Diagnosis of Tetrahydrobiopterin (BH_4) Responsive Mild Phenylketonuria in Japan over the Past 10 Years

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Abstract

Background: A novel therapeutic strategy for phenylketonuria (PKU) has been initiated in Japan. Hyperphenylalaninemia (HPA) results from a phenylalanine hydroxylase (PAH) enzyme deficiency or a deficiency of its cofactor, tetrahydrobiopterin (BH_4). BH_4 can normalize blood phenylalanine levels in BH_4 deficiency, but typically not in PKU. However, since 1999 it has been reported that many HPA patients (serum phenylalanine <20 mg/dL) showed a gradual decrease of serum phenylalanine levels after 24 hours from BH_4 loading. The BH_4 responsiveness seems to be regulated in mild PKU by PAH mutations, and affected by the BH_4 dose and administration period. Methods and Results: In 2002 we formulated a provisional diagnostic criteria for patients with BH_4-responsive PAH deficiency, and newly diagnosed 19 patients in 100 HPA cases between 2002 and 2006. The incidence in the recent 5 years for BH_4-responsive mild PKU among patients with PAH deficiency was 25 %. Conclusion: A total of 31 patients was detected in the past 10 years, and the incidence detected using the provisional diagnostic criteria had increased to 25% among PAH deficient patients. BH_4 treatment for BH_4-responsive mild PKU is a new and effective pharmacotherapy, which replaces or liberalises the phenylalanine-restricted diets for a considerable number of mild PKU patients.

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Key words: BH_4-responsive PAH deficiency, Biopterin, Tetrahydrobiopterin

Introduction

Tetrahydrobiopterin (BH_4) can normalise blood phenylalanine levels in BH_4 deficiency, but typically not in phenylalanine hydroxylase (PAH) deficiency. However, in 1999 Kure et al reported 4 patients with PAH deficiency showed a decrease in blood phenylalanine elevations after BH_4 loading.[1] In 2000, Shintaku et al found that 5 out of 15 patients with mild PKU (serum phenylalanine <20 mg/dL) showed a gradual decrease of serum phenylalanine at 24 hour with BH_4 loading, although no patients with classical phenylketonuria (PKU: serum phenylalanine >20 mg/dL) responded to BH_4.[2] Shintaku et al examined 12 patients with BH_4-responsive PAH deficiency discovered by PKU screening and evaluated the responses in the BH_4 loading tests and formulated a provisional diagnostic criteria, which was the percent decline in serum phenylalanine from initial values after single-dose (>20%), four-dose (>30%), and 1-week BH_4 (>50%) loading tests.[3] We administered this provisional diagnostic criteria to neonatal PKU screening between 2002 and 2006 in Japan and detected 19 patients with BH_4-responsive mild PKU.

Materials and Methods

In all of the 100 patients with HPA detected by neonatal PKU screening, we examined biopterin metabolism by pteridine analysis, dihydropteridine reductase assay and single-dose BH_4 loading test. Four-dose and 1-week BH4 loading tests were conducted in patients who had normal BH_4 metabolism and had decreases in plasma phenylalanine concentrations by over 20% in the single-dose test.

An oral BH_4 (Asubio Pharma, Tokyo, Japan) loading test was performed after demonstrating serum phenylalanine concentrations of greater than 6 mg/dL upon instituting a normal diet, which was maintained during loading tests. In the single-dose BH_4 loading test, BH_4 (10 mg/kg) was administered before breakfast; blood samples were collected

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Table 1. Incidence of BH4-responsive mild PKU in Neonatal Mass-screening in Japan

Before and after implementing a provisional diagnostic criteria in 2002		BH_4-responsive HPA	HPA	Incidence
Total	1995-2006	31	234	13%
Before	1995-2001	12	134	9%
After	2002-2006	19	100	19%

at 0, 4, 8, and 24 hours after loading. In the four-dose BH_4 loading test, BH_4 was administered at doses of 10, 10, 5, and 5 mg/kg at 0, 24, 36, and 48 hours, respectively. Blood samples were obtained at 0, 4, 8, 24, and 52 hours after loading. In the 1-week BH_4 loading test, BH_4 was administered for 1 week at 20 mg/kg/day divided into 3 doses daily. Blood samples were obtained before loading and after 4 and 7 days respectively. Serum phenylalanine concentrations were determined by using an automated amino acid analyser (L-8800; Hitachi, Tokyo, Japan). Serum pteridine was measured by high performance liquid chromatography (LC-10; Shimazu, Kyoto, Japan) after iodine oxidation. Dihydropteridine reductase (DHPR) activity was measured in Guthrie card specimens as described previously.[4]

Results and Discussion

Among these 100 patients, 19 patients had normal biopterin metabolism, and their mean values of percentage decline in serum phenylalanine from initial values were 40, 43, and 52 after single-dose, four-dose, and 1-week BH_4 loading tests respectively. The incidence of BH_4-responsive mild PKU in neonatal PKU screening in Japan was 19% between 2002 and 2006 (Table 1). Before 2002, 12 patients with BH_4-responsive mild PKU were detected among 134 patients with hyperphenylalaninemia (HPA). The diagnosis of BH_4-responsive mild PKU was made using the provisional diagnostic criteria and the incidence of BH_4-responsive mild PKU in neonatal PKU screening increased from 9% to 19% after 2002. A total of 31 patients with BH_4-responsive mild PKU were detected in 234 HPA cases and the incidence was 13% in neonatal PKU screening in Japan over the past 10 years. However, among the 100 patients found by neonatal PKU screening between 2002 and 2006, 4 were affected by BH_4 deficiency and 21 were affected other diseases (for example, neonatal hepatitis), and the remaining 75 patients were affected by PAH deficiency. Therefore the incidence of BH_4-responsive mild PKU among PAH deficiency in neonatal screening was 25% (19 out of 75) between 2002 and 2006. The incidence has increased to 25% after implementing the provisional diagnostic criteria, so that BH_4 treatment is thought to be available for a considerable number of mild PKU cases.

Conclusion

Between 2002 and 2006, 19 patients with BH_4-responsive mild PKU were newly detected by using the provisional diagnostic criteria described above, and during this period, the incidence among patients with PAH deficiency was 25%. A total of 31 patients were detected in the past 10 years in Japan, and the incidence of BH_4-responsive mild PKU detected using the provisional diagnostic criteria has increased to 25%. BH_4 treatment for mild PKU is a new and effective pharmacotherapy, which replaces or liberalises the phenylalanine-restricted diets for a considerable number of mild PKU patients.

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