J Inherit Metab Dis (2007) 30:153–158
DOI 10.1007/s10545-007-0556-4

ICIEM 2006

Double blind placebo control trial of large neutral amino acids in treatment of PKU: Effect on blood phenylalanine

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Received: 10 January 2007 / Submitted in revised form: 30 January 2007 / Accepted: 31 January 2007 / Published online: 27 February 2007

Summary Large neutral amino acids (LNAA) have been used on a limited number of patients with phenylketonuria (PKU) with the purpose of decreasing the influx of phenylalanine (Phe) to the brain. In an open-label study using LNAA, a surprising decline of blood Phe concentration was found in patients with PKU in metabolic treatment centres in Russia, the Ukraine, and the United States. To validate the data obtained from this trial, a short-term double-blind placebo control study was done using LNAA in patients with PKU, with the participation of three additional metabolic centres – Milan, Padua and Rio de Janeiro. The results of the short trial showed significant lowering of blood Phe concentration by an average of 39% from baseline. The data from the double-blind placebo control are encouraging, establishing proof of principle of the role of orally administered LNAA in lowering blood Phe concentrations in patients with PKU. Long-term studies will be needed to validate the acceptability, efficacy and safety of such treatment.

Communicating editor: Verena Peters

Competing interests: None declared

References to electronic databases: Phenylketonuria (PKU; OMIM 261600)

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Abbreviations

BBB	blood–brain barrier
LNAA	large neutral amino acids
Phe	phenylalanine
PKU	phenylketonuria
VIL	valine, isoleucine and leucine

Introduction

Phenylketonuria (PKU) is caused by deficient activity of the enzyme phenylalanine hydroxylase (PAH) (Folling 1934; Jervis 1953; Kaufman 1971). The treatment of PKU with diet restricted in phenylalanine (Phe) has become a standard care following the early trials of Bickel and colleagues (1953).

Experience with the treatment of PKU indicated efficacy of the low-Phe diet with the possibility of diet relaxation in older children. Studies showing decline in intellectual

performance when blood Phe concentrations were high resulted in reassessment of the policy of diet relaxation. Gradually the concept of 'Diet for Life' emerged on the basis of subsequent studies (Azen et al 1991; Fisch et al 1997; Gleason et al 1992; Michals et al 1985; Walter et al 2002). Decline of intellectual performance when blood Phe concentrations are elevated is the basis for continued diet in PKU. When blood Phe concentrations are high, individuals with PKU often have problems with poor school performance, decline in executive functioning, and changes in white matter of the brain. (Burgard et al 1997; Diamond 2001; Fisch et al 1995; Griffiths et al 1995; Lou et al 1985; Michals et al 1988; Pietz et al 1998; Ris et al 1994; Schmidt et al 1994; Smith et al 1978, 1991; Scriver and Kaufman 2001; Seashore et al 1985; Thompson et al 1990, 1994). In order to prevent blood Phe from exceeding acceptable concentrations, different modes of therapy have been advocated (Scriver and Kaufman 2001).

Centres that treat PKU have advocated different blood Phe concentrations for young children or adults, so that uniformity of acceptable Phe concentration has been lacking. A consensus conference organized by NIH (NIH 2001) with experts from the United States, the United Kingdom, Germany, France and other countries resulted in guidelines suggesting blood Phe concentrations of 120–360 μmol/L for children from birth to 13 years of age. Those who are 13 years and older are recommended to have blood Phe concentration not exceeding 900 μmol/L, with concentration below 600 μmol/L preferred. In Europe, in some centres, blood Phe concentration of 1200 μmol/L is allowed. In the UK, specific guidelines were developed (MRC Working Party on Phenylketonuria 1993), although some centres in the UK accept 1200 μmol/L. Even with these higher blood Phe recommendations it is still difficult to attain desired blood Phe concentrations (NIH Consensus Report on Phenylketonuria 2001).

The discovery that (6*R*)-L-*erythro*-5,6,7,8-tetrahydrobiopterin (BH_4) could reduce blood Phe in some patients with PKU (Kure et al 1999) was met with enthusiasm. Subsequent studies (Blau and Scriver 1997; Blaue and Trefz 2002; Erlandsen et al 2004; Lassker et al 2002; Lindner et al 2003a,b; Matalon et al 2003, 2004; Muntau et al 2002; Spaapen et al 2000; Trefz et al 2000, 2001; Weglage et al 2002) confirmed the findings of Kure and colleagues. However, the response to BH_4 is primarily limited to patients with mild PKU.

Large neutral amino acids (LNAA) have been suggested for use in treatment of PKU because of the competition with Phe at the blood–brain barrier (BBB). Oldendorf and Szabo (1976) showed that LNAA cross the BBB with the same transporter protein that is also shared by cationic amino acids. The large neutral amino acids and the cationic amino acids (phenylalanine, tyrosine, tryptophan, threonine, isoleucine, leucine, valine, methionine, lysine, arginine histidine and other cationic amino acids) share a common transporter to the brain and compete with one another (Choi and Pardridge 1986; Hargreaves and Pardridge 1988; Hidalgo and Borchardt 1990; Pardridge 1977, 1982; Pardridge and Oldendorf 1975). Pardridge (1982) showed that the transport of LNAA and movement of amino acids across the BBB depend on the affinity of each amino acid for the carrier protein.

Large neutral amino acids and cationic amino acids cross the intestinal mucosa by a carrier protein similar to that of BBB, except that the affinity of the amino acid for the intestinal carrier has a K_m two orders of magnitude higher than that of the BBB, so that under physiological conditions high concentrations of LNAA need to be present in the GI tract in order to compete with Phe. Recently, we have shown that blood Phe concentration in patients with PKU, as well as in mice with PKU, decline significantly when treated with LNAA (Matalon et al 2006).

We report the results of a study using LNAA in patients with PKU carried out by several metabolic centres in different countries. The data from open-label studies showing decline of blood Phe concentration in patients with PKU were encouraging and indicated that a mixture of LNAA (NeoPhe) can successfully lower blood Phe concentrations in patients with PKU (Matalon et al 2006). This double-blind study confirms previous findings that LNAA can be used for all patients with PKU who need reduction in blood Phe concentration.

Patients and methods

Tablets of large neutral amino acids (NeoPhe) were obtained from Prekulab, Korsor, Denmark. The composition of LNAA is the same as described in the open-label study (Matalon et al 2006). The placebo tablets were supplied by the same company and were identical in size and appearance. The placebo contained lactose monohydrate, microcrystalline cellulose and colloidal hydrated silica.

Patients with PKU were recruited from six centres: Department of Pediatrics, University of Texas Medical Branch, Children's Hospital, Galveston, Texas, USA; Institute of Clinical Genetics, Kharkiv State Medical University, Kharkiv, Ukraine; Department of Clinical Genetics, Institute of Pediatrics and Child Surgery, Moscow, Russia; University of Milan, Italy; Inherited Metabolic Disease Unit and Department of Neuroscience, Neurological Clinic, University Hospital of Padua, Italy; Diagnósticos Laboratoriais Especializados and Centro Ambulatorial de Prevenção/APAE- Rio de Janeiro, Brazil. The patients enrolled in the study had to have PKU and be old enough to swallow pills. Each patient signed an institutionally approved informed consent prior to enrollment.

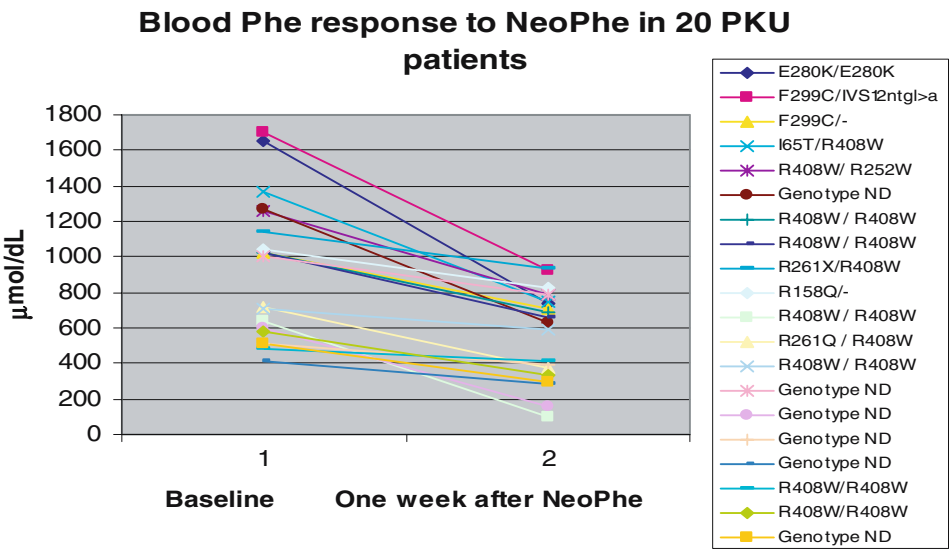

Fig. 1 Blood Phe response to NeoPhe in 20 PKU patients after one week. Average blood Phe concentration at baseline was 932.9 µmol/L, which dropped to 568.4 µmol/L (average drop of 364.5, SD = 232.2, $p < 0.0001$). Seven patients with mean baseline of 531.6 µmol/L, which dropped to 281.5 µmol/L (average drop of 250.1, SD = 73.7, $p = 0.009$)

There were 20 patients in the study, aged 11 to 32 years, 12 female and 8 male. There were 4 patients from the USA, 5 from Ukraine, 1 from Milan, 2 from Padua, 2 from Brazil, and 6 from Russia. With the exception of one patient, the 20 patients had classical PKU as indicated by the clinical classification based on initial Phe concentration and genotype.

The patients were instructed to continue their diet as prior to enrolling in the trial. The dosage of LNAA was as 0.5 g/kg per day in three divided doses to be taken with meals, which is about one tablet per kg per day. The placebo tablets were given in the same dosage. This dosage was acceptable and patients complied with the treatment. The order of the placebo and experimental treatments was random and was unknown to the patient and the treating physician. Compliance and randomization were supervised by the metabolic centres. Baseline Phe was determined on three separate occasions prior to active participation in the study. The baseline is the point of comparison of the patient's current treatment and the double-blind study. Pills containing either placebo or NeoPhe were administered and blood Phe was determined twice weekly. Each centre took blood at the same time for each visit, usually two hours after meals. The patients had a one-week washout period prior to the next week of the double-blind crossover trial. Blood Phe was again assayed at the beginning and twice weekly during the second phase.

Paired *t*-tests were used to assess changes from baseline measurements with the *t*-test procedure using SAS statistical software (SAS Institute, 2004).

Results

At the end of the double-blind trial the results were unmasked. Blood Phe concentration in the 20 patients from all the participating centres dropped significantly while on 0.5 g/kg per day of LNAA (NeoPhe). The average blood Phe concentration at baseline, taken on three separate occasions, was 932.9 µmol/L. During the week of NeoPhe (LNAA), the average blood Phe concentration dropped to 568.4 µmol/L (average drop of 364.5, SD = 233.2), a decline of 39%, which was highly statistically significant ($p < 0.0001$) as indicated in Fig. 1. Seven patients with classical PKU who adhered to treatment with their PKU formula also showed a drop in blood Phe concentration from baseline of 531.6 µmol/L, which dropped to 281.5 µmol/L (average drop of 250.1, SD = 173.7, $p = 0.009$) when NeoPhe was given (Fig. 1).

The results of the placebo trial showed minor changes in blood Phe concentrations when compared to baseline levels. The average blood Phe concentration changed from 932.9 µmol/L to 882.66 µmol/L, a decline of 5.4%, which was not statistically significant ($p = 0.07$), as indicated in Fig. 2.

Discussion

In a recent report of a one-week open-label trial with LNAA, blood Phe concentrations decreased without any change in the dietary practice of the patients with PKU (Matalon et al 2006). These results were encouraging and seemed to raise the possibility of a new modality for the treatment of PKU. As patients with PKU grow older, their dietary adherence erodes, and this is associated with neuropsychological deficits (Burgard et al 1997; Diamond 2001; Fisch et al 1995; Griffiths et al 1995; Lou et al 1985; Michals et al 1988; Pietz et al 1998; Smith et al 1978; Thompson et al 1990). According to the NIH conference (NIH 2001), the blood Phe concentration should be 120–600 µmol/L in adolescents, a

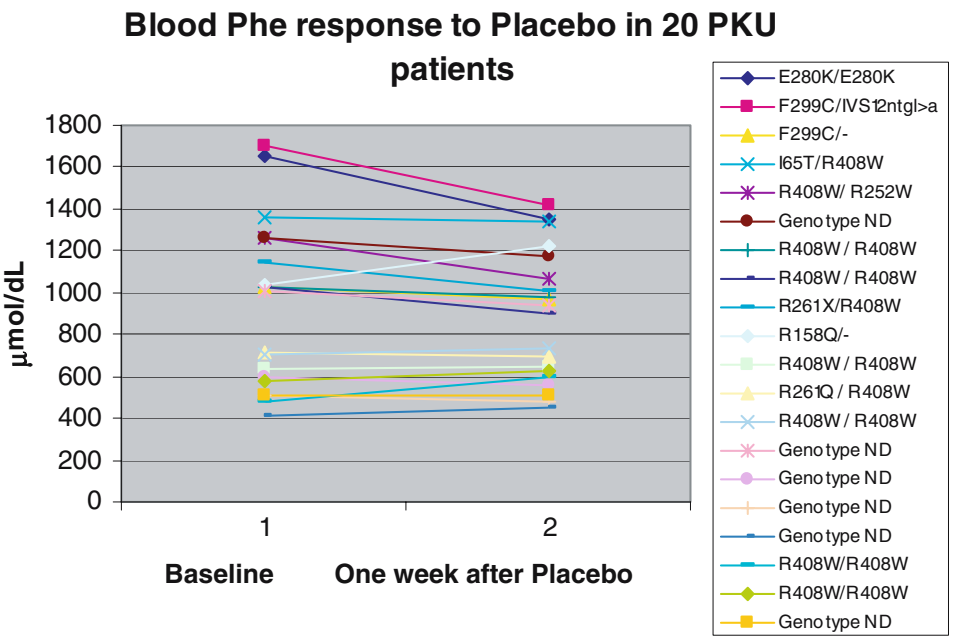

Fig. 2 Blood Phe response to placebo in 20 PKU patients after one week of washout. Average blood Phe concentration at baseline was 932.9 µmol/L, which dropped to 882.6 µmol/L, a decline of 5.4% (not statistically significant)

level difficult to attain in older children and adults (Walter et al 2002). Tetrahydrobiopterin (BH_4) can lower blood Phe in some patients; however, most patients will still require dietary Phe restriction and only mild PKU patients will benefit from BH_4 as the sole method of treatment (Blau and Trefz 2002; Kure et al 1999; Lindner et al 2003a,b; Matalon et al 2002, 2004; Muntau et al 2002; Trefz et al 2000, 2001; Weglage et al 2002).

In the past, trials with LNAA in the treatment with PKU have focused on the transport of Phe to the brain. Trials with tyrosine, 160 mg/kg, to patients with PKU showed increased attention span and neurotransmitter synthesis, as judged by neurotransmitter metabolites in the CSF (Lou et al 1985). However, Pietz and colleagues (1995) gave 100 mg/kg tyrosine to 24 early-treated PKU patients for four weeks and showed no improvement in neuropsychological tests.

Studies using valine 150 mg/kg, isoleucine 150 mg/kg, and leucine 200 mg/kg (VIL) resulted in substantial lowering of Phe in the CSF, but tyrosine was also lowered. The first study of LNAA supplementation in the treatment of PKU was conducted using a formula of LNAA without lysine, such as PreKUnil (Dotremont et al 1995). Four patients were treated for one month using a formula with 0.8 g/kg LNAA and a low-protein diet, 0.6 g/kg. The treatment led to negative nitrogen balance due to lysine deficiency, indicating that such a formulation was not adequate for treatment of PKU.

The current study with LNAA gave consideration to the transport of Phe in the GI tract, where the K_m of the carrier protein in the GI tract is two orders of magnitude higher than that in the BBB. It is interesting that lysine and arginine are also transported by the same carrier protein (Hidalgo and Borchardt 1990; Larsen et al 1964; Pardridge 1982). Experiments by Hidalgo and colleagues (1990) using human intestinal-epithelial cells, Caco-2-cells, in monolayers with a buffer containing 10 µmol/L Phe, showed significant inhibition of Phe transport requiring 100-fold (1 mmol/L) LNAA, as dictated by the K_m equation for affinity of LNAA to the GI carrier protein. For example, at such concentrations, leucine inhibits Phe transport by 55%, tyrosine by 45% and the cationic amino acid lysine by 50%. Competition of LNAA with Phe is likely to occur in the GI tract only if LNAA is given in high concentration. We have shown in PKU mice that when 16.7% of LNAA was added to the normal chow a statistically significant decrease in blood Phe concentration was observed (Matalon et al 2003.)

The success of the open-label trial with LNAA (Matalon et al 2006) in lowering blood Phe concentration in patients with PKU suggests that the carrier protein of the GI tract can be inhibited in the transport of Phe to the blood. The results of the double-blind study clearly show a significantly lowering of the blood Phe concentration while on LNAA (NeoPhe). The reduced concentration of Phe was found in subjects from six participating metabolic centres. The lower blood Phe concentration was observed in seven patients with classical PKU who were on protein-free food and PKU formula, who had an average blood Phe concentration of 531.6 µmol/L which dropped to 281.5 µmol/L (average drop of 250.1 µmol/L, SD $= \pm$ 173.7, $p = 0.009$) (Fig. 1). The reduction of blood Phe in the seven patients was 47%, which is statistically significant.

It is possible that LNAA may contribute to better utilization of Phe, or another anabolic effect. However, an anabolic effect has not been observed in long-term treatment of LNAA in mice with PKU.

It is important to underscore, that LNAA can lower blood Phe concentration in all patients with PKU. Such results are not likely to occur on treatment with BH_4, where the response is mainly in those with mild or atypical PKU. Therefore, LNAA offer a new modality of treatment of PKU when routine treatment with protein-free food and PKU formula are not successful in lowering blood Phe concentrations.

The data presented suggest that long-term studies with LNAA (NeoPhe) are required to establish safety, long-term efficacy and long-term compliance. The number of pills seems high; it is now possible that, with improved technology from the food industry, LNAA can be given in powder form, or chewable forms, and be made more palatable, so that taking LNAA will be more acceptable to patients.

Acknowledgement This work was supported in part by the Genetics Research Trust, the Mid-Atlantic Connection for PKU and Allied Disorders (MACPAD), the South Texas Association for PKU and Allied Disorders (STAPAD), and PKU and Allied Disorders of Wisconsin (PADOW). The authors would like to acknowledge the generous donation of PreKUNil and NeoPhe by PreKU lab, Denmark.

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