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Future treatment strategies in phenylketonuria ☆

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ABSTRACT

Phenylketonuria (PKU) was the first inherited metabolic disease in which treatment was found to prevent clinical features of the disorder; dietary management was established almost 60 years ago. The institution of a low-phenylalanine (Phe) diet in the first few weeks of life was made possible by Guthrie neonatal screening, which further increased effectiveness of therapy. Indeed, neonatal diagnosis of PKU followed by institution of a low-Phe diet has been a remarkable success in preventing the devastating brain damage associated with untreated PKU. Nevertheless, significant difficulties exist in caring for PKU patients, including problems with adhering to the prescribed dietary regimen and the presence of neurocognitive deficits despite therapy. During the past few years, several ideas for new treatment strategies have emerged. This review aims to address these treatment strategies based on theoretical considerations of the biochemistry and pathogenesis of PKU. Recent times have seen the introduction of a wide array of novel treatments currently in clinical use, including more palatable medical foods, glycomacropeptide, large neutral amino acids, and tetrahydrobiopterin. Human trials are underway using an enzymatic therapeutic approach, while preclinical work continues in the fields of gene and cellular therapy. These therapeutic strategies propose to treat PKU at various levels, including nutritional intake, gut, liver, and blood–brain barrier, and have the potential to further improve outcome in PKU.

Introduction

Phenylketonuria (McKusick 261600, PKU) is one of the best-known examples of inherited metabolic diseases. It was the first disorder in which a toxic agent, phenylalanine (Phe), was identified to cause severe mental retardation. It was also the first known defect in amino acid metabolism that involved the discovery of Phe-to-tyrosine metabolism and the primary enzyme deficiency of hepatic phenylalanine hydroxylase (PAH; EC1.14.16.1). More importantly, PKU was the first inherited metabolic disease in which treatment was found to prevent clinical features of the disorder. The dietary management of PKU was established almost 60 years ago [1], with the first effects of treatment published in 1953 [2]. Many reviews have emerged since then, with one of the most recent reviews addressing the sizeable collection of knowledge accumulated to date [3].

During the past few years, several ideas for new treatment strategies have emerged. This paper aims to address these treatment strategies based on theoretical considerations of the biochemistry and pathogenesis of PKU. These therapeutic strategies aim to treat PKU at various levels, including nutritional intake, gut, liver, blood–brain barrier (BBB), and other areas in the body (i.e., gene therapy) (Fig. 1).

The successes and challenges of dietary treatment

High levels of Phe are considered toxic, and since Phe is not synthesized by the body, the low-Phe diet was introduced. The effectiveness of treatment was further increased by starting the low-Phe diet in the first few weeks of life. This was made possible by the introduction of Guthrie neonatal screening, which screened for high Phe concentrations in blood taken by heel puncture [4]. In principle, the diet restricts the amount of Phe intake to the amount needed for protein anabolism. In other words, the amount

Abbreviations: PKU, phenylketonuria; Phe, phenylalanine; PAH, phenylalanine hydroxylase; BBB, blood–brain barrier; IQ, intelligence quotient; GMP, glycomacropeptide; PAL, phenylalanine-ammonia lyase; LNAA, large neutral amino acids; BH_4, tetrahydrobiopterin; FDA, Food and Drug Administration; EMEA, European Medicines Agency; rAAV, recombinant adeno-associated virus.

☆ *References to electronic databases:* Phenylketonuria, McKusick 261600. Phenylalanine hydroxylase, EC 1.14.16.1, phenylalanine-ammonia lyase (PAL, EC 4.3.1.5).*Conflict of interest disclosure:* Dr. van Spronsen is a member of the scientific advisory board for phenylketonuria, which is supported by Merck Serono S.A. Geneva, Switzerland. Dr. Enns reports serving on the BioMarin Pharmaceutical Inc. data safety monitoring board for clinical trials involving Kuvan® and PEGylated phenylalanine-ammonia lyase and also is on the BioMarin Pharmaceutical Inc. speaker's bureau for Kuvan®.*Funding statement:* Dr. Enns was supported by the Arline and Pete Harman Scholarship.

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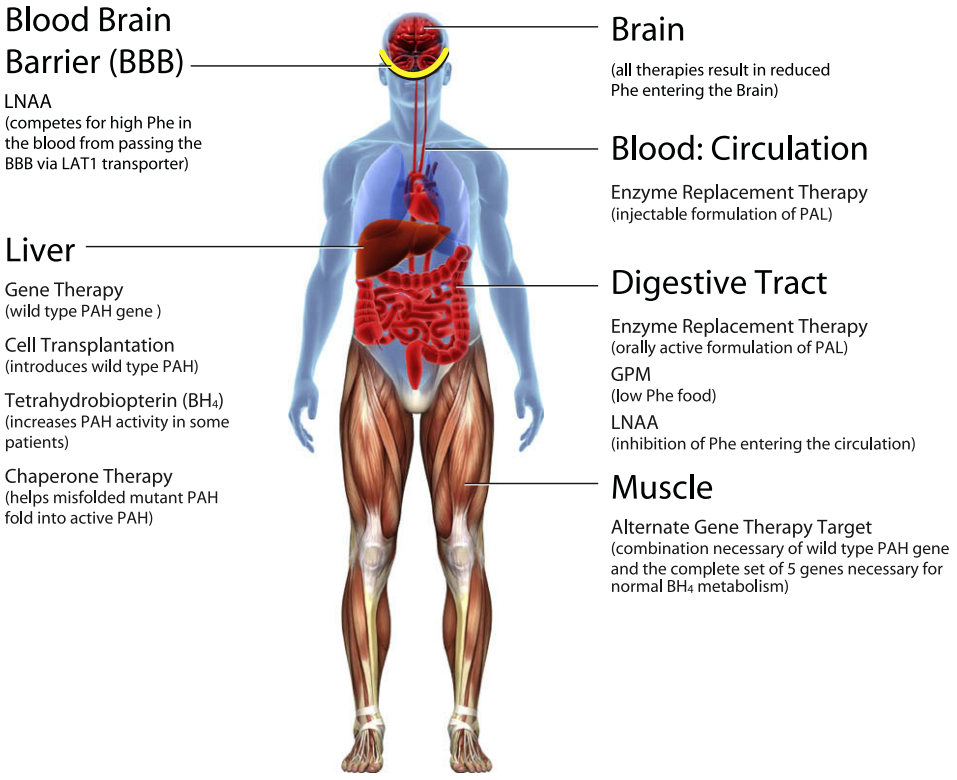

Fig. 1. The figure presents the different treatment options available in practice or in theory, looking at different locations in the body.

of Phe necessary for protein synthesis is provided by normal nutritional protein intake. The amount of Phe normally converted into tyrosine, which can represent up to 90% of Phe intake, is replaced by medical food substitutes. This treatment has proven to result in grossly normal cognition. However, when compared to siblings or healthy age-related control groups, there is always a small but consistent gap in intelligence quotient (IQ) scores [5–7]. In addition, neuropsychometric testing has identified problems particularly in executive functioning at various ages [8–13], although the hypothesized central role that prefrontal lobe damage plays in such findings has been questioned [14]. There is also a risk of regression of neurocognitive function if patients are not treated in adulthood, possibly related to brain white matter abnormalities or impaired synthesis of brain catecholamines [15–17]. Therefore, there is increasing consensus for the need of lifelong dietary treatment. Despite such consensus, dietary management practices are notoriously variable throughout the world. Although Phe restriction is the mainstay of PKU treatment worldwide, significant differences exist in recommended blood Phe levels in both children and adults [18–23].

Apart from the fact that the diet does not result in a completely normal outcome, the diet is not easy to comply with, resulting in a social burden, which may result in various social problems [24]. Additionally, treatment with special medical foods is associated with risks of nutritional deficiencies, especially concerning vitamin B_{12}, vitamin D, calcium, iron, and unsaturated long chain fatty acids [25–27]. Such deficiencies may result in neurological problems and bone density-related issues [28,29]. Therefore, we need to consider additional therapeutic possibilities in the treatment of PKU patients.

At the level of dietary intake

During the past few years, many issues concerning protein substitutes used to treat PKU have been improved, including content, palatability, and practical use of the products. Apart from this, there is now some experience with a protein that is almost free of Phe. This protein, glycomacropeptide (GMP), is derived from goat milk during cheese making and has a better taste than protein substitutes. A drawback of this treatment is that it cannot completely replace the need for supplemental protein substitute because it is not completely free of Phe. In addition, some amino acids must be added because natural GMP does not include other amino acids such as tyrosine and tryptophan [30]. Still, it is a very interesting and palatable product that is a good addition to the PKU treatment arsenal, especially given how difficult it is for many patients to adhere to the low-Phe diet [31,32].

Another advancement that has the potential to improve dietary adherence is the development of a portable Phe monitoring device [33,34]. PKU patients often have to wait days to weeks to obtain the results of a blood Phe test. The ability to monitor blood Phe levels in real time could serve as an important tool to help motivate patients to follow dietary recommendations more closely.

At the gut level

Although the gut does not contain the essential pathways for Phe metabolism, it may help to treat PKU in two different ways. First, the enzyme phenylalanine-ammonia lyase (PAL, EC 4.3.1.5) is active at this site if taken orally in a non-absorbable and protected form [35–37]. To increase the activity and mask PAL from the host immune system, the injectable form of PAL is PEGylated. PEGylation is the process of attachment of polyethylene glycol polymer chains to PAL [38,39]. In its present PEGylated formulation the maximal effect is likely in the vascular space. PAL is an enzyme that metabolizes Phe into the non-toxic compound trans-cinnamic acid and ammonia. The small amount of ammonia released is easily converted into urea. Preclinical studies in murine models of PKU using gavage feeding or intraperitoneal injection of PAL or subcutaneous injection of a PEGylated enzyme preparation (PEG-PAL conjugates)

have been promising, showing lowered Phe levels in both brain tissue and vascular space and reduced manifestations of disease [36,38]. In addition, PEGylation allows for subcutaneous depot injection of PEG-PAL, a form of enzyme delivery that has the potential to be relatively long-acting. Even with such enzyme therapy tyrosine remains an essential amino acid; the amount of required supplementation of this amino acid during PAL treatment is a matter for further study. The first clinical trial of PEG-PAL was started in the USA in 2008 (PAL-001, NCT00634660).

Second, supplementation with large neutral amino acids (LNAA) decreases the blood Phe concentration [40]. This is a secondary effect of LNAA, while the primary effect is to decrease the brain Phe concentration. The concept behind LNAA treatment is that Phe and other LNAA share the same transport system, creating competitive inhibition of the transport of LNAA with each other [40,41]. Therefore, LNAA supplementation counteracts the effects of high Phe exposure to the transport system that delivers LNAA across membranes from gut to blood or from blood to brain [42]. In the gut, Phe and other LNAA compete for transport from the apical to the basolateral membrane [43]. When PKU patients are off dietary therapy, the blood Phe concentration is very high. In such instances, large amounts of LNAA may reduce the influx of Phe to the brain, as was clearly shown by the study of Pietz and coworkers [41]. However, at the gut level, the Phe concentration is more or less normal, so that supplying large amounts of LNAA may not decrease Phe transport to the blood appreciably, although a recent study has shown a significant drop in blood Phe (39%) following treatment of PKU patients with LNAA for one week [44]. Therefore, the effect of LNAA supplementation on blood Phe concentration might be a result of other factors, including stimulating anabolism or potentially improving the competitive effect of LNAA resulting from decreased natural protein intake, rather than directly influencing transport mechanisms [45]. This is in line with the finding that the blood Phe concentration decreases when amino acid supplements are given more frequently in conjunction with non-LNAA [46,47].

In the liver

The essential part of Phe metabolism takes place in the liver. This was conclusively shown to be true after a boy with PKU and unrelated end-stage liver disease underwent liver transplantation; complete correction of the metabolic phenotype followed [48]. Normally, the entire set of enzymes is active in hepatocytes, including PAH as well as the five enzymes involved in the synthesis and recycling of (6*R*)-L-erythro-5,6,7,8-tetrahydrobiopterin (BH_4). In the first years of treatment, although dietary therapy was successful in preserving intelligence in the vast majority of PKU patients, some were found to respond poorly to dietary intervention despite adequate control of their blood Phe levels. These patients were later shown to have BH_4 deficiency due to a defect in either the synthesis or recycling of BH_4 [49,50]. A BH_4 loading test was developed to distinguish the patients with a defect in BH_4 metabolism from patients with deficient PAH activity [51]. The patients with a defect in BH_4 metabolism could also be detected by analysing urine pterins and measuring the activity of one of the enzymes involved with the recycling of BH_4 [50,52]. In 1999, however, two things changed: the effectiveness of BH_4 increased by a change in the formulation, while the dose was also increased from 10 to 20 mg/kg body weight. This resulted in detecting a third group of patients, the so-called BH_4-responsive patients. These patients neither have a defect in BH_4 metabolism nor BH_4 deficiency, but do exhibit an increase of *in vivo* PAH capacity with large doses of BH_4.

Since then, some patients with a deficiency of the PAH enzyme were found to be responsive to BH_4 [53–55]. These patients usually have a milder form of PAH deficiency. While a large number of patients with a mild degree of PAH deficiency are responsive to BH_4, a smaller number of patients with more severe PAH deficiency also clearly show responsiveness [56,57]. The exact proportion of BH_4-responders who have a more severe phenotype is not yet known, due to selection bias associated with initial clinical trials [58,59]. However, even some patients with a mutation in the gene encoding PAH, resulting in a clear lack of enzyme activity, might show some response [60]. Therefore, while it is of importance to make clear to families that many patients will not be responsive, every classic PKU patient should be offered testing [57]. Experience shows that most of the patients responsive to BH_4 still need at least some restriction of natural protein and continued use of low-Phe medical foods. Following the initial demonstration of BH_4 efficacy in mild PKU patients in 1999 [53], 6*R*-BH_4 (sapropterin dihydrochloride, Kuvan®) has been developed from a chemical compound into an FDA and EMEA approved drug (December 2007 and 2008, respectively). The product has been launched in the US and in a growing number of European countries.

At the level of genes and cells

After the detection of the PAH gene by Kwok et al. [61] and the studies of Ledley et al. [62] showing that only about 10% enzyme activity is necessary for normal Phe metabolism in mice, further experimental protocols emerged to treat PKU with gene therapy [61–63]. However, after the unfortunate death of a patient with another inborn error of metabolism (ornithine transcarbamylase deficiency) [64], it became clear that there were important issues to be addressed before a gene therapy strategy could be used widely in PKU patients. Eighteen hours following gene transfer, the subject developed a systemic inflammatory response syndrome with multiple organ system failure, ultimately leading to death [64]. Nevertheless, more recent studies in murine models of PKU are promising. Long-term correction of murine hyperphenylalaninemia associated with no adverse effects has been achieved by the administration of a recombinant adeno-associated virus (rAAV) vector [65]. AAV vectors appear to be less prone to cause an immune reaction that would result in rejection of transfected hepatocytes when compared to the initial vectors used in gene therapy experiments [66]. Such advances in viral vector design, especially related to improvements in AAV technology, have led to human gene therapy trials for other inborn errors of metabolism, including Canavan disease, late-infantile neuronal ceroid lipofuscinosis, α_1-antitrypsin deficiency, and lipoprotein lipase deficiency [67].

Hepatocyte transplantation in PKU is also under investigation, but, in order to be effective, donor cells need to have a selective growth advantage over native hepatocytes [66]. Hepatocyte transplantation has been attempted in a number of preclinical studies using various animal models of inborn errors of metabolism, as well as in humans with metabolic disorders such as urea cycle defects, glycogen storage disorders, α_1-antitrypsin deficiency, and Refsum disease. This cellular approach has not yet been attempted in PKU. Cell-based therapies using stem cells or more differentiated progenitor cells may represent the future of cell transplantation for treatment of metabolic liver disease, and eventually may be a viable option for treating PKU patients [68].

In the muscle

Apart from the reports on liver transfection, there are some innovative studies on muscle as a target for gene therapy [69]. In order to introduce the Phe hydroxylating system into tissue other than the liver, gene delivery must include not only the PAH enzyme but also transgenes that encode the complete enzyme

system necessary to synthesise and recycle BH_4. Despite such a daunting technical challenge, Ding et al. [69] have shown this to be possible in mice. One tremendous advantage of muscle gene therapy is the ease of administration of the therapeutic vectors when compared to liver-directed gene therapy.

At the brain level

Notwithstanding the good results of treatment and the clear relationship between blood Phe concentrations and cerebral outcome, the pathogenesis of brain damage in PKU is still largely not understood [70,71]. The fact that the deleterious effects of PKU are almost completely restricted to the brain, in addition to the fact that the brain, unlike other tissues, is separated from the blood by the BBB, lends support to the hypothesis that the BBB is of central importance to disease pathogenesis.

The studies of Möller et al. [72], Weglage et al. [73], Koch [74], and Moats et al. [75] showed that some patients with untreated classical PKU who consequently have very high blood Phe concentrations do not show evidence of significantly impaired cognitive function. Despite high blood Phe concentrations, such individuals showed almost normal cerebral Phe concentrations [73–75]. These findings again point to the possible importance of the BBB in the pathogenesis of brain disease in PKU. In particular, polymorphisms in the gene encoding the LNAA transport system (the neutral amino acid transporter 1, LAT1) might play a major role in determining susceptibility to brain injury in PKU patients. For the LNAA transport across the BBB, alterations in the 4F2hc/LAT1 complex have been suggested to be a possible explanation for the phenomenon found by Weglage et al. [72,73]. However, no sequence variants that would be expected to result in a structural change of this transporter were detected in 13 PKU patients who had demonstrated a low ratio of brain to blood Phe concentrations [72], so the cause of the discordance between blood and brain Phe levels in some patients is still unknown.

As already mentioned above, LNAA supplementation is an approach that may lower blood Phe levels in some instances, although the major effect appears to be on brain Phe levels [76]. Christensen [77] proposed in 1953 that high blood Phe concentrations could interfere with transport of other LNAA into the brain. In 1976, Andersen et al. [78] demonstrated the potential for LNAA to lower brain Phe levels in a rat model. Although other studies also concentrated on altering brain amino acid and catecholamine composition in PKU through a modified dietary approach [79–81], it was not until the groundbreaking work of Pietz et al. [41] that LNAA supplementation was studied in earnest in PKU patients. Provision of large amounts of LNAA in the diet resulted in clearly decreased brain Phe concentrations and improved neurophysiological function [41]. A recent prospective, double-blind, crossover study using LNAA supplementation showed no correlation between plasma and brain Phe levels. However, LNAA supplementation improved executive functioning, especially in the domains of verbal generativity and cognitive flexibility. The authors concluded that use of LNAA in patients who are compliant with diet therapy is of limited value, but LNAA supplementation may benefit those who are unable to comply with the dietary restrictions necessary to treat PKU [82].

The list of known amino acid transporters continues to grow, so new studies are necessary to identify genes and polymorphisms that have the potential to affect brain Phe levels. Once novel transporter genes are found, our understanding of the phenotypic variability in patients, including which patients may need either less strict treatment or no treatment at all, should increase.

Finally, BH_4 appears to have the potential to cross the BBB, at least at high doses, and hence might directly affect neurotransmitter levels. Peripherally administered BH_4 was found to enter the brain in rats and monkeys [83,84]. When BH_4 or 6-methyltetrahydrobiopterin was given orally along with ascorbate or intravenously to patients with defective BH_4 synthetic pathways at doses of 10–20 mg/kg, substantial amounts were detected in the cerebrospinal fluid [85]. On the other hand, a recent study of murine tyrosine hydroxylase activity during BH_4 supplementation showed that only high doses (100 mg/kg) resulted in a significant increase of brain tyrosine hydroxylase activity [86]. Therefore, Thöny et al. [86] considered it unlikely that current therapeutic regimens using BH_4 to treat PKU in humans (with doses typically <20 mg/kg) would have much of an effect on brain neurotransmitters.

Conclusion

Neonatal diagnosis of PKU followed by institution of a low-Phe diet has been a remarkable success in preventing the devastating brain damage associated with untreated PKU. Nevertheless, significant difficulties exist in caring for PKU patients, including problems with adhering to the prescribed dietary regimen and the presence of neurocognitive deficits despite therapy. Recent times have seen the introduction of a wide array of novel treatments currently in clinical use, including more palatable medical foods, GMP, LNAA, and BH_4. Human trials are underway using an enzymatic therapeutic approach (PEG-PAL), while preclinical work continues in the fields of gene and cellular therapy. Taken together, the future of PKU treatment has never looked brighter.

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