Screening for Biopterin Defects in Newborns with Phenylketonuria and Other Hyperphenylalaninemas*

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ABSTRACT

The newborn screening for phenylketonuria (PKU) has become a common practice in many countries. The success of this type of mass screening is the result of the reliability of the Guthrie test in the identification of high blood phenylalanine levels. Recently, new syndromes other than PKU have been recognized that give high blood phenylalanine levels. These syndromes are referred to as “atypical” PKU. While in classical PKU phenylalanine hydroxylase is the deficient enzyme, in the atypical forms of PKU the deficiency is in the enzymes affecting the production of tetrahydrobiopterin, the cofactor required for the activity of phenylalanine hydroxylase. Two types of atypical forms of PKU are now known. One is caused by a deficiency of dihydropteridine reductase, and the other is caused by defects in the synthetic pathways of biopterin, termed as “biopterin synthetase deficiency.” In addition, a transient form of biopterin synthetase deficiency occurs in the newborn period which has to be distinguished from the permanent defect in biopterin synthesis. Analysis of oxidized pterins in urine by high performance liquid chromatography identifies classical PKU, dihydropteridine reductase deficiency, and biopterin synthetase defects. The early recognition of these cofactor defects is essential for the successful treatment of patients with the atypical forms of hyperphenylalaninemas.

Introduction

The enzyme phenylalanine hydroxylase, which catalyzes the conversion of phenylalanine to tyrosine, requires tetrahydrobiopterin as a cofactor for this reaction.\textsuperscript{7,8,9} Tetrahydrobiopterin is also a cofactor of other hydroxylases, such as tryptophan hydroxylase and tyrosine hydroxylase. Enzyme defects affecting the production of this cofactor result in the inactivity of these hydroxylases, which is reflected in high blood phenylalanine levels.
Recently, two categories of cofactor defects affecting tetrahydrobiopterin have been described.\textsuperscript{1,2,3,4,10,11,12,13,14,15,16,17,18,19} The first reports dealt with dihydropteridine reductase deficiency.\textsuperscript{11,19} In this enzyme deficiency the dihydropteridine formed is not reduced back to the active cofactor, tetrahydrobiopterin. The result of such a deficiency is the deterioration of the affected child despite good dietary treatment for classical PKU.

The second type of defect to be identified is in the synthetic pathways of tetrahydrobiopterin.\textsuperscript{2,10,15} This enzyme deficiency has been termed as biopterin synthetase deficiency. The clinical features of this condition are similar to the dihydropteridine reductase deficiency. Patients identified as having dihydropteridine reductase deficiency or biopterin synthetase deficiency require the addition of levo-dihydroxyphenylalanine (L-dopa) and 5-hydroxytryptophan to their diet. Such patients may also benefit from treatment with tetrahydrobiopterin.

More recently, a transient form of biopterin synthetase deficiency has been encountered in a newborn with classical PKU.\textsuperscript{13} Biopterin levels returned to normal over a six-week period and no special treatment was required except for the phenylalanine restricted diet.

Several methods are in use in order to recognize the atypical forms of PKU. One is the oral administration of tetrahydrobiopterin, in cases of elevated blood phenylalanine levels.\textsuperscript{5,15} If the patient has classical PKU, blood phenylalanine is expected to remain high over a four to six hour period. If the deficiency is in the generation of the cofactor, the blood phenylalanine level will drop sharply.

The second method is the use of urinary pterine analysis.\textsuperscript{14,16} Using this method, as developed by Fukushima and Nixon,\textsuperscript{5,6} urinary pterins are oxidized to neopterin (N) and biopterin (B). The separation and quantitation of these compounds can then be achieved using high performance liquid chromatography. According to this method, normal individuals and patients with classical PKU have similar neopterin and biopterin ratio in the urine which ranges between 0.2 to 2.0 in most cases. Patients with a biopterin synthetase defect have very high neopterin and extremely low biopterin levels, resulting in a very high N/B ratio while patients with dihydropteridine reductase deficiency have a low neopterin and very high biopterin peak resulting in a low N/B ratio.\textsuperscript{16} Variation in these ratios can be followed and problems of enzyme maturation in the newborn can be resolved by follow up analyses.\textsuperscript{13}

**Materials and Methods**

Urine samples from newborns identified as having high blood phenylalanine levels above four mg per dl by the Guthrie test and subsequently confirmed by the fluorometric method were analyzed for pterins according to the method of Fukushima and Nixon.\textsuperscript{5,6} Urine has to be freshly collected and immediately acidified with HCL then oxidized with one percent I\textsubscript{2} in two percent KI. The oxidized urine is then passed on Dowex 50 (H\textsuperscript{+}) column and pterins eluted with 1.0 N NH\textsubscript{4}OH. The eluate is then applied to Dowex 1 (OH\textsuperscript{-}) and the oxidized pterins eluted from the column with 1N acetic acid.

The oxidized pterins are fractionated on a high performance liquid chromatography system using the Waters 6000A solvent delivery system, Model 420 fluorescence detector and Model U 6K Injector.\textsuperscript{*} An isocratic solvent of five percent methanol in water is used. Separation of pterins involved the use of a Bondapak C\textsubscript{18} column using the Radial Compression System (RCM-100). Oxidized pterins\textsuperscript{†} were detected by a continuous flow fluorometer.

\* Waters Associates Inc., Milford, MA.

\† Standard neopterin and biopterin were purchased from B. Schricks Laboratories, Wettswilla, Switzerland.
TABLE I

<table>
<thead>
<tr>
<th>Neopterin (N)</th>
<th>Biopterin (B)</th>
</tr>
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<tbody>
<tr>
<td>Age 2-4 wks.</td>
<td></td>
</tr>
<tr>
<td>PKU (n = 28)</td>
<td>7,377±2,601</td>
</tr>
<tr>
<td>Normal controls</td>
<td>5,078±2,170</td>
</tr>
<tr>
<td>Age 6 mos. to 16 yrs.</td>
<td>3,435±1,374</td>
</tr>
<tr>
<td>PKU (n = 23)</td>
<td>4,872±1,860</td>
</tr>
<tr>
<td>Normal controls</td>
<td>592± 310</td>
</tr>
</tbody>
</table>

Results

There is a wide variation in the quantities of oxidized pterins in the urine of newborns and in table I are presented the neopterin and biopterin levels of 28 newborns with PKU as compared with six normal newborns. Despite the variation in the quantities of these compounds, neopterin to biopterin ratio (N/B) is similar in the normal and the PKU newborns and usually does not exceed 2.0 and is not below 0.2. Similarly the N/B ratio of older children with classical PKU and normal control falls in the same range. In one newborn with a high blood phenylalanine level of 31 mg per dl, an initial screen of urinary pterins indicated an elevated N/B ratio of 7.8. As seen in table II, the abnormally high N/B ratio returned to normal levels at age 40 days.

At 30 days of age, the baby was given a tetrahydrobiopterin load. Blood phenylalanine level was brought to 18 mg per dl, then an oral dose of L-tetrahydrobiopterin (2 mg per Kg) was given. There was no appreciable change in blood phenylalanine. An additional load of phenylalanine, 25 mg per Kg was given orally at 3.5 hours. The levels of blood phenylalanine and tyrosine are shown in table III. These results indicate no profound defect in the cofactor for phenylalanine hydroxylase, since blood phenylalanine did not drop sharply.

In all these experiments, the neopterin and biopterin peaks fractionated by high performance chromatography were compared to authentic neopterin and biopterin.

Discussion and Conclusions

The determination of urinary pterins, neopterin and biopterin, has been important in the differential diagnosis of the hyperphenylalaninemias. In patients with classical PKU, the urinary neopterin and biopterin and N/B ratios are similar to normal individuals of the same age. In the absence of dihydropteridine reductase activity, the urinary pterins in their oxidized form give an extremely high level of biopterin fraction resulting in N/B ratio below 0.1. When such results are obtained, then further confirmation may require the direct enzymic measurement of dihydropteridine reductase on cultured skin fibroblasts or white blood cells and platelets pellet.

The biosynthetic steps leading to tetrahydrobiopterin are conveniently termed as “biopterin synthetase.” A block in any of these steps leads to very low levels of biopterin and high neopterin level. In two such cases, one infant had N/B ratio of 8.0 and the other 207.3. When such cases are encountered, an oral load with tetrahydrobiopterin will be required in order to confirm the diagnosis of biopterin synthetase deficiency.

The finding of a case with classical PKU and transient biopterin synthetase defi-
iciency, as indicated by N/B ratio of 7.8 at 16 days of age, adds a new dimension to the need for careful follow-up of such patients. Such follow-up should include serial urinary pterin determinations and trial dose of tetrahydrobiopterin.

The estimation of urinary pterins should be a part of the routine work-up of all patients with elevated blood phenylalanine levels. High performance liquid chromatography methods for pterin estimations have proven to be most reliable for such purposes. However, interpretation of results require careful follow-up in order to take into account enzyme maturation processes in the biosynthesis of tetrahydrobiopterin.

References


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**TABLE III**

Blood Phenylalanine and Tyrosine Following Oral Load with Tetrahydrobiopterin

<table>
<thead>
<tr>
<th>Time Minutes</th>
<th>Phenylalanine mg/dl</th>
<th>Tyrosine mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>0'</td>
<td>18.0</td>
<td>1.0</td>
</tr>
<tr>
<td>90'</td>
<td>19.1</td>
<td>1.2</td>
</tr>
<tr>
<td>210</td>
<td>17.4</td>
<td>1.1</td>
</tr>
<tr>
<td>*</td>
<td>18.5</td>
<td>1.0</td>
</tr>
<tr>
<td>240</td>
<td>21.3</td>
<td>0.9</td>
</tr>
<tr>
<td>360</td>
<td>21.6</td>
<td>1.0</td>
</tr>
<tr>
<td>420</td>
<td>20.9</td>
<td>1.1</td>
</tr>
</tbody>
</table>

*A load of 25 mg/kg of phenylalanine given orally.*