment of biopterin biosynthesis. The response of serum biopterin (Crithidia factor) following Phe loading was not found and dihydropteridine reductase activity in skin fibroblasts was normal. The metabolic pathway of biopterin biosynthesis is not yet clear in man. The analyses of pterins in the patient's urine suggest an impaired conversion of neopterin to biopterin (Table 3). These quantitative findings obtained by HPLC corresponded

Table 2. Plasma phenylalanine levels (mg/100 ml) during phenylalanine loading

	Time after load (hours)					
	Before	1	2	3	4	24
Patient	15	32	31	32	31	27
Classic PKU	32	51	48	46	44	18
Control	1.5	9.1	8.0	6.0	4.3	1.1

These values were measured simultaneously with serum biopterin

Table 3. Excretion of urinary pterin compounds

Condition	Amount of pterins (μg/ml of urine)					
	P-COOH	NP	MP	B	IXP	Pterin
Before load	0.36	11.4	0.76	0.06	0.02	0.09
After load[a]	0.83	17.8	1.41	0.08	0.03	0.26
Normal control[b]	0.24 ± 0.13	0.90 ± 0.08	0.25 ± 0.09	0.27 ± 0.04	0.06 ± 0.01	0.16 ± 0.04

[a] L-phe (100 mg/kg, per os) was given
[b] Value is mean ± SD of four normal age-matched controls (basal level)
P-COOH, 6-carboxypterin; NP, *erythro*-neopterin; MP, *threo*-neopterin; B, biopterin; IXP, isoxanthopterin

well with the qualitative result reported by Curtius et al. [7]. The trace amount of biopterin also corresponded well with the low level of *Crithidia* activity. These findings suggest that BH_2 synthetase might be deficient or repressed in this patient.

Progressive neurologic damage in patients with atypical PKU is explained by a lack of neurotransmitters, such as dopamine, serotonin and norepinephrine, because BH_4 participates as a co-factor in their biosynthesis [13, 30]. BH_4 itself is the most logical therapeutic agent and it has been reported that administration of BH_4 decreases plasma Phe in patients with atypical PKU [7, 11, 12, 29]. However, it has been thought that BH_4 administrated intravenously or orally does not penetrate the blood-brain barrier [12, 19, 28]. There is no evidence that patients with BH_4 dificiency can be treated by BH_4 alone. These patients should be treated by supplementation with precursors of the deficient neurotransmitters (L-dopa and 5-HTP) in combination with a peripheral aromatic amino acid decarboxylase inhibitor (carbidopa or benserazide). The decarboxylase inhibitor is necessary because it decreases the peripheral conversion of 5-HTP to serotonin and L-dopa to dopamine, allowing higher circulating levels and greater availability for crossing the blood-brain barrier, so that the dosages of L-dopa and 5-HTP can be lower. This agent can prevent their adverse effects [1].

Our patient clearly benefited from neurotransmitter therapy. The biochemical investigation in this case indicate that the low CSF level of serotonin and 5-hydroxyindoleacetic acid are in accordance with the predicted decrease in BH_4 deficiency. Severe deficiency of serotonin in cortical brain tissue has been reported in a case of dihydropteridine reductase deficiency [5]. Soon after therapy was started, serotonin in our patient's CSF rose to more than twice the normal level. However, 5-hydroxyindoleacetic acid was still lower than normal and no adverse effects were observed, such as diarrhea, vomiting or dyskinesia. The high levels of serotonin which were measured might, in part, represent undifferentiated serotonin from the precursor (5-HTP) by the method employed in this study [23].

The clinical benefits of the neurotransmitters therapy were remarkable in our patient, although the beginning of the therapy was delayed until 14 months of age. In the cases reported in the literature there was minimal improvement if the start of this therapy was delayed [2, 9]. In cases where there was a good clinical response, the therapy had been started before 13 months of age [3, 9, 14]. Thus early diagnosis of BH_4 deficiency is desirable because the prognosis depends more on an early treatment with neurotransmitters than on the type of molecular defect. It has been reported that monitoring the urinary excretion of 5-hydroxyindoleacetic acid or the BH_4 challenge test is useful in screening for this disease [7, 10, 14]. Although long term follow-up is required, the recovery of developmental progression in our patient has been remarkable, and the results of this treatment are very encouraging. It has been recommended that the daily doses of L-dopa, 5-HTP and carbidopa should be 7.5–10 mg/kg, 2–8 mg/kg and 1 mg/kg, respectively, divided into three equal doses [9]. Our patient has been given 11 mg/kg/day of L-dopa, 13 mg/kg/day of 5-HTP and 2.7 mg/kg/day of the decarboxylase inhibitor (benserazide). Phe intake has been also controlled (about 100 mg/kg/day), since the injurious effects of high Phe levels may still occur. The replacement of these neurotransmitters may prove to be good, but incomplete, treatment. The doses should be adjusted for the requirement of each patient with monitoring for adverse effects. Further studies are required to determine the role of biopterins in human metabolism.

BH_4 deficiency is a rare disorder. The incidence in Japan is unknown but there have been seven reported cases: a case with impaired biopterin biosynthesis [26], three siblings with dihydropteridine reductase deficiency [25], two siblings with low urinary biopterin levels [21], and a case under investigation [18].

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