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TETRAHYDROBIOPTERIN-RESPONSIVE PHENYLALANINE HYDROXYLASE DEFICIENCY IN ITALY

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We describe the results of the BH_4 loading test performed in 57 Italian PKU patients. Patients with classical (26/57), moderate (8/57), mild (6/57) PKU and 17 subjects with MHP were orally given BH_4 20 mg/kg. Blood Phe and Tyr were determined at 0 h, 4 h and 8 h. Since 2004 our protocol has been modified with measurement of Phe and Tyr also after 24 h. From the 63 patients included in this study, 9 showed a positive response (reduction $>30\%$ at 8 h): 5 MHP patients, one with moderate, and 3 with mild PKU. In these 9 patients a defect in the synthesis or recycling of tetrahydrobiopterin was excluded by analysis of urinary pterins and DHPR activity (Dr N. Blau-Zurich).

One children with moderate and one with mild PKU are currently on BH_4 treatment (10 mg/kg/day in two doses). In the first patient combined BH_4 and dietary treatment is effective to keep Phe levels within the therapeutic range, in the second patient a good metabolic control is achieved with BH_4 therapy without any dietary restriction.

Our findings show: (1) the therapeutic potential of BH_4 in some patients with mild and/or moderate PKU; (2) the largest incidence of patients with biopterin-responsive phenylalanine hydroxylase deficiency in the group of the patients with mild PKU (50%); (3) the ineffectiveness of the pharmacological doses of tetrahydrobiopterin in patient with classical PKU.

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PHARMACOKINETIC OF ORALLY ADMINISTERED BH_4 IN PATIENTS WITH PHENYLALANINE HYDROXYLASE DEFICIENCY

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Tetrahydrobiopterin (BH_4) was measured in dried blood spots from patients with hyperphenylalaninemia after oral administration according to following protocols: (A) 20 mg/kg BH_4 sampling times 0, 4, 8, and 24 h ($n = 64$); (B) 20 mg/kg BH_4 sampling times 0, 2, 4, 8, 12, 24, and 32 h ($n = 34$); (C) 100 mg/kg Phe+20 mg/kg BH_4 sampling times –3, 0, 4, 8, and 24 h ($n = 35$); and (D) 2×20 mg/kg BH_4 sampling times 0, 4, 8, 24, and 48 h ($n = 11$). Maximal BH_4 blood concentrations (C_{max}) were compared with Phe levels at different time points and responsiveness (% of Phe reduction) was calculated for each group of patients. BH_4 peaked at 4 h after BH_4 administration in 58/64 patient (median = 22.7 nmol/mg Hb), 6 patients had C_{max} levels at 8 h (median = 13.5 nmol/mg Hb). When compared with C_{max} at 4 h, BH_4 concentrations at 2 and 12 h were 28% and 74% lower, respectively. Almost normal BH_4 levels were reached 24 to 32 h after single BH_4 administration. Repeated administration of BH_4 (2×20 mg/kg at T_0 and T_{24}) resulted in a second BH_4 peak at 32 h (38% lower compared to 4 h). In the combined Phe/BH_4 loading test, BH_4 concentrations increased 3 h after Phe administration ($\sim 51\%$), probably due to induction of GTP cyclohydrolase, but BH_4 profile was not significantly different from those of other protocols. There was a significant reduction of blood Phe after a combined Phe+BH_4 challenge in all patients. There was no significant effect of BH_4 concentration on the outcome of the loading test in all protocols.

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THE DIFFERENCE OF SEIZURE BETWEEN PATIENTS WITH TETRAHYDROBIOPTERIN (BH_4) DEFICIENCY AND PHENYLKETONURIA (PKU)

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Objective: To better understand the difference of seizure between patients with tetrahydrobiopterin (BH_4) deficiency and phenylketonuria (PKU). **Method**: The time of onset, clinical manifestation, electroencephalogram (EEG) and outcome of seizure were compared between two groups. **Results**: In the last twelve years, 98 PKU and 12 BH_4 deficient patients experienced seizure out of 391 late-treated patients with hyperphenylalaninemia, excluding other causes such as high fever, hypocalcemia and neonatal asphyxia. Seizure of the 98 PKU cases occurred at the age of 10.7 ± 2.6 ($4.5 \sim 27.1$) months, displaying various patterns, mostly infantile spasm (55/98). Of the 32 cases who had EEG detection, 31 were found to have epileptiform discharge and 15 have hypsarrhythmia. EEG follow-up after treatment further revealed that abnormalities were still present but less severe. Administration of sodium valproate and other anticonvulsant drugs to control seizure resulted in a long-term and tough course. In comparison, seizure of the 12 BH_4 deficient cases occurred at the age of 5.1 ± 1.9 ($2.7 \sim 11.0$) months, presenting paroxysmal spasm of limbs, sometimes with fixed eyes. EEG was carried out in 10 cases, among which 3 were found to have slight epileptiform discharge, and the other 7 have normal EEG. EEG tracing revealed that change with age after treatment was not specific. The symptom was under control immediately after the administration of levodopa. **Conclusion**: The onset time, clinical, electrographic manifestation and control of seizure in BH_4 deficiency were quite different from those in PKU. There should be substantial difference in the mechanism of seizure between BH_4 deficiency and PKU.

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RELATIONSHIP BETWEEN GENOTYPE AND INTELLECTUAL PHENOTYPE IN UNTREATED PHENYLKETONURIC PATIENTS

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The aim of this study was to explore correlation between phenylalanine hydroxylase (PAH) genotype and intellectual phenotype in untreated patients with phenylketonuria (PKU). Genomic DNA was isolated from whole-blood samples of 165 untreated PKU patients. PAH gene mutation screening analyses were performed by polymerase chain reaction (PCR) of exon 3, 5, 6, 7, 10, 11 and 12, followed by single strand conformational polymorphism (SSCP) or denaturing high-performance liquid chromatography (DHPLC). The genotypes were determined by direct sequencing at last. Eighty-six untreated PKU patients whose two mutant alleles were both defined were involved in this study. The DQ/IQ of these patients were tested by Gesell development schedules. Genotypes were classified according to Guldberg's classification (Am J Hum Genet 63:71–79, 1998). Among 86 patients, 8 (9.3%) were mild retardation, 31 (36.1%) were moderate, the severe mental retardation accounted for 47 (54.6%). The relationship between genotype and intellectual phenotype in this group was examined. It was found that the intellectual phenotypes of 63 (73.3%) patients were compatible with genotypes and not well matched in 23 (26.7%) cases. The result indicates that the intellectual phenotype was well matched with genotype in untreated PKU patients. Genotype determination is useful in the prediction of clinical phenotype in PKU patients.