SCREENING OF 6-PYRUVOYL-TETRAHYDROPTERIN SYNTHASE ACTIVITY DEFICIENCY AMONG HYPERPHENYLALANINEMIC PATIENTS

DURDI QUJEQ

Department of Biochemistry and Biophysics, Babol University of Medical Sciences, Babol, Iran

ABSTRACT

A deficiency of the phenylalanine hydroxylase activity or its cofactor tetrahydrobiopterin may lead to hyperphenylalaninemia and as a result, loss of IQ, poor school performance, and behavior problems occurs. Deficiency in 6-pyruvoyl-tetrahydropterin synthase activity is the major cause of tetrahydrobiopterin deficient phenylketonuria. In this study, blood specimens from 165 healthy volunteers and 127 children with phenylketonuria were used to determine the 6-pyruvoyl-tetrahydropterin synthase activity. It was found that the activity of 6-pyruvoyl- tetrahydropterin synthase was decreased in comparison with control [23.46 +/- 2.94, (mean +/- SD, mmol/ml/h, n=127) vs. 127.63 +/- 4.52, n=165], p<0.05]. Results of this study indicate that examination of 6-pyruvoyl-tetrahydropterin synthase activity is helpful and may lead to the diagnosis cause of hyperphenylalaninemia.

Key Words: 6-pyruvoyl-tetrahydropterin synthase, phenylketonuria, tetrahydropterin

INTRODUCTION

Deficiency in 6-pyruvoyl-tetrahydropterin synthase (PTPS) activity is the major cause of tetrahydrobiopterin (BH4) deficient phenylketonuria (1). Hyperphenylalaninemia (HPA) may be caused by deficiency of phenylalanine hydroxylase or BH4, the essential cofactor for the aromatic amino acid hydroxylase. PTPS deficiency is a major cause of BH4 deficient HPA (2). The relationship between blood phenylalanine concentrations and serum or erythrocyte biopterin and neopterin concentrations has been reported in 20 phenylketonuric patients with different dietary compliance (3). Some cases of primary hyperphenylalaninemia are not caused by the lack of phenylalanine hydroxylase, but are caused by the lack of its cofactor tetrahydrobiopterin. These patients are not clinically responsive to a phenylalanine-restricted diet, but need specific substituted therapy (4). BH4 deficiency in HPA babies has to be recognized rapidly and disease requires a specific treatment (5). The deficiency of PTPS is the most common cause of BH4 deficiency (6). The cofactor for various enzymes includes the hepatic phenylalanine hydroxylase. Inherited PTPS deficiency leads to BH4 deletion, causes HPA, and requires cofactor replacement therapy for treatment (7). BH4 deficiencies are highly heterogeneous disorders, with more than 30 molecular lesions identified in the past 2 years in the GTP cyclohydrolase 1 and PTPS gene.

Compound heterozygous or homozygous mutations spread over all six exons encoding the PTPS and causes an autosomal recessively inherited variant of HPA that is mostly accompanied by a deficiency of dopamine and serotonin (8). BH4 deficiency, a variant of HPA, may be caused by deficiency of one of the following enzymes: Guanosine triphosphate cyclohydrolase 1,6-pyruvoyl-tetrahydropterin synthase, dihydropteridine reductase and pterin-4a-carbinolamine dehydrogenase (9). Autosomal recessive mutations in the PTPS synthase gene are the most common reason for HPA due to BH4 deficiency (10). Four naturally occurring mutants with single amino acid alterations in human in which PTPS synthase are overexpressed have been characterized in vitro (11). The purpose of this study was to find a very sensitive indicator for hyperphenylalaninemia patients with enzyme deficiency, other than of phenylalanine hydroxylase, a variant of hyperphenylalaninemia. This report may leads to a better understanding for detection of PKU.

MATERIALS AND METHODS

Reagents: Neopterin, Biopterin and D-erythro dihydroneopterin triphosphate were obtained from Sigma Chemical Company St. Louis, MO., USA. All other reagents and solvents were of analytical grade and were purchased from Merck, Germany. Characteristics of subjects: Control blood (3 ml) were obtained from 165 healthy volunteers (92

Correspondence: D. Qujeg, Department of Biochem. & Biophys., Babol University of Medical Sciences, Babol, Iran.
males and 73 females) aged 2-8 year (mean 5.8+/−
2.7 SD ), and 127 patients with PKU (69 males
and 58 females), aged 1-7 year (mean 4.7+/−2.9
SD). Blood was centrifuged at 2500 g for 5 min,
and the separated serum was stored at -20°C until
analyzed. Details of age, sex, clinical presentation
and diagnostic findings were recorded in routine
way.

Procedure: Blood specimens from subjects were
employed for determination of 6-pyruvoyl-
tetrahydropterin synthase activity. The activity of
6-pyruvoyl-tetrahydropterin synthase was
determined in serum by converting the
dihydroneopterin triphosphate to 6-pyruvoyl
tetrahydropterin. The buffer, which was used for
the assay of 6-pyruvoyl-tetrahydropterin synthase,
was 70 mM Tris-HCl with pH 7.25, containing 15
mM MgCl₂. Ten microliters of serum in this assay
buffer was incubated with 4 mM NADPH,
sepiapterin reductase, and 80 μM freshly prepared
dihydroneopterin triphosphate with a final volume
of 150 μL for 60 min in dark. The reaction was
started by addition of dihydroneopterin
triphosphate and was stopped with 15 μL of a
mixture of 20 μL of 1 M HCl and 15 μL of 0.2 M
Iodide (I₂), dissolved in 0.30 M potassium iodide.
After a 60 min incubation in the dark, 10 μL of
ascorbic acid was added and the mixture was
stored at -20°C until analyzed for biopterin
content. Ten μL of blood from each subject was
applied separately across 8.0 cm in a 0.5 cm band
in whatman 3mm paper (60 cm x 50 cm), samples
were applied 3 cm apart, and were separated by
high voltage electrophoresis (HVE). HVE was
performed at 500 V for 60 min using acetate buffer
of pH 3.5. All samples were applied under a
stream of nitrogen. The paper was dried in an oven
at 120°C for approximately 20 min. Fluorescent
compounds were located by viewing the paper
under ultraviolet light at 370 nm. As amino acids
fluoresce after heating on paper, ninhydrin (0.3 %
W/V) was used to demonstrate the separation of
fluorescent compounds. For the purpose of
comparison of consecutive HVE separations,
quinox sulfate (5 mg per ml) in distilled water
was applied at intervals across each paper. Migration
of compounds was compared with migration of
quinox sulfate (RQ=migration of compound
/migration of quinox sulfate). The material at RQ
2.0 was eluted overnight with water, freeze-dried
and dissolved in 4 ml of 0.1 M NaOH.
Fluorescent spectra reading at the maximum
wavelengths for excitation (400nm) and
fluorescence (480 nm) was used for quantitation.
Standards were prepared by treating 10, 20, 30, 40,
50, 60, 70, 80, 90, 100 μl of a biopterin and
neopterin solution as above. The percent recovery
of the method was determined by comparing the
fluorescence of the standards and solutions of 10,
20, 30, 40, 50, 60, 70, 80, 90, 100 μl of the above
neopterin and biopterin solution which were
diluted to 5 ml with 0.2 M NaOH. All solutions
were diluted 1 in 5 to confirm the linearity of
fluorescent response over the range that was used.

RESULTS
Calibration curve for measurement of 6-pyruvoyl-
tetrahydropterin synthase activity is shown in
figure 1. The measuring range of substrate
concentration that extends from 0.0 to 1.2 mmol of
1.6-pyruvoyl-tetrahydropterin synthase activity of
blood in normal and patients with PKU is shown
in figure 2. Serum levels of biopterin and neopterin from patients with hyperphenylalanine and healthy subjects are given in figure 3.

DISCUSSION

Hyperphenylalaninemia is not always the result of phenylalanine hydroxylase deficiency. Non-responsiveness to diet therapy of PKU has led to the discovery of hyperphenylalaninemia which is caused by enzyme defects other than phenylalanine hydroxylase. In accordance with other investigations 6-pyruvoyl-tetrahydropterin synthase in PKU patients was decreased in comparison with normal volunteers. These results suggest that it is important to distinguish between the hyperphenylalaninemia caused by phenylalanine hydroxylase deficiency and the one that is caused by defect of BH4 metabolism since clinical management of those types of disease is very different. Phenylalanine hydroxylase deficiency is treated by the use of a low phenylalanine diet whereas BH4 deficiency due to severely reduced serotonin and dopamine synthesis requires additional therapy to overcome the neurotransmitter deficiency. In defect of the BH4 metabolism pathway due to the GTP cyclohydrolase, it appears that there is a normal production of dihydروpterin triphosphate but a failure of its conversion to BH4. This results in accumulation of neopterin and a deficiency of biopterin in serum (figure 3). These findings are supported by findings of other investigator (3) and suggest that examination of this enzyme is helpful for diagnosis of the causes of hyperphenylalaninemia, a defect in phenylalanine hydroxylase or cofactor synthesis. It is important to distinguish between the hyperphenylalaninemia that is caused by phenylalanine hydroxylase deficiency or by defect of BH4 metabolism.

REFERENCES