Original Article

Alternative Therapies for PKU

Journal of Inborn Errors of Metabolism & Screening
2017, Volume 5: 1–5

DOI: 10.1177/2326409816685734
journals.sagepub.com/home/iem
SAGE

Norma Spécola, MD[1] and Ana Chiesa, MD[2]

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: normaspecola@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 biopterin 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.

Responsiveness 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 biopterin 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 $\geq$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 formula[17,18] 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

amino acid formula increases the likelihood of its acceptance later in life if needed.[20]

Large Neutral Amino Acids Treatment

The brain pathology of PKU is related to imbalance of LNAAs (tyrosine, phenilalanine, 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 (NeoPhePrekulab, 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, LNAA 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 LNAA 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 LNAA 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 glycophosphopeptide 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 L-phenylalanine.[32] It converts excess PHE into transcinamic acid and negligible amounts of ammonia. Transcinamic 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

the PKU mouse model encouraged efforts to adjust treatment for human application.[33]

Experimental studies observed that repeated intravenous (iv) administration of PAL diminished its half-life significantly. Unfortunately, immune response was elicited with iv delivery and protein gastric degradation affected orally administered PAL. Polyethylene glycol (PEG) linked to PAL's lateral lysine chains helped to reduce immunogenic response and lower brain PHE levels in PKU mouse models. The PAL-PEG conjugated products were able to revert hypopigmentation, reduce mortality, and increase weight gain in these animals.[34]

Regarding oral administration, neither encapsulation of the enzyme nor its delivery with yeast was able to augment enzyme bioavailability.[35,36]

Recently, an alternative administration of PAL, the infusion of red blood cells loaded with rAv-PAL enzyme, was tested in ENU2 PKU mouse. A persistent reduction in blood PHE levels that was not affected by the generation of antidrug antibodies was observed.[37]

In humans, phase I studies demonstrated that subcutaneous administration of rAvPAL-PEG in a single dose of 0.1 mg/kg was safe and well tolerated in adult patients with PKU and led to mean reductions of 54% in blood PHE levels. The PHE levels became stable after 6 days of administration but return to baseline by 21 days. All patients developed antibodies.[38]

In phase II studies, patients with a stable diet were treated for at least 1 year, and blood PHE levels reduced by an average of 68% from baseline.

In both phases, adverse events, present in almost all patients, were injection site or disseminated skin reactions, dizziness, and joint pains. In long-term treatment, injection site and hypersensitivity reaction rates decreased. There was no laboratory evidence of liver or kidney injury.

Phase III studies to evaluate the efficacy and safety of self-administered injections of rAv-PAL-PEG for adults with PKU were recently initiated.[39]

Conclusion

In recent years, alternative treatments for PKU have been developed and their use has been suggested for some patients who become candidates for options other than traditional treatments.

Novel therapies (BH4 and LNAA) provide more physiopathologic insight to neuropsychological symptoms and it is possible that the use will improve outcomes. Nevertheless, a PHE-restricted diet has proven to be efficient in all patients and remains the preferred approach for most patients. In this regard, GMP as a source of low PHE natural protein will be extremely useful.

On the other hand, studies indicate that the diet is difficult to follow and that compliance decreases with age.

In Latin America, other aspects to be consider include the availability of new drugs and costs, as new treatments are even more expensive than traditional treatment. Public health efforts to supply treatment for all patients may be a burden.

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.

References

1. Scriver SC, Kaufman S. Hyperphenylalaninemia: phenylalanine hydroxylase deficiency. In Scriver SC, Beaudet AL, Valle D, Vogelstein B, Sly WS, eds. *The Metabolic and Molecular Basis of Inherited Disease*. New York, NY: McGraw-Hill; 2001.
2. Enns G, Koch R, Brumm V, Blakely E, Suter R, Jurecki E. Suboptimal outcome in patients with PKU treated early with diet alone. *Mol Genet Metab*. 2010;101(2-3):99-101.
3. Feillet F, van Spronsen FJ, MacDonald A, et al. Challenges and pitfalls in the management of phenylketonuria. *Pediatrics*. 2010; 126(2):333-341.
4. Aharing K, Belanger-Quintana A, Dokoupil K, et al. Blood phenylalanine control in phenylketonuria: a survey of 10 European centres. *Eur J Clin Nutr*. 2011;65(2):275-278.
5. Gonzalez MJ, Gutierrez AP, Gassio R, Fusté ME, Vilaseca MA, Campistol J. Neurological complications and behavioral problems in patients with phenylketonuria in a follow-up unit. *Mol Genet Metab*. 2011;(suppl 104):S73-S79.
6. Gentile JK, Ten Hoedt AE, Bosh A. Psychosocial aspects of PKU: hidden disabilities – a review. *Mol Genet Metab*. 2010;99(suppl): S64-S67.
7. MacDonald A, Gokmen-Ozel H, van Rijn M, Burgard P. The reality of dietary compliance in the management of phenylketonuria. *J Inher Metab Dis*. 2010;33(6):665-670.
8. Kure S, Hou DC, Ohura T, et al. Tetrahydrobiopterin-responsive phenylalanine hydroxylase deficiency. *J Pediatr*. 1999;135(3): 375-378.
9. van Spronsen FJ. Future treatment strategies in phenylketonuria. *Mol Genet Metab*. 2010;(suppl 99):S90-S95.
10. Harding CO. New era in treatment for phenylketonuria: pharmachologic therapy with sapropterindihydrochloride. *Biologics*. 2010;4:231-236.
11. Blau N, Hennermann J, Langenbeck U, Lichter-Konecki U. Diagnosis, classification and genetics of phenylketonuria and tetrahydrobiopterin (BH4) deficiencies. *Mol Genet Metab*. 2011;(suppl 104):S2-S9.
12. Blau N, Belanger-Quintana A, Demirkol M, et al. Optimizing the use of sapropterin (BH4) in the management of phenylketonuria. *Mol Genet Metab*. 2009;96(4):158-163.
13. Burton BK, Nowacka M, Hennermann JB, et al. Safety of extended treatment with sapropterindihydrochloride in patients

with phenylketonuria: results of a phase 3b study. *Mol Genet Metab*. 2011;103(4):315-322.
14. Gordon P, Thomas J, Suter R, Jurecki E. Evolving patient selection and clinical benefit criteria for sapropterindihydrochloride (Kuvan®) treatment of PKU patients. *Mol Genet Metab*. 2012; 105(4):672-676.
15. Feillet F, Chery C, Namour F, et al. Evaluation of neonatal BH4 loading test in neonates screened for hyperphenylalaninemia. *Early Hum Dev*. 2008;84(9):561-567.
16. Specola N, Nunez M, Muschietti L, et al. BH4 loading test for evaluation of neonatal hyperphenylalaninemia *JIEMS*. 2015; (suppl):Abstract P070:42.
17. Vockley J, Andersson HC, Antshel KM, et al; American College of Medical Genetics and Genomics Therapeutics Committee. Phenylalanine hydroxylase deficiency: diagnosis and management guideline. ACMG Practice Guidelines. *Genet Med*. 2014; 16(2):188-202.
18. Scala I, Concolino D, Della Casa R, et al. Long-term follow-up of patients with phenylketonuria treated with tetrahydrobiopterin: a seven years' experience. *Orphanet J Rare Dis*. 2015;10:14-26.
19. Grange DK, Hillman RE, Burton BK, et al; Phenylketonuria Demographics Outcomes and Safety (PKUDOS) Registry; Maternal Phenylketonuria Observational Program (PKU MOMS) Sub-Registry. Sapropterindihydrochloride use in pregnant women with phenylketonuria: an interim report of the PKU MOMS Sub-Registry. *Mol Gent Metab*. 2014;112(1):9-16.
20. Cunningham A, Bausell H, Brown M, et al. Recommendations for the use of sapropterin in phenylketonuria. *Mol Genet Metab*. 2011;106(3):269-275.
21. vanVliet D, Bruinenberg VM, Mazzola PN, et al. Large neutral amino acid supplementation exerts its effect through three synergistic mechanisms: proof of principle in phenylketonuria mice. *PLOS ONE*. 2015;10(12):1-18.
22. Lou HC, Toft PB, Andersen J, et al. Unchanged MRI of myelin in adolescents with PKU supplied with non-PHE essential amino acid after dietary relaxation. *Acta Paediatr*. 1994;83(12):1312-1314.
23. Rocha JC, Martel F. Large neutral amino acids supplementation in phenylketonuric patients. *J Inherit Metab Dis*. 2009;32(3): 472-480.
24. Matalon R, Michals-Matalon K, Bhatia G, et al. Large neutral amino acids in the treatment of phenylketonuria (PKU). *J Inherit Metab Dis*. 2006;29(6):732-738.
25. Ahring KK. Large neutral amino acids in daily practice. *J Inherit Metab Dis*. 2010;33(suppl 3):S187-S190.
26. Ney DM, Gleason ST, van Calcar SC, et al. Nutritional management of PKU with glycomacropeptide from cheese whey. *J Inherit Metab Dis*. 2009;32(1):32-39.
27. Ney D, Blank R, Hansen K. Advances in the nutritional and pharmacological management of phenylketonuria. *Curr Opin Clin Nutr Metab Care*. 2014;17(1):61-68.
28. Solverson P, Murali SG, Litscher SJ, Blank RD, Ney DM. Low bone strength is a manifestation of phenylketonuria in mice and is attenuated by a glycomacropeptide diet. *PLoS One*. 2012;7(9): e45165.
29. van Calcar SC, MacLeod EL, Gleason ST, et al. Improved nutritional management of phenylketonuria by using a diet containing glycomacropeptide compared with amino acids. *Am J Clin Nutr*. 2009;89(4):1068-1077.
30. Lim K, van Calcar SC, Nelson KL, Gleason ST, Ney DM. Acceptable low phenylalanine foods and beverages can be made with glycomacropeptide from cheese whey for individuals with PKU. *Mol Genet Metab*. 2007;92(1-2):176-178.
31. Macleod EL, Ney DM. Nutritional management of phenylketonuria. *Ann Nestle Eng*. 2010;68(2):58-69.
32. Fritz RR, Hodgins DS, Abell CW. Phenylalanine ammonia-lyase. Induction and purification from yeast and clearance in mammals. *J Biol Chem*. 1976;251(15):4646-4650.
33. Sarkissian CN, Shao Z, Blain F, et al. A different approach to treatment of phenylketonuria: phenylalanine degradation with recombinant phenylalanine ammonia lyase. *Proc Natl Acad Sci U S A*. 1999;96(5):2339-2344.
34. Ikeda K, Schiltz E, Fujii T, et al. Phenylalanine ammonia-lyase modified with polyethylene glycol: potential therapeutic agent for phenylketonuria. *Amino Acids*. 2005;29(3):283-287.
35. Sarkissian CN, Kang TS, Gamez A, Scriver CR, Stevens RC. Evaluation of orally administered PEGylated phenylalanine ammonia lyase in mice for the treatment of phenylketonuria. *Mol Genet Metab*. 2011;104(3):249-254.
36. Kang TS, Wang L, Sarkissian CN, Gámez A, Scriver CR, Stevens RC. Converting an injectable therapeutic protein into an oral form: phenylalanine ammonia lyase for phenylketonuria. *Mol Gent Metab*. 2010;99(1):4-9.
37. Yew NS, Dufour E, Przybylska M, et al. Erythrocytes encapsulated with phenylalanine hydroxylase exhibit improved pharmacokinetics and lowered plasma phenylalanine levels in normal mice. *Mol Genet Metab*. 2013;109(4):339-344.
38. Longo N, Harding CO, Burton BK, et al. Single-dose, subcutaneous recombinant phenylalanine ammonia lyase conjugated with polyethylene glycol in adult patients with phenylketonuria: an open-label, multicentre, phase 1 dose escalation trial. *Lancet*. 2014;384(9937):37-44.
39. Blau N, Longo N. Alternative therapies to address the unmet medical needs of patients with phenylketonuria. *Editorial Expert Opin Pharmacother*. 2015;6(6):791-800.