Alternative Therapies for PKU

Norma Spécola, MD¹ and Ana Chiesa, MD²

Abstract
The phenylalanine (PHE)-restricted diet has improved in quality and diversity over time and has proven to be effective in all patients. Nevertheless, this treatment imposes a heavy social and economic burden to patient and family and impacts quality of life. Sustained adherence to PHE restriction is difficult to maintain. Moreover, even patients with phenylketonuria (PKU) with normal intelligence quotient (IQ) have lower IQ than matched individuals without PKU and can have deficits in multiple other aspects of neuropsychological function, including cognitive and executive function, working memory. They can also have behavior problems, depression, and low self-esteem. In recent years, alternative treatments for PKU have been developed and their use has been indicated for some patients who are candidates for options besides traditional treatment. Sapropterindihydrochloride, large neutral amino acids, and glycomacropeptide are alternative treatment options in use for selected patients. The aim of this article is to review the current knowledge of these new approaches to PKU treatment.

Keywords
phenylketonuria, new treatment, adherence, glycomacropeptide, BH4, PAL

Introduction
Phenylketonuria (PKU; OMIM 261600) is a metabolic disorder caused by an inherited deficiency of hepatic enzyme phenylalanine hydroxylase (PAH; EC 1.14.16.1), that converts phenylalanine (PHE) to tyrosine, leading to an accumulation of PHE and, if untreated, subsequent neurocognitive dysfunction.¹ Early detection by newborn screening can prevent intellectual disability if a PHE-restricted diet is started soon after birth. A PHE-restricted diet has been applied in patients with PKU for decades and during that time quality and diversity of the diet have improved.

However, patients with PKU, even with normal intelligence quotient (IQ) scores have lower IQ than matched individuals without PKU and can have deficits in multiple additional aspects of neuropsychological function, including cognitive and executive function, working memory as well as behavior problems, depression, and low self-esteem.²⁻⁶ The PKU diet imposes a heavy social and economic burden for patients and their families and impacts quality of life. For all these reasons, sustained adherence to a PHE-restricted diet is difficult to maintain, decreasing progressively as age increases.⁷

After the successes of newborn screening and PHE-restricted diet for preventing severe mental disability but taking into consideration the aforementioned limitation, new therapeutic options have been developed and others are under evaluation to improve neuropsychological outcome, adherence to treatment, and quality of life for patients with PKU.

Sapropterindihydrochloride, large neutral amino acid (LNAA) mixtures, and glycomacropeptide (GMP) are options currently available for selected patients and use of pegylated phenylalanine ammonia-lyase (PAL) is under investigation.

The aim of this article is to review the current knowledge of these new approaches to PKU treatment. This article also summarizes the different treatment approaches in selected Latin American countries.

Tetrahydrobiopterin Therapy
Tetrahydrobiopterin (BH4) is a natural cofactor of PAH. Starting in the late 1970s, synthetic biopterin compounds were made

¹ Unidad de Metabolismo, Hospital de Niños de La Plata, Buenos Aires, Argentina
² División de Endocrinología. Hospital de Niños R, FEI, Gutiérrez, Buenos Aires, Argentina

Received June 17, 2016, and in revised form October 31, 2016. Accepted for publication October 31, 2016.

Corresponding Author:
Norma Spécola, Unidad de Metabolismo, Hospital de Niños de La Plata, calle 14 N 1730, La Plata, Buenos Aires 1900, Argentina.
Email: normaspeca@gmail.com
available for the treatment of atypical PKU caused by deficit in BH4 synthesis and recycling. In 1999, Kure reported that oral administration of BH4 to patients with mild PKU, by PAH deficiency, produced a significant reduction in blood PHE level without a change in PHE intake.8

Sapropterindihydrochloride is a synthetic version of the naturally occurring pterin cofactor, BH4 (Kuvan; BioMarin Pharmaceutical Inc, Novato, California). It was approved in 2007 by the Food and Drug Administration and in 2008 by the European Medicines Agency (EMA) as a drug for PKU patients older than 4 years without BH4 metabolism defects.9,10 Later, in 2015, the EMA approved the extension of Kuvan use for all ages in responsive patients with PKU.

The molecular mechanism of BH4 responsiveness is related to its action as a chemical chaperon stabilizing mutant PAH monomers. Some specific PAH mutations are known to affect the affinity of the PAH enzyme for its biotinoprotein cofactor and others give rise to unstable and misfolded proteins. In both cases, high doses of BH4 can improve PAH activity.10 Tetrahydrobiopterin responsiveness occurs only in patients who carry at least 1 mutant PAH allele, yielding some enzyme activity. The PAH genotype is a tool to predict BH4 responsiveness in a given patient, but it has limitations related to the diversity of mutations, their combination, and negative intraallelic complementation.11 In addition, in many Latin American countries, genetic studies are difficult to obtain.

Response to sapropterin varies in patients with PKU according to their clinical and molecular form. Approximately 80% of patients with non-PKU hyperphenylalaninemia, 50% of patients with mild and moderate PKU, and in about 10% of patients with classical PKU respond to this treatment.12 The response is dose related and is sustained over time. Doses between 5 and 20 mg/kg/d have no significant adverse effects. Mild side effects include gastrointestinal disorders, cough, and headache.13

Clinical evaluation of sapropterin responsiveness in patient with PKU needs to be assessed by either a short- or prolonged-loading test; the most widely accepted positive response is defined as at least of 30% reduction in basal blood PHE level at BH4 loading. Recently other parameters, such as patient behavior and increased PHE tolerance, have been considered as response parameters.14

Ideally, all patients with PKU should be tested with sapropterin before starting treatment. Three principal types of tests have been developed and standardized:

- An 8- to 24-hour BH4 overload with 20 mg/kg/d has been primarily used in newborns who screen positive for PKU to detect early BH4 deficiencies. In addition, this test may detect BH4-responsive patients with PKU. The test is performed at first newborn appointment after basal PHE, tyrosine, and biotinoprotein blood samples have been taken. This is a practical approach because, during the newborn period, the PHE levels are high and patients are on a normal diet. The PHE concentration must be determined at 4, 8, and 24 hours after the oral BH4 load. The test is only valuable if positive; negative results do not exclude BH4 responsiveness.15,16
- The 48-hour BH4 overload with 20 mg/kg/d is usually used in Europe to test BH4 responsiveness in PKU children older than 4 years, previously under dietary treatment with PHE, with blood levels within therapeutic range (2-6 mg/dL or 120-360 μmol/L). To obtain an optimal BH4 action on PHA, the basal PHE blood concentrations should be ≥8 mg/dL or 480 μmol/L, which is obtained after an increase in natural protein intake (eg, adding milk powder), for the purpose of the test only. Once the PHE level is stabilized, oral BH4 overload is administered. Samples of blood PHE must be obtained during the 2 days of BH4 administration at T0 (just before BH4) and after 8, 16, and 24 hour, each day. Although useful in identifying responders, a partial result (between 20% and 30%) does not exclude late responders.12
- Long BH4 tests are mainly used in the United States for patients with PKU on diet after the neonatal period. Sapropterin responsiveness is commonly determined by obtaining a baseline blood PHE level on the day when medication is started (baseline) and to prescribe a sapropterin single daily dose of 20 mg/kg. Blood PHE levels are obtained at regular intervals, usually at 24 hours, and then once a week for 1 month. For patients with higher blood PHE levels (6-10 mg/dL or 360-600 μmol/L) and who consume a stable diet, a significant and rapid decline in blood PHE is expected in responders but occasionally a delay of 2 to 4 weeks is observed. In patients with a baseline PHE level lower than 3 mg/dL (180 μmol/L), responsiveness must be determined by adding additional PHE to the diet.17 A low-PHE diet must be maintained throughout the test. Once BH4 responsiveness has been determined, treatment is initiated with 10 mg/kg/d and the PHE intake is increased progressively. Kuvan (BioMarin Pharmaceutical Inc) is available in 100 mg tablets and doses are taken once a day with food.

For less responsive patients, BH4 dose is adapted between 10 and 20 mg/kg/d. In either case, diet must be adapted to cover all nutrients adequately. For patients with high response (>40%), diet and amino acid formula supply can be reduced and even stopped. But for lesser responses, a milder PHE-restricted diet must be continued with the addition of amino acid formula17,18 or GMP as a protein source.

Patients should maintain regular PHE level and nutritional supervision and PHE tolerance should be reassessed when there are changes in body mass or lifestyle.17

Indications of use. Sapropterin is indicated for BH4-responsive patients with PKU at any age, including during pregnancy.19 Published documented experience related to the safety of starting treatment in infancy is increasing every day.17,20 In cases of newborn or infancy, the introduction of a small amount of
Large Neutral Amino Acids Treatment

The brain pathology of PKU is related to imbalance of LNAAs (tyrosine, phenylalanine, tryptophan, methionine, isoleucine, threonine, valine, leucine, and histidine) within the brain caused by the competition between PHE with LNAAs with the transporter 1 (LAT1) for access to the blood–brain barrier, which is the predominant transport system for all LNAAs. Transporter LAT1 is normally >95% saturated and has a high affinity for PHE. High blood PHE level increases brain PHE influx while decreasing transport of other LNAAs. High PHE concentration is neurotoxic and low availability of non-PHE LNAAs affects protein and monoaminergic neurotransmitter (dopamine and serotonin) synthesis.21

The role of LNAAs in brain amino acids transport was studied from 1950 but was not proposed for use as a therapy until 1994, when the John F. Kennedy Institute in Denmark decided to use it for adult patients with PKU noncompliant to the PHE-restricted diet.22,23 The formula used was PreKunil (Nilab, Denmark) comprising L-forms of tyrosine, tryptophan, methionine, isoleucine, threonine, valine, leucine, histidine, and arginine.

After 2006, a new formulation (NeoPhePrekula, Denmark) included lysine and resulted in a reduction in plasma PHE concentration as a consequence of the competition for absorption at the gastrointestinal level.23,24

Supplementation with LNAAs causes:

- Decreased central nervous system (CNS) concentration of PHE,
- increased CNS concentration of tyrosine and tryptophan,
- improved CNS protein synthesis by reestablishing balance of brain amino acids, and
- lower PHE gut absorption with decreased blood PHE level.

Indications

- adults and adolescents with low adherence to low-PHE diet and
- late diagnosed patients with PKU.

During LNAAs treatment, the blood PHE levels are higher than those recommended for pregnancy. Therefore, to avoid a teratogenic effect, LNAAs therapy is contraindicated for reproductive-aged women and those who are pregnant. Additionally, this therapy is not recommended during childhood because the effect of elevated blood PHE level on growth is not yet completely understood.

The LNAAs must be accompanied with diet, ensuring that all necessary nutrients are covered. Adult recommendation of protein intake is 1 g/kg of ideal body weight, thus 25% to 30% of protein must be supplied by LNAAs and 70% to 75% by natural food proteins. Tablets contain 0.5 g of LNAA and initial indication is 0.5 g/kg/d. The daily amount of powder or tablets for a given patient is divided and taken with main meals 3 or 4 times a day. If the planned diet does not cover the daily requirements, PHE-free amino acid mixture or low-protein food should be prescribed.25

The LNAAs therapy has some pitfalls as PHE level loses its value as a control tool, even though the PHE blood level must be maintained lower than 20 mg/dL (1200 μmol/L). Patients need continuous dietary supervision to avoid inadequate protein intake; some patients present aversion to proteins of high biological value, while others are prone to higher intake.

Glycomacropeptide

Glycomacropeptide (GMP) is a 64-amino acid glycoprophosphopeptide derived from cheese whey and naturally low in PHE (less than 2 mg PHE per protein gram).

It has been purified and manufactured for use with patients with PKU together with other treatment alternatives required by a PHE-restricted diet.

The product is rich in valine, isoleucine, and threonine but has a very low amount of tyrosine, tryptophan, arginine, cysteine, and histidine; thus, supplementation with those essential amino acids is necessary to provide an intact protein with high biological value.26,27

Studies in PKU mice show that GMP feeding reduces metabolic activity and attenuates immune responses due to high PHE levels. The murine model receiving GMP, lowered PHE levels in blood and brain, and showed increased lean mass and improved bone strength.28

In patients with PKU, GMP has shown better palatability than the usual amino acid formula. It reduces ureagenesis, improves protein retention and PHE utilization, and provides more satiety.29

In view of these observations, GMP seems to be a valid alternative in conjunction with other treatment for patients with PKU who need PHE restriction, extending the variety of foods and allowing better adherence, especially in adolescence when compliance fails.30,31 Nevertheless, data on long-term consumption of GMP and its effects on immune response are not yet available and need further evaluation.

Phenylalanine ammonia-lyase

Phenylalanine ammonia-lyase (PAL) is a monomeric enzyme that requires no cofactors. It is present in plants and yeast and is an alternative for obtaining carbon and nitrogen from 1-phenylalanine.32 It converts excess PHE into transcinnamic acid and negligible amounts of ammonia. Transcinnamic acid is rapidly converted into hippuric acid and excreted in urine.

Enzyme substitution therapy using PAL has also been suggested as a possible therapeutic approach for PKU.

Evidence of PHE level reduction with the administration of PAL either in enteric-coated gelatin capsules or as injection in
In Latin America, other aspects to be considered include the phenotype, genotype, age, family, and lifestyle. Nevertheless, the accurate prescription of new therapies for patients with PKU, either alone or combined with diet or other drugs, could allow a more individualized treatment for each patient with PKU, which takes into account phenotype, genotype, age, family, and lifestyle.

**Declaration of Conflicting Interests**

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

**Funding**

The author(s) received no financial support for the research, authorship, and/or publication of this article.

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