Sapropterin hydrochloride: enzyme enhancement therapy for phenylketonuria

Robin Lachmann

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Abstract: Phenylketonuria (PKU) is an inherited disorder of amino acid metabolism caused by deficiency of the enzyme phenylalanine hydroxylase (PAH). Historically PKU was a common genetic cause of severe learning difficulties and developmental delay, but with the introduction of newborn screening and early dietary management, it has become a treatable disease and people born with PKU should now have IQs and achievements similar to their peers. Dietary treatment, however, involves lifestyle changes that pervade most aspects of daily life for an individual and their family. A simple pharmacological treatment for PKU would have a great appeal. Sapropterin hydrochloride is a synthetic form of tetrahydrobiopterin, the cofactor for PAH. A proportion of mutant PAH enzymes show enhanced activity in the presence of pharmacological doses of sapropterin and, for some patients with milder forms of PKU, sapropterin can effectively lower plasma phenylalanine levels. This article discusses the potential place for sapropterin in the management of PKU and how this expensive orphan drug is being integrated into patient care in different healthcare systems.

Keywords: dietary therapy, enzyme enhancement therapy, orphan drugs, phenylketonuria, sapropterin hydrochloride, tetrahydrobiopterin

Phenylketonuria

Phenylketonuria (PKU) was one of the first treatable genetic diseases. The development of dietary therapy, 50 years ago, led to the introduction of universal newborn screening for PKU in developed countries in the late 1960s and early 1970s. Institution of a low-protein diet in the newborn period transformed PKU from the most common genetic cause of severe mental retardation to a lifelong condition compatible with normal health and educational achievement [van Spronsen, 2010]. Forty years on, one of the major genetic and public health success stories of the 20th century is, however, largely unrecognized: whilst every newborn baby has a blood spot collected, most parents and many healthcare professionals have no idea for what the blood is being screened, let alone the implications of a positive test. Certainly in the UK, they do not even expect to get a result from the test.

PKU is caused by deficiency in the activity of the enzyme phenylalanine hydroxylase (PAH) [Scriver *et al.* 2008]. This leads to an accumulation of the substrate of the enzyme, phenylalanine (Phe) and a deficiency of its product, tyrosine. Between them, these lead to the phenotype of

developmental delay, learning difficulties and occasionally, other neurological features, such as epilepsy and Parkinsonism. The pathophysiology of PKU is not fully understood, but Phe is certainly directly neurotoxic and tyrosine deficiency in the brain would be expected to lead to alterations in catecholaminergic neurotransmission, which might also have a role to play [van Spronsen *et al.* 2009]. Deficiency of tyrosine also results in reduced melanin formation, which explains the fair-skinned, blond-haired phenotype of untreated PKU.

As with most inherited metabolic diseases, there is a spectrum of disease severity in PKU, which relates, at least in part, to the residual activity of the mutant PAH enzyme(s) expressed in any patient. The degree of neurological impairment seen is directly related to Phe levels (in fact, it is the Phe level in the brain that is important [Weglage *et al.* 2001]). The observation that there was a degree of hyperphenylalaninaemia where neurodevelopment was normal suggested that this disease might be treatable if a way of reducing Phe levels could be developed, an approach that could be described as substrate reduction therapy.

Correspondence to:
Robin Lachmann
Charles Dent Metabolic
Unit, Box 92, National
Hospital for Neurology and
Neurosurgery, Queen
Square, London WC1N
3BG, UK
[robin.lachmann@
uclh.nhs.uk](mailto:robin.lachmann@uclh.nhs.uk)

Dietary therapy for PKU

Phe in the blood is, in the fed state, derived from dietary protein (in fasting, or other catabolic states, plasma Phe is increasingly derived from the breakdown of endogenous protein). The dietary treatment of PKU was developed in the 1950s [Bickel, 1996]. The aim is to lower plasma Phe levels by restricting dietary Phe [Levy, 1989]. In practical terms, this means restricting dietary protein. Patients must avoid high protein foods, such as meat, fish, eggs, most dairy products, nuts and pulses. Staples such as potatoes, wheat, rice and maize, which all contain significant amounts of protein, are severely restricted. Most fruit and vegetables can be eaten freely, and special low-protein products (e.g. bread, pasta, rice, flour, milk) help to make up calorie requirements. Protein requirements are met by the use of synthetic, Phe-free amino acid mixtures and these also normally contain all the vitamins and trace elements that are missing from a diet low in natural, high-quality protein [Macdonald *et al.* 2004].

Early studies demonstrated that such a diet was effective in controlling blood Phe levels and, if instituted in the neonatal period, could prevent the neurodevelopmental effects of PKU [Bickel, 1969]. It became clear that final IQ was related to the degree of metabolic control, with children who obtained levels below 400 $\mu\text{mol/l}$ in early and middle childhood having near normal final IQ [Burgard, 2000]. For every 300 $\mu\text{mol/l}$ rise in average Phe levels, IQ decreased by half a standard deviation.

Interestingly, by the age of 10 years, IQ appears to be fixed and remains stable independent of the quality of dietary control of Phe [Burgard, 2000]. The rationale for continuing dietary treatment into adolescence and adulthood is based on the study of more subtle neurocognitive effects in individuals with early treated PKU. Many studies have looked at executive function, 'higher-order cognitive abilities that facilitate the flexible modification of thought and behaviour in response to changing cognitive or environmental demands' [Christ *et al.* 2010]. Although many studies show defects in executive function, and some suggest that this is related to Phe levels, there is remarkably little agreement on the precise neurocognitive phenotype seen in older children and adults with PKU [Christ *et al.* 2010]. The most robust data concern measures of reaction time [Albrecht *et al.* 2009]. Where groups of people

with PKU have been compared with non-PKU controls, reaction time seems to be related to plasma Phe level in children and adolescents, but in adults, although PKU sufferers appear to react slightly slower than controls, this deficit is independent of Phe levels.

Patients with early treated PKU also have white matter abnormalities on magnetic resonance imaging (MRI) scans and it has been suggested that these might be related to the various neurocognitive deficits that have been described. The significance of these MRI lesions is unclear; it has been suggested that they may represent dysmyelination or simply oedema [Anderson and Leuzzi, 2010]. Longitudinal studies have suggested that they are at least in part reversible if Phe levels are reduced [Cleary *et al.* 1995].

Therefore, in adults with PKU, there is no good evidence for any neurological damage due to high Phe levels, and the evidence for reversible neurocognitive effects is inconsistent and of uncertain clinical relevance.

The fact that there are measurable neuropsychological changes in people with PKU who started dietary treatment early and who have been continuously treated has led to the proposal that tight control of Phe levels should be maintained throughout adult life [Gentile *et al.* 2010]. In reality, however, many older people with PKU choose to discontinue dietary treatment [Walter *et al.* 2002]. There have been very few studies examining how this group of patients perform on neuropsychological testing, but the majority are leading normal lives and, although there are some who choose to return to diet [Gassió *et al.* 2003], most do not recognize any adverse effects from their high Phe levels.

Sapropterin hydrochloride: a small molecule therapy for PKU

The reason that many adults choose to discontinue treatment currently is that the low-protein diet is arduous and pervades all aspects of their lives. If a safe and effective pharmaceutical or biological treatment was available, then it seems likely that it would be much easier to persuade people with PKU to accept lifelong treatment.

For most inherited diseases, the majority of patients are compound heterozygotes and will possess at least one allele that contains a missense mutation rather than a nonsense or other

null mutation. These missense mutations, by definition, are associated with the production of a mutant protein. They are pathogenic because this protein either has reduced activity or is unstable, mistargeted or fails to associate correctly with other molecules. Where such mutant molecules exist, enzyme enhancement therapy is a potential therapeutic approach.

In enzyme enhancement therapy, the aim is to maximize the activity of the mutant protein within the cell. For most processes in the body, a large excess of capacity exists and, therefore, in many cases of enzyme deficiency it may only be necessary to provide 10–20% of wild-type activity to restore normal cellular function. Where there is a mutation that affects a crucial residue in the active site of the enzyme, this may not be possible, but where enzymes are misfolded or unstable, then it is possible to use small molecules to enhance their stability and activity [Fan, 2008].

Sapropterin hydrochloride is a synthetic form of tetrahydrobiopterin (BH4), the natural cofactor of PAH [Burnett, 2007]. Its use in treating PKU was based on the hypothesis that for some mutant forms of the enzyme, providing pharmacological doses of its cofactor might enhance enzyme activity. Hence there would be a subgroup of patients with PKU who would be 'responsive' to sapropterin therapy, much in the same way that some patients with classical homocystinuria respond to large doses of pyridoxine, the natural cofactor for cystathionine beta synthase, the enzyme that is deficient in homocystinuria.

In vitro work has shown that sapropterin can indeed enhance the activity of a range of mutant PAH enzymes [Blau and Erlandsen, 2004]. The mutant enzymes that are significantly enhanced by sapropterin are for the most part those that have considerable residual activity anyway, although the mutations themselves can occur anywhere in the protein. The mechanism of action of sapropterin is not fully understood but it appears to stabilize the active PAH quadramers.

Assessing sapropterin responsiveness

The clinical efficacy of sapropterin is judged by its ability to reduce plasma Phe levels. In clinical trials a reduction of 30% in Phe was set as the cut-off for responsiveness, but this arbitrary

figure simply represents a point in the spectrum of responses that can be seen. How one tests for sapropterin responsiveness in clinical practice remains controversial. Regulatory authorities have recommended that patients be given sapropterin daily at a dose of 10 mg/kg for 1 week. Responsiveness is defined as a drop of $\geq 30\%$ in plasma Phe levels from baseline. If patients do not respond to 10 mg/kg/day, then a dose of 20 mg/kg/day can be given for up to another 3 weeks.

The problem with this approach is that over such a long period, the introduction of sapropterin is not the only thing that will influence Phe levels. Although the recommendations are to ensure that patients do not alter their diet during the testing period, in practice this is difficult to monitor, as patients are at home. Conversely, an intercurrent infection can lead to Phe levels rising and give a false-negative result. It has been suggested that responsiveness testing should be done in a double-blinded manner with a period of exposure to the active drug and another to placebo.

An alternative approach is to use a shorter protocol and assess the response to a single dose of sapropterin. In many parts of Europe, a BH4 loading test has been part of the routine assessment of neonates with hyperphenylalaninaemia detected on newborn screening with the aim of detecting the rare forms due to defects of bioppterin synthesis rather than PAH deficiency [Feillet *et al.* 2008]. The test is either performed before the babies are commenced on a low-protein diet or, if protein restriction is already in place, an oral Phe load is given before the test. Although it would seem sensible to test all neonates for responsiveness to sapropterin, sapropterin hydrochloride is only licensed for use in children over the age of 4 years and, for the present, routine testing has to be delayed. Nevertheless, there is no reason why a similar protocol should not be used in older patients and the European working group for PKU has recently proposed a protocol that can be performed over 36 h [Blau *et al.* 2009]. Baseline levels are established in the first 24 h and then a single dose of 20 mg/kg sapropterin is given. Phe levels are measured at 8, 16 and 24 h. If levels do not fall by $\geq 30\%$, a further dose of sapropterin can be given in order to pick up 'late responders'. This protocol can be performed at home, with patients collecting blood spots on to Guthrie

cards, but could probably still be manipulated by dietary modification.

Owing to its mechanism of action, the patients who are most likely to respond to sapropterin are those with lower baseline Phe levels and 'milder' disease. When 485 patients were screened for responsiveness (defined as a $\geq 30\%$ decrease in Phe levels) in the original clinical trials of sapropterin, it was shown that the probability of being a responder was 25% if untreated levels were between 600 and 900 $\mu\text{mol/l}$, but only 10% if they were $>900 \mu\text{mol/l}$ [Burton *et al.* 2007]. In postmarketing reports of clinical use in much smaller cohorts of patients, much higher rates of response have been reported, up to 100% in patients with baseline levels $<1200 \mu\text{mol/l}$ and 27% in those with classical PKU and baseline levels $\geq 1200 \mu\text{mol/l}$ [Vernon *et al.* 2010]. These discrepancies highlight the need for standardized definitions and protocols to test for responsiveness. The use of a cut-off of 30% reduction in Phe in the initial studies was to some extent arbitrary and there will be higher rates of responsiveness if lower decreases are accepted.

Efficacy of sapropterin in reducing Phe levels

In the initial phase III clinical trial, 89 patients with PKU who were responsive to sapropterin were randomized to receive either sapropterin 10 mg/kg/day or placebo [Levy *et al.* 2007]. These subjects were predominantly adolescents and adults who had 'relaxed or abandoned a strict low-phenylalanine diet'. Even without strict dietary treatment, Phe levels at baseline were between 800 and 900 $\mu\text{mol/l}$, indicating that this group of patients mostly had what would normally be termed hyperphenylalaninaemia rather than classical PKU. After 6 weeks of treatment, levels in the placebo group were unchanged whilst Phe levels in subjects treated with sapropterin had fallen by an average of 236 $\mu\text{mol/l}$ to a mean level of 607 $\mu\text{mol/l}$ [Levy *et al.* 2007]. It is worth noting, however, that even amongst this group of patients who had already undergone responsiveness testing and been shown to be sapropterin responders [Burton *et al.* 2007], only 44% had a reduction of $\geq 30\%$ in Phe levels after 6 weeks of sapropterin treatment, and 17% actually had an increase in their Phe levels after 6 weeks of therapy. The authors observed that these patients may not have been truly sapropterin responsive, and yet they did achieve reductions of $\geq 30\%$ in the initial screening.

Eighty patients from the placebo-controlled study went on to participate in an open-label, dose titration study [Lee *et al.* 2008]. In this study, response rates ($\geq 30\%$ reduction in Phe again) after 2 weeks of treatment with 5, 10 and 20 mg/kg/day, were 25%, 46% and 55%, respectively. Thus, whilst doubling the dose of sapropterin from 5 mg/kg to 10 mg/kg led to a 46% increase in response rate, a further doubling to 20 mg/kg only resulted in a further 20% of patients responding. Responses were maintained through a 10-week treatment period. Treatment with sapropterin was safe and well tolerated.

These clinical trials showed that for a minority of patients with PKU, most of whom have 'milder' disease with baseline Phe levels in the hyperphenylalaninaemia range, treatment with sapropterin can produce significant and sustained decreases in plasma Phe concentrations. Based on these data, sapropterin was licensed for use in PKU in both USA and Europe in conjunction with a low-protein diet.

If patients could buy an over-the-counter vitamin pill that might improve their metabolic control and was unlikely to do them any harm, most of us would not hesitate to suggest they try it. When that vitamin is a licensed drug, and it costs £50,000–100,000 to treat an average adult patient for a year, issues other than its potential efficacy have to be taken into account. Dietary treatment, which is universally effective, costs in the region of £12,000 annually. Only in the orphan drug industry could profits be made from the introduction of a new agent which, although much less effective, was an order of magnitude more expensive than the universally accepted gold standard treatment. Can sapropterin really provide sufficient benefit to justify the additional cost?

Efficacy of sapropterin in increasing protein tolerance

The argument being made for the use of sapropterin is not that it improves metabolic control, but that it allows some patients to relax their diets whilst maintaining metabolic control and thus provides significant benefits in terms of quality of life. This may be true, but there is little evidence to support such claims. One study has been conducted to assess Phe tolerance in children treated with sapropterin [Trefz *et al.* 2009]. Forty-five children between 4 and 12 years old were studied. The subjects were

randomized in a 3:1 ratio to take either sapropterin 20 mg/kg/day or placebo. They were studied for a 10-week period during which time they were asked not to alter their dietary treatment. Blood Phe levels were monitored and oral Phe supplements were given, the dose of Phe being titrated so as to maintain blood Phe levels at $\leq 360 \mu\text{mol/l}$.

After 10 weeks of treatment, the Phe intake of those taking sapropterin increased 2.6-fold from 16.3 mg/kg/day to 43.8 mg/kg/day, although there was a wide range of individual responses. Phe intake for the placebo group increased 1.4-fold from 16.3 mg/kg/day to 23.5 mg/kg/day. This is a significant increase and the sapropterin-treated children would have been able to increase their natural protein intake to almost 1 g/kg/day.

In their report of introducing sapropterin in a clinical setting, Vernon and colleagues described dietary liberalization in 14 patients. Phe tolerance increased by an average of 20 (range 2–83) mg/kg/day and two patients were able to go on to an unrestricted diet [Vernon *et al.* 2010]. In this study, however, no attempt was made to modify the diet before sapropterin was introduced. Many patients on a protein-restricted diet have been following the same diet since childhood. Our experience in a large, adult metabolic clinic is that when patients are transferred from paediatric services, it is often possible to liberalize substantially the diet, sometimes doubling natural protein intake, whilst still maintaining Phe levels within the target range. It is possible, therefore, that not all of the dietary liberalization reported by Vernon and colleagues was actually due to the introduction of sapropterin.

There will be a small group of babies diagnosed on newborn screening with hyperphenylalaninaemia who, if treated with sapropterin, would be able to avoid going on a low-protein diet at all. Clearly these patients should be treated, especially because with the relaxation of targets for plasma Phe levels with age, most would be able to come off all treatment in later childhood or adolescence. Unfortunately, as sapropterin is only licensed for use in children over 4 years, currently we are not able to identify and treat these babies.

A further group of patients, with higher untreated Phe levels, will need some dietary treatment in

childhood, but would be able to maintain target Phe levels in the adult range with the use of sapropterin alone, allowing them to return to an unrestricted diet. Such patients would need careful monitoring: food preferences are determined early in life and people who have not eaten meat during childhood may never develop a taste for it, often finding the texture unpleasant. A proportion of people with PKU, if allowed to increase their natural protein intake, will choose foods of low nutritional quality (e.g. cakes, confectionary, potato snacks, fries) and, as they concomitantly stop taking their amino acid supplements, they can be at risk of problems related to vitamin B12 and other nutritional deficiencies [Brenton and Pietz, 2000]. Nonetheless, this group of patients would gain a clear benefit in quality of life from the use of sapropterin.

For the remainder of sapropterin-responsive patients who might be able to relax rather than come off their low-protein diets, the benefits of being on sapropterin are less clear. A lot more data are required before it can be concluded that the introduction of sapropterin significantly improves quality of life by allowing a relaxation of the low-protein diet.

Sapropterin in the clinical setting

Currently, even in countries that can afford to treat patients with an expensive orphan drug like sapropterin, there are major differences in its uptake. These seem to relate more to the mechanisms for providing healthcare than to clinical factors. In the USA, it has always been difficult to persuade insurers to support the costs of dietary treatment due to the fact that the products used are not licensed pharmaceuticals and therefore are not automatically covered [Brown *et al.* 2002]. In contrast, it has been much easier to get insurers to pay for sapropterin, and it is now being widely and enthusiastically used. In contrast, in Europe the amino acid supplements and low-protein foods required for dietary treatment are readily available and fully funded in most countries. In countries where there is significant state funding of healthcare, commissioners are asking why it is necessary to use a highly expensive orphan drug that will only work in a minority of patients, when dietary therapy has greater efficacy and represents better value for money. In the UK in particular, the National Health Service (NHS) has to date refused to fund treatment of PKU with sapropterin.

Conclusion

There can be very few diseases in which it would be possible to introduce a new drug that was less effective than the current gold standard treatment and would only have any effect in those with relatively mild disease. When this new treatment is a highly expensive orphan drug, costing many times more than the available, highly effective and safe treatment, it is difficult to see how it would gain any market share. However, when the gold standard therapy is a diet, a lifestyle rather than a drug, then there are obviously quality-of-life arguments to be made. With sapropterin, these arguments have not yet been made and its current use relates as much to models of healthcare as to its proven benefits in PKU.

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