Introduction

Tetrahydrobiopterin (BH₄) can normalise blood phenylalanine levels in BH₄ 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 BH₄ loading. 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 levels after 24 hours from BH₄ loading. The BH₄ responsiveness seems to be regulated in mild PKU by PAH mutations, and affected by the BH₄ dose and administration period. Methods and Results: In 2002 we formulated a provisional diagnostic criteria for patients with BH₄-responsive PAH deficiency, and newly diagnosed 19 patients in 100 HPA cases between 2002 and 2006. The incidence in the recent 5 years for BH₄-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. BH₄ treatment for BH₄-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.
at 0, 4, 8, and 24 hours after loading. In the four-dose BH₄ loading test, BH₄ 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 BH₄ loading test, BH₄ 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 BH₄ loading tests respectively. The incidence of BH₄-responsive mild PKU in neonatal PKU screening in Japan was 19% between 2002 and 2006 (Table 1). Before 2002, 12 patients with BH₄-responsive mild PKU were detected among 134 patients with hyperphenylalaninemia (HPA). The diagnosis of BH₄-responsive mild PKU was made using the provisional diagnostic criteria and the incidence of BH₄-responsive mild PKU in neonatal screening increased from 9% to 19% after 2002. A total of 31 patients with BH₄-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 BH₄ 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 BH₄-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 BH₄ treatment is thought to be available for a considerable number of mild PKU cases.

**Conclusion**

Between 2002 and 2006, 19 patients with BH₄-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 BH₄-responsive mild PKU detected using the provisional diagnostic criteria has increased to 25%. BH₄ 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.

**REFERENCES**