Minireview

Optimizing the use of sapropterin (BH4) in the management of phenylketonuria

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A B S T R A C T

Phenylketonuria (PKU) is caused by mutations in the phenylalanine hydroxylase (PAH) gene, leading to deficient conversion of phenylalanine (Phe) to tyrosine and accumulation of toxic levels of Phe. A Phe-restricted diet is essential to reduce blood Phe levels and prevent long-term neurological impairment and other adverse sequelae. This diet is commenced within the first few weeks of life and current recommendations favor lifelong diet therapy. The observation of clinically significant reductions in blood Phe levels in a subset of patients with PKU following oral administration of 6R-tetrahydrobiopterin dihydrochloride (BH4), a cofactor of PAH, raises the prospect of oral pharmacotherapy for PKU. An orally active formulation of BH4 (sapropterin dihydrochloride; Kuvan) is now commercially available. Clinical studies suggest that treatment with sapropterin provides better Phe control and increases dietary Phe tolerance, allowing significant relaxation, or even discontinuation, of dietary Phe restriction. Firstly, patients who may respond to this treatment need to be identified. We propose an initial 48-h loading test, followed by a 1–4-week trial of sapropterin and subsequent adjustment of the sapropterin dosage and dietary Phe intake to optimize blood Phe control. Overall, sapropterin represents a major advance in the management of PKU.

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Phenylalanine (Phe) is an essential amino acid which cannot be synthesized by the human body. Its net blood level is dependent on a number of processes, including dietary and caloric intake, endogenous protein turnover, catabolism, and incorporation into proteins. After its absorption by the digestive tract, Phe is converted to tyrosine, by Phe hydroxylase (PAH) and its cofactor, 6R-tetrahydrobiopterin (BH4); this is the major metabolic pathway of dietary Phe [1,2].

Phenylketonuria (PKU), an autosomal recessive inherited disorder characterized by defective or deficient PAH, is the cause of almost all (about 98%) cases of hyperphenylalaninemia (HPA). A minority of cases arise from disorders of BH4 synthesis or regeneration [2,3]. The PAH mutation knowledgebase (hPAHdb) currently describes 532 known mutations of this gene, mostly missense mutations (61% of all mutations), deletions (14%), splice variants (11%), silent mutations (6%), and nonsense mutations (5%) [4]. A systematic review identified 29 mutations that are particularly prevalent among patients with PKU in Europe [5]. Different mutations affect the activity of PAH to different extents.

If left untreated, PKU leads to the development of a variety of clinical problems including mental retardation, microcephaly, autistic behavior, eczema, and seizures [6]. The term, PKU, is reserved for the most severe forms of PAH deficiency “classic PKU” (Phe level >1200 μmol/L). Less severe forms are mild PKU (Phe level <600–1200 μmol/L) and mild HPA (Phe level <600 μmol/L). Notwithstanding our huge experience with PKU, the distinction between PKU and mild PKU is not always that clear and differing protocols for the age of screening add to the confusion in using initial untreated, screening Phe results to classify the type of PKU.

The more severe forms present with more severe neurological diseases, if untreated. The prevalence of PKU varies by country and ethnic group, ranging from approximately 1 in 4000 births in Northern Ireland or 1 in 6500 births in Turkey to 1 in 71,000 births in Finland, and the overall estimates fall within the range of 1 case per 10,000–20,000 births in Europe and the USA [7–9].

The observation that levels of Phe can be reduced significantly by administration of exogenous BH4 in a subset of patients with PKU raises the prospect of pharmacologic management of this disease...
A tablet formulation of BH4 (dihydrochloride) has been available for three decades. Although this formulation has been used extensively in experimental studies, it has not been evaluated in formal clinical trials and was not registered. A newer formulation of BH4 (sapropterin dihydrochloride, Kuvan®) that is more stable at room temperature is now available for the treatment of PKU in the USA and Europe [11]. This review describes the current and potential future therapeutic use of sapropterin in the management of PKU.

Dietary management of PKU

To date, the management of all patients with PKU has focused firmly on restriction of dietary Phe, accompanied by regular monitoring of circulating levels of Phe. Current recommendations on target Phe levels are 120–360 μmol/L for the first 10–12 years of life [12]. These recommendations, however, differ from country to country. Dietary Phe restriction, ideally begun within 1–2 weeks after birth, is effective in protecting the developing central nervous system from the toxic effects of HPA, although differences in cognitive function, behavior, or educational achievement have been observed between early-treated subjects with PKU and control populations [13–15]. There is an increasingly held view that dietary treatment for this condition should be lifelong [16,17]. Long-term outcome studies showed that adults with non-restricted diets may have some brain MRI disturbance or speed processing deficiencies [18–21]. The optimal Phe level in adulthood is widely debated. The behavior of some adult patients improves when they return to an appropriate diet (maternal PKU), but the burden of the regimen is still difficult to support. Treatment with BH4 not only helps to improve Phe levels, but also eases the burden of dietary management and should thereby improve dietary compliance issues in a subset of PKU patients who are BH4-responders.

Adhering to a low-Phe diet is onerous. The diet is supplemented with Phe-free protein substitutes consisting of essential and non-essential amino acids, and it excludes many natural high-protein foods such as dairy products, meat, and fish [22–24]. Commercial Phe-free amino acid supplements, designed for use by individuals with PKU, may have an unappealing taste or smell, and the Phe-restricted diet has been associated with adverse feeding behaviors in young subjects [25,26]. Nutritional deficiencies with clinical relevance have also been observed in diet-treated patients with PKU [23,27]. Newer protein substitutes may offer better tolerability and convenience for patients, but the burden of the diet remains a major cause for the loss of compliance, as observed in patients beyond childhood with PKU [24,28,29].

Therapeutic use of tetrahydrobiopterin in the management of PKU

Rationale

Clinically significant reductions in blood Phe in response to oral administration of exogenous BH4 (using the unregistered formulation) have been observed in about 80% of patients with mild HPA, in about 50% of patients with mild PKU, and in <10% of patients with classical PKU. A decrease in blood Phe of at least 30% is often used as a cut-off value to determine treatment response, although this is arbitrary [30,31]. Continued administration of this 6R-BH4 preparation (up to 5 years) has been shown to maintain reductions in blood levels of Phe without adverse effects [32,33].

Therapeutic profile of sapropterin in patients with PKU

Controlled clinical trials have evaluated the efficacy of a pharmaceutical formulation of sapropterin (Kuvan®), in patients with PKU (Table 1) [34–38]. Overall, the results of these trials indicated that about 20–50% of patients with PKU achieved a reduction in blood Phe of >30%. A study in 489 patients (mean age: 22 years; range 8–49 years) showed that 8 days of sapropterin (10 mg/kg/day) reduced mean plasma Phe by ≥30% in about one-fifth of patients (Fig. 1A), with a mean change in blood Phe of −392 ± 185 μmol/L [34]. An analysis of responders to treatment in this trial confirmed the efficacy of sapropterin versus placebo (Fig. 1B) [35]. Further studies showed that the effects on blood Phe are dose-related (Fig. 2) and are durable over time (Fig. 3) [35].

One study, performed in a pediatric population, recorded the amount of Phe supplementation possible while maintaining blood Phe at <360 μmol/L. These data were consistent with earlier data from a 2-year evaluation of the unregistered preparation of BH4, in which daily Phe tolerance increased from 18 mg/kg before treatment to 40 mg/kg during treatment [33].

Sapropterin is effective in reducing plasma Phe concentrations in a dose-dependent manner and is well tolerated at doses of 5–20 mg/kg/day over 22 weeks in BH4-responsive patients with PKU [39]. Headache, upper respiratory tract infections, and rhinorrhea were the most common side-effects observed in sapropterin-treated patients with PKU in clinical trials [35,37,38].

Optimizing sapropterin therapy

Who to test?

Sapropterin will be used for the treatment of HPA in patients with PKU who have been shown to be responsive to such treatment. Thus, all patients with PKU should undergo a sapropterin oral-response test before treatment initiation [40]. In Europe, the BH4-loading test is mostly performed in the neonatal period. In the neonatal period Phe levels are high and it is practical to perform the test. In instances where the child is under strict dietary control, a Phe ‘challenge’ (100 mg/kg) must precede the BH4 administration; however, there is no clear recommendation how to interpret the data without a preceding single Phe load. Also, it is contentious whether one should perform such a challenge at all. A number of different protocols have been followed using the unregistered BH4 formulation and sapropterin [31,34,41,42]. These studies have included using a normal diet or a Phe-restricted diet, different doses of BH4, different time periods to assess the effect on blood Phe (a 24-h test may detect slower responders more effectively than an 8-h test), single dose or multiple dose treatment administration, and the measurement of blood Phe levels or the half-life of decreases in blood Phe [40]. It should be noted that a ≥30% reduction in blood Phe is often considered to represent a clinically significant response to treatment; however, it is important to note that this threshold is arbitrary and some medical professionals consider smaller reductions to be clinically significant. Clearly, a simple and universal loading test would facilitate the identification of responders to sapropterin. Such a test must be practical in its application, being sufficiently predictive for BH4 responsiveness while restraining the number of measurements that need to be made.

Current protocol for treatment initiation

Fig. 4A shows the algorithm approved by the US Food and Drug Administration (FDA) for initiating therapy with sapropterin in patients above 4 years of age [37]. The prescribing Information for this product does not provide a specific cut-off value for a clinically significant reduction in blood Phe [37]. According to the FDA-approved algorithm, a measurement of blood Phe is followed by an initial daily dose of sapropterin, 10 mg/kg/day, given for 1 week, at the end of which a repeat blood Phe measurement is taken. If
A sufficient reduction in blood Phe is not observed, the dose can be increased to 20 mg/kg/day, and blood Phe levels are followed for a total initial treatment period of up to 1 month. At this time, treatment is stopped for non-responders, while responders enter a dose-optimization phase where the daily dosage of sapropterin is adjusted, usually within the range of 5–20 mg/kg/day, according to blood Phe levels. It should, however, be taken into account that this method may still create false positive and false negative results if patients adjust their diet during the trial. For this reason, a double-blinded trial could be also considered.

**Optimized protocol**

A recent expert commentary proposed an alternative test for identifying responders to BH4 therapy [40]. This involved adminis-

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**Table 1**

**Summary of clinical trials of sapropterin dihydrochloride in patients with phenylketonuria.**

<table>
<thead>
<tr>
<th>Ref</th>
<th>Design</th>
<th>N</th>
<th>Patients</th>
<th>On diet?</th>
<th>Dose</th>
<th>Duration</th>
<th>Key findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>[34] M, OL, U</td>
<td>489</td>
<td>8–49 years Phe &gt;450 µmol/L</td>
<td>No</td>
<td>10 mg/kg</td>
<td>8 days</td>
<td>＞30% reduction in Phe in 20% (mean plasma Phe was reduced by 392 ± 185 µmol/L in these patients).</td>
<td></td>
</tr>
<tr>
<td>[36] R, DB, P</td>
<td>88</td>
<td>Responders from above trial</td>
<td>No</td>
<td>10 mg/kg</td>
<td>6 weeks</td>
<td>Sapropterin reduced Phe by 236 ± 257 µmol/L versus placebo (P &lt; 0.001); 44% on sapropterin versus 9% of controls demonstrated a reduction in Phe of ＞30%. A significant difference was evident at week 1.</td>
<td></td>
</tr>
<tr>
<td>[35,37] OL, U</td>
<td>80</td>
<td>Extension to above trial</td>
<td>No</td>
<td>5–20 mg/kg</td>
<td>22 weeks</td>
<td>Dose-related reduction of Phe with sapropterin during initial 6 weeks of treatment, 4 weeks of fixed dose at 10 mg/kg/day, followed by optimized dose over a further 12 weeks.</td>
<td></td>
</tr>
<tr>
<td>[35,37,38] OL, U</td>
<td>89</td>
<td>4–12 years Phe &lt;480 µmol/L</td>
<td>Yes</td>
<td>20 mg/kg</td>
<td>8 days</td>
<td>Mean blood Phe reduced from 317 ± 173 µmol/L to 108 ± 70 µmol/L. Reduction in Phe of ＞30%, together with final Phe &lt;300 µmol/L in 56%.</td>
<td></td>
</tr>
<tr>
<td>[35,37,38] R, DB, P</td>
<td>45</td>
<td>Responders from above trial</td>
<td>Yes</td>
<td>20 mg/kg</td>
<td>3 weeks</td>
<td>Patients receiving sapropterin tolerated 7-fold higher levels of Phe intake (21 ± 15 versus 3 ± 4 mg/kg/day) while maintaining Phe &lt;360 µmol/L. Sapropterin reduced Phe by 149 µmol/L versus placebo.</td>
<td></td>
</tr>
</tbody>
</table>

DB, double-blind; M, multicenter; OL, open-label; P, placebo-controlled; R, randomized; U, uncontrolled; Phe, plasma phenylalanine. N refers to the total number of patients in analyses. Diet refers to phenylalanine restriction. Dose refers to the daily dose of sapropterin (Kuvan®). Means ± SD where applicable.

**Fig. 1.** Efficacy of sapropterin dihydrochloride in the management of phenylketonuria. (A) Response rates (%) according to blood phenylalanine levels before sapropterin treatment (10 mg/kg/day) over a period of 8 days. Derived from data published by Burton et al. [34]. (B) Randomized comparison of the effect of sapropterin dihydrochloride and placebo on blood phenylalanine levels in responders to sapropterin therapy (10 mg/kg/day) over a period of 6 weeks. Derived from data published by Levy et al. [36]. Bars are 95% CI.

**Fig. 2.** Dose relationship of the effect of sapropterin dihydrochloride on blood phenylalanine levels in patients with phenylketonuria who previously responded to sapropterin therapy [35,37]. Bars are 95% CI.

**Fig. 3.** Durability of the effect of sapropterin dihydrochloride on phenylalanine in patients with phenylketonuria who previously responded to sapropterin therapy [35,37]. Patients were responders from a previous dose–response trial: columns show mean changes from baseline in blood phenylalanine in an extension to this trial during which sapropterin treatment was adjusted individually, based on blood phenylalanine responses. Bars are 95% CI.
tation of a single dose of BH4, 20 mg/kg, followed by serial measurements of blood Phe during the following 24 h. This test considered a reduction in blood Phe of at least 30% as the primary determinant of BH4 responsiveness [40,41]. Using this test (single 20 mg/kg administration with blood Phe monitoring over 24 h and with the 30% cut-off), the overall prevalence of BH4 responsiveness in patients with PKU was found to be 46% [31]. It should be noted, however, that this test of BH4 responsiveness is not consistent with the FDA-approved Prescribing Information for Kuvan® (sapropterin dihydrochloride) [37]. Fig. 4B is the optimized protocol proposed by the European working group for phenylketonuria (authors of this article). This 48-h protocol follows the current practice in Europe and is based on experience with the unregistered BH4 formulation over the last 8 years. It aims to relax, or even discontinue, dietary Phe restriction, if indicated as appropriate by the BH4 response. An option would be to perform the 24-h test first to determine the initial responders and to do a second (24-h) test later for patients who showed a rather slow responsiveness (<30%) in the first test. In addition to the recommended blood sampling at 0, 8, 16, and 24 h after sapropterin administration, a 4-h sample may be useful in the detection of BH4-deficient patients. In some instances, the blood sampling can be done at home. The decision to combine sapropterin with a low-Phe diet, or to introduce a monotherapy with sapropterin, is based on the individual Phe tolerance and on the targeted therapeutic range for blood Phe levels. Regardless of the protocol used, only the long-term follow-up of initial responders with a constant therapeutic control of blood Phe levels can justify the application of sapropterin.

**Genotyping**

Genotyping represents another potential means of identifying patients suitable for treatment with sapropterin, and a series of specific mutations in the PAH gene have been associated with partial BH4 responsiveness [43–46]. One study showed that patients with PKU and the same specific mutations in the PAH gene differed in their responses to a BH4-loading test [47]. However, it was reported later that one of these patients was initially loaded with an old formulation of BH4 and that both siblings showed the same response to BH4 [48]. The authors concluded that factors other than the PAH genotype (e.g. BH4 pharmacokinetics) contributed to BH4 responsiveness in PKU [49]. Moreover, the use of genotyping to predict BH4 responsiveness is complicated by the fact that most patients with PKU resulting from PAH mutations present as compound heterozygotes [43]. While genotyping may be useful to some extent in predicting a higher or lower probability of BH4 responsiveness [45], this approach requires further investigation before it can be used as a definitive diagnostic test for this phenomenon.
Practical aspects

Patients diagnosed as BH4-responsive need a careful and frequent follow-up of blood Phe levels while adjusting or discontinuing dietary regime. Sapropterin dosage is usually adjusted according to the actual Phe tolerance and therapeutic blood Phe target levels (initial dosage, 10 mg/kg/day) [50]. Patient doses are subsequently up- or down-titrated (range: 5–20 mg/kg/day).

Table 2 summarizes important information relating to the therapeutic use of sapropterin. The preparation is available as 100 mg soluble tablets, taken as a single dose with food in the morning. No formal drug interaction studies have been performed with this agent in humans; however, pharmacokinetic or pharmacodynamic interactions may occur with certain agents that influence BH4 metabolism, such as methotrexate or trimethoprim. If the long-term response to sapropterin is inadequate, the physician should explore the possibility of poor compliance with sapropterin treatment or with diet before adjusting the dose. Although, levodopa is listed as a precaution in Table 2, patients with BH4 deficiency can be safely treated with combination therapy (BH4 + levodopa). In Europe, sapropterin is indicated for the treatment of HPA in adult and pediatric patients with PKU (>4 years of age) or BH4 deficiency (all ages) who have been shown to be responsive to such treatment.

Conclusions

Treatment with sapropterin has been shown to markedly reduce blood Phe levels in a substantial proportion of patients with PKU. In this article, we propose a simple BH4-loading test, allowing fast discrimination between responders and non-responders. Individual responsiveness should be correlated to the patient’s genotype and, in the case of discrepancy between the responsiveness and genotype, sapropterin pharmacokinetics should be investigated.

Although loading-test data from well-designed clinical studies are now available, further studies are required in order to assess the relevance of the simple BH4-loading test to identify candidates for potential sapropterin treatment. Sapropterin significantly improves the management of patients with milder or moderate forms of PKU who respond to this treatment and it is crucial to have unique guidelines on how to identify responsive patients and how to improve their quality of life through avoiding or reducing the burden of the low-Phe diet.

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References


Table 2

Practical aspects relating to the administration of sapropterin dihydrochloride for the management of phenylketonuria [37,51].

Adminstration
- Dosage is based on body weight.
- Round the calculated dose up or down to the nearest 100 mg.
- Dissolve 100 mg sapropterin dihydrochloride tablets in water (or apple juice) according to Prescribing Information and take within 15–20 min.
- Take as a single dose with food at the same time each day; preferably in the morning (treatment for BH4 deficiency may require divided doses).
- For doses <100 mg, dissolve one 100 mg tablet in 120 mL of water and administer the appropriate volume.
- Monitor blood phenylalanine to keep this parameter within its normal range.
- Use with caution in patients prone to convulsions or in patients receiving inhibitors of dihydrololate reductase (e.g. methotrexate, trimethoprim), agents that affect metabolism or action (e.g. nitric oxide donors, molsidomin, phosphodiesterase-5 inhibitors, minoxidil), or levodopa.


