Early Human Development (2008) **84**, 561–567

available at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/earlhumdev

Evaluation of neonatal BH4 loading test in neonates screened for hyperphenylalaninemia

François Feillet [a,b,*], Céline Chery [a,b], Fares Namour [a,b], Antoine Kimmoun [a], Elisabeth Favre [a], Elisabeth Lorentz [a], Shyue-Fang Battaglia-Hsu [b], Jean-Louis Guéant [a,b]

[a] *Reference Centre for Inborn Errors of Metabolism, Department of Pediatry and Department of Biochemistry-molecular biology-nutrition-metabolism, CHU of Nancy, Allée du Morvan, Vandoeuvre les Nancy, 54500, France*
[b] *INSERM U724, Faculté de Médecine de Nancy, Nancy-Université, Rue de la Forêt de Haye, Vandoeuvre les Nancy, 54500, France*

Received 23 October 2007; received in revised form 12 December 2007; accepted 7 January 2008

KEYWORDS
Hyperphenylalaninemia;
Phenylketonuria;
Tetrahydrobiopterin;
Phenylalanine hydroxylase;
Neonatal screening

Abstract

Background: The outcome in phenylketonuria is related to the early diagnosis and management due to neonatal screening.
Aims: To assess the interest of tetrahydrobiopterin (BH4) loading test and phenylalanine hydroxylase (PAH) genotyping in the management of neonates with hyperphenylalaninemia (HPA).
Study design: We evaluate the effectiveness of a BH4 loading test (20 mg/kg) in ten neonates screened for HPA. We evaluated the time required to reach a target plasma Phenylalanine (Phe) level below 300 µmol/l. We compared these ten BH4-loaded patients to the 10 previous neonates non-loaded with BH4. In all these patients, the PAH genotype was determined.
Results: One loaded patient had biopterin synthesis deficiency and has been retrieved from statistical analysis. All others patients have PAH deficiency. Between the BH4 loaded group (L) and the BH4 non-loaded group (NL), a statistically significant difference was observed in the average time required to reached the target Phe level (13.56 ± 4.30 (L) vs. 20.6 ± 7.59 days (NL) [$p<0.02$]). Results of the genotyping from all but one of these 19 patients indicated that among all mutations present in this patient population, there were 4 known PAH mutations associated with BH4 responsiveness (p.R261Q, the p.V388 M, the p.E390G and the p.Y414C). These mutations were found in 4 non-loaded and 6 loaded patients. Two patients had a more than 90% reduction in their plasma Phe level within 24 h after the load. One of these patients had a PTPS deficiency. The other fully responsive patient (p.Y414C and IVS10-11G>A) has been treated with BH4 from birth with an excellent metabolic control for three years now.

* Corresponding author. Reference Centre for Inborn Errors of Metabolism, Hôpital d'Enfants, CHU Brabois, Allée du Morvan, Vandoeuvre les Nancy, 54500, France. Tel.: +33 3 83 15 46 01; fax: +33 3 83 15 45 29.
E-mail address: f.feillet@chu-nancy.fr (F. Feillet).

0378-3782/$ - see front matter
doi:10.1016/j.earlhumdev.2008.01.003

Conclusion: BH4 loading test improves the management of HPA. It allows an immediate identification of the children fully responsive to BH4. Our results therefore suggest the incorporation of BH4 loading test in the management of neonates screened for HPA.

1. Introduction

Phenylketonuria (PKU, MIM# 261000) is one of the most common inborn errors in amino acid metabolism due to phenylalanine hydroxylase (PAH) deficiency. Because of phenylalanine (Phe) accumulation, phenylketonuria (PKU) is responsible for mental retardation, hypopigmentation and sometimes severe behavioral problems. To prevent mental retardation, diagnosis and treatment are performed by neonatal screening [1]. Today in France, the neonatal screening is performed at the third day of life by Guthrie's test. A good patient compliance to an early Phe restriction in diet allows a normal outcome in brain development [2].

Tetrahydrobiopterin (BH4) is the cofactor for Phe hydroxylation by PAH. BH4 loading test has first been used to detect BH4 deficiency [3]. In 1999, Kure et al showed that patients with PAH deficiency could be BH4 responsive [4]. Since then, the responsiveness to BH4 in PAH deficient patients has been extensively studied in various populations [5–8]. This responsiveness to BH4 is probably associated with mutations in the *PAH* gene that encode a variant form of the enzyme with some residual activity and additional BH4 enhances the PAH activity and increases the half-life of the mutant enzyme [5–8]. The PAH-mutation genotype is the main determinant of the metabolic phenotype [9,10].

Typically after a single loading dose at 20 mg/kg, the responsiveness of BH4 to HPA can be determined by a reduction of 30% or more in blood phenylalanine concentration. Clearly, for BH4 responsive PAH deficient patients, administering BH4 can be considered as an alternative treatment method to the phenylalanine restricted diet in managing PKU [11–14]. All these published results in BH4 loading prompted us to evaluate the benefits of a systemic BH4 loading in neonates screened for PKU. During the past four years, ten neonates screened for HPA were given BH4 loading. Here we report the results of the comparison made between these 10 loaded patients and the 10 neonates previously screened for HPA (without BH4 load).

2. Materials and methods

2.1. Screening and neonatal BH4 loading test

Neonatal BH4 loading test in patients with hyperphenylalaninemia (HPA) began in 2003 in our centre. Since then, this procedure was carried out in ten newborns. In France, the neonatal HPA screening begins with a blood sample drawn at the third day of life. The phenylalanine level is determined on the 5th or 6th day of life. When Phe level is above 180 μmol/l, the concerned family is alerted and the baby is hospitalized the next morning on the 6th or 7th day of life. The BH4 loading test is performed as soon as the baby had been admitted to the hospital. Briefly, the procedure is as follows. Tetrahydrobiopterin (BH4) was obtained from Schirks Laboratories (Jona, Switzerland). The standard BH4 loading test included blood Phe measurements at time zero just before the BH4 dose (T0) and at 2, 4, 6, 8, 12, and 24 h (T2, T4, T6, T8, T12 and T24) after a single BH4 oral dose of 20 mg/kg. The BH4 responsiveness is defined as a 30% phenylalanine reduction cut-off after a 24-hour challenge using 20 mg/kg of BH4 in a single dose. During the loading test, the patients were not subject to the Phe restricted diet. The blood tests were sampled on a Guthrie card and were analyzed with a fluorimetry method [15]. When we received the results the next day around 12 am, the patients were allowed to start the diet or BH4 treatment. Additionally, at T0 a urine sample was collected together with the T0 blood sample to look for putative abnormalities in biopterin metabolism [16]. We classified our HPA patient according to the established criteria: >1200 μmol/l, classical PKU; 600–1200 μmol/l, mild PKU; <600 μmol/l, moderate HPA [10] and biopterin deficiency [16].

2.2. Time lapse to reach metabolic control

We evaluate the lapse time, starting from the time of management to the time at which the plasma Phe reaching a level of 300 μmol/l. We compared the time lapse difference between the groups of 10 loaded patients just mentioned and of 10 non-loaded patients. As we found, in the loaded group, a patient with HPA due to biopterin synthesis defect (PTPS deficiency), we retrieved him from the statistical analysis.

2.3. PAH genotype

The genotype of almost all patients recruited in both groups was determined (one patient in the non loaded group refused the genetic analysis). Briefly, the DNA in leukocytes was isolated from the blood samples using Nucleon DNA Extraction Kit (Amersham Life Sciences). PCR amplification of all 13 exons of the PAH gene (Gene ID 5053) was performed using thirteen pairs of primers, each designed to span a specific exon with the adjacent intronic 5' and 3' regions. DHPLC analysis was carried out on the WAVE system with a DNASep column (Transgenomic). Following DHPLC screening, fragments showing an abnormal DHPLC pattern were sequenced in order to determine the nature of the sequence change. Direct sequencing was performed using the BigDye terminator v1.1 Cycle Sequencing Kit (Applied Biosystems) along with an automatic sequencer (ABI Prism 3100 Genetic Analyzer, Applied Biosystems).

2.4. Statistical analysis

Data analyses were mainly performed with Statview software. The *U* Mann–Whitney nonparametric test was used to compare the time lapse difference to reach Phe control between the two groups and between the classical PKU patients and the mild PKU patients in the loaded group.