New treatment options in PKU and future role of dietary treatment

Michael Staudigl (physician), Katharina Dokoupil (nutritionist), Esther M. Maier (physician, head of department)

Dr. von Hauner Children’s Hospital, Department of Inborn Errors of Metabolism, University of Munich, Munich, Germany

Introduction
Phenylalanine hydroxylase (PAH; EC 1.14.16.1) catalyzes the hydroxylation of the aromatic amino acid phenylalanine to tyrosine. Mutations in the PAH gene lead to the most common inherited disorder of amino acid metabolism in the European-descended population, namely Phenylketonuria (PKU; OMIM 261600) (Zschocke, 2003). PAH deficiency leads to an elevation of phenylalanine concentrations in blood resulting in severely impaired cognitive and motor development if untreated. The implementation of newborn screening in the 1960s has helped to identify newborns with PKU within the first days of life and allowed early initiation of treatment to prevent these deleterious effects. For over 60 years, the mainstay of PKU treatment has been a phenylalanine-restricted diet. Yet, with ongoing research, new therapeutic options have emerged. We provide a short overview of current and future treatment options for PKU patients and the future role of dietary treatment.

Current Treatment Options
Phenylalanine-restricted diet. Until today, a phenylalanine-restricted diet remains the main treatment option for PKU patients. Yet, following a strict diet is burdensome and may hamper compliance. In addition, the nutritional management of PKU has become more complex in order to optimize patients’ growth, development and diet compliance (Feillet & Agostoni, 2010). Therefore, companies specialized in medicinal foods have made great efforts to develop new products and improve existing products to comply with today’s needs. As these products have become more palatable, therapeutic compliance has increased but regular monitoring of blood phenylalanine levels and assessment of nutrient intake and biochemical markers of nutrition remain. Additionally, repeated dietary education aiming to avoid protein malnutrition and other nutrient deficiencies is continuously needed.
Large neutral amino-acids (LNAAs). LNAAs inhibit the transport of phenylalanine across the gastrointestinal and the blood brain barrier by competing with the same transporter (Matalon et al.,
2006). Unfortunately, the effects of LNAAs have been assessed only for a short time and in a limited number of patients using variable dosages and different formulations (Blau, Hennermann, Langenbeck, & Lichter-Konecki, 2011). Evidence to support the efficacy of LNAA supplementation in significantly reducing blood phenylalanine levels in PKU patients is therefore still limited and further research is needed (Strisciuglio & Concolino, 2014).

Pharmacological chaperone treatment. In 1999, Kure et al. discovered that pharmacological doses of 6R-L-erythro-5,6,7,8-tetrahydrobiopterin (BH₄), the natural cofactor of PAH, lead to a reduction in phenylalanine concentrations of patients with PAH deficiency (Kure et al., 1999). Further studies showed, that BH₄ reduces blood phenylalanine concentrations and increases enzyme activity and phenylalanine tolerance (Muntau et al., 2002). These findings led to the description of a new clinical phenotype, namely BH₄-responsive PAH deficiency. Recent studies have shown, that PKU is a protein misfolding disease with loss-of-function (Gersting et al., 2007), and BH₄ acts as a pharmacological chaperone stabilizing misfolded proteins (Gersting et al., 2010) (Lagler et al., 2010). In 2008, Sapropterin-dihydrochloride (KUVAN®) was approved for the treatment of BH₄-responsive PAH deficiency in Europe. As PKU is an inborn error of metabolism with high genetic variability, allelic heterogeneity and frequent compound-heterozygosity, prediction of the clinical phenotype leading to individualized treatment decisions is difficult (Danecka et al., 2015). Current studies aim to understand the functional mechanisms underlying the loss-of-function phenotype by analysis of genotype-specific activity landscapes which allow insights into the interplay between the genotype, the metabolic state and pharmacological treatment with BH₄ (Danecka et al., 2015; Staudigl et al., 2011).

Future Treatment Options
In the past years, new alternatives to current treatment strategies have evolved. Glycomacropeptide, enzyme substitution, gene therapy, and hepatocyte transplantation seem to be promising approaches undergoing continuous research.

Glycomacropeptide (GMP). GMP occurs naturally in bovine milk within the whey fraction during cheese production (Ney, Blank, & Hansen, 2014). It represents a new dietary treatment option for PKU patients as pure GMP contains no aromatic amino acids, including phenylalanine (Etzel, 2004). In addition, GMP contains up to three times the amount of LNAAs as compared with other dietary proteins (van Calcar & Ney, 2012). In order to provide a complete source of protein, GMP must be supplemented with essential amino acids (van Calcar & Ney, 2012). Studies in PKU mice showed,
that GMP is suitable to support normal growth and body composition (Solverson et al., 2012). Until today, an increasing number of foods and beverages have been developed using GMP as a protein source with reduced phenylalanine content (van Calcar & Ney, 2012). Currently, a phase 2 study will evaluate the metabolic and nutritional features of a GMP diet in comparison to an amino acid diet in PKU (www.clinicaltrials.gov; NCT01428258).

**Enzyme substitution.** Phenylalanine ammonia lyase (PAL) is a non-mammalian protein found in plants, yeast, and bacteria. PAL converts phenylalanine to trans-cinnamic acid, a harmless organic acid being rapidly excreted in urine, and insignificant levels of ammonia (Sarkissian et al., 2008). Weekly subcutaneous injection of the polyethylene glycol (PEG)-modified recombinant PAL (rAvPAL-PEG) to Pahnu2 mice led to complete and sustained correction of blood phenylalanine levels (Harding & Blau, 2010). These results led to the initiation of clinical trials of injectable rAvPAL-PEG. Currently, patients are being recruited for an open-label Phase 3 study to evaluate safety and tolerability over a time frame of 36 weeks (www.clinicaltrials.gov). In addition, experiments have been performed with corn-root-derived PAL for a potential oral therapeutic use (López-Villalobos et al., 2014).

**Gene therapy.** PKU has been a target disease for gene therapy for many years (Fang et al., 1994). Different studies have used Adeno-associated hepatocyte-directed viral vectors to correct blood phenylalanine concentrations. Yet, these vectors do not achieve a permanent metabolic correction as continuous hepatocyte regeneration eventually eliminates the vector genomes, thus leading to a loss of PAH expression (Harding & Blau, 2010). Reinjection is ineffective due to antibody-mediated destruction of the vector (Harding & Blau, 2010). More promising results have been demonstrated by intramuscular injection in PKU mice (Ding et al., 2008). Until today, research is continuing, improving hepatocyte- or muscle-directed gene therapy for PAH deficiency.

**Hepatocyte transplantation.** In PKU, liver organ transplantation is not an option. Therefore, repopulation of the liver with wild-type hepatocytes or stem cells has been explored as a potential therapy in both animal models and humans (Harding & Gibson, 2010). Transplantation of wild-type hepatocytes in PKU mice demonstrated a complete correction of blood phenylalanine concentrations in animals with at least 10% liver repopulation (Hamman et al., 2005). As in gene therapy, the major limitation to hepatocyte transplantation is the lack of a selective growth advantage of PAH-expressing cells over native PAH-deficient hepatocytes (Harding & Blau, 2010)(Laconi & Laconi, 2002).

**Conclusion**
In the past years, promising new therapeutic options for PKU patients have evolved i.e. GMP, enzyme substitution, gene therapy, and hepatocyte transplantation. However, further research and clinical studies are needed to determine the safety and the efficacy of these developing treatment strategies. Until then, the available treatment options for patients with PKU consist of phenylalanine-restricted diet and treatment with KUVAN®. The approval of KUVAN® for BH₄-responsive PAH deficiency has led to an increased quality of life for an increasing number of patients. Yet, these patients still show alterations with respect to a healthy, well balanced diet. They developed special eating habits and preferences in their lifetime leading to deficits in vitamin and mineral intake (Lambruschini et al., 2005). Thus, continuous nutritional follow-up with education, monitoring, and supplementation is needed. For many patients, current and future treatment options might not put an end to dietary treatment but only alleviate its requirements. Therefore, the development of new and improvement of existing phenylalanine-free amino acid supplements and low protein products is mandatory. Irrespective of the preferred treatment, PKU remains a lifelong condition requiring medical care and attention with continuing research in different fields to provide individualized therapeutic strategies for our PKU patients.
References


