Chapter 6

Large neutral amino acids in the treatment of PKU: from theory to practice

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Abstract

Notwithstanding the success of the traditional dietary phenylalanine restriction treatment in phenylketonuria (PKU), the use of large neutral amino acid (LNAA) supplementation rather than phenylalanine restriction was suggested. This treatment modality deserves attention as it might improve cognitive outcome and quality of life in patients with PKU. Following various theories about the pathogenesis of cognitive dysfunction in PKU, LNAA supplementation may have multiple treatment targets: a specific reduction of brain phenylalanine concentrations, a reduction of blood (and consequently brain) phenylalanine concentrations, an increase of brain neurotransmitter concentrations, and an increase of brain essential amino acid concentrations. These treatment targets imply different treatment regimes. This review summarizes the treatment targets and the treatment regimes of LNAA supplementation, and discusses the differences in LNAA intake between the classical dietary phenylalanine restricted diet and several LNAA treatment forms.

Introduction

In phenylketonuria (PKU, OMIM 261600), dietary restriction of phenylalanine (Phe) has been the cornerstone of treatment for over 50 years. The result of dietary treatment is a near-normal cognitive outcome, although mild neuropsychological disturbances may still occur (1). Large neutral amino acids (LNAAs) have been suggested as an alternative treatment to further improve outcome. Possible LNAA treatment targets include reduction of brain Phe concentrations (2-4), reduction of blood Phe concentrations (5), augmentation of cerebral neurotransmitter synthesis (6-9), and/or elevation of brain non-Phe LNAA concentrations (10). In this article, we summarize the reports of the clinical trials of LNAAs, and the rationales behind the use of LNAAs.

Apart from Phe, LNAAs include tyrosine, tryptophan, threonine, methionine, valine, isoleucine, leucine, and histidine (10). In healthy individuals, all of these except tyrosine are essential amino acids. In patients with PKU, tyrosine has become an essential amino acid. LNAA treatment as an alternative to dietary Phe restriction was suggested as early as 1948 (11) and first studied in rats in 1976 (12). Since then, different combinations of LNAAs have been designed, based on different rationales and treatment targets. Some 'LNAA' combinations contain arginine and/or lysine, both of which are not LNAAs.

LNAA treatment is further addressed here through the work discussed by Bodamer and Hung, and Ney in this journal. Together, they show not only the relevance but also the diversity in the use of LNAAs in theory and treatment strategies for patients with PKU.

Rationale A: LNAA supplementation to decrease cerebral Phe concentrations

The underlying rationale for this therapeutic strategy is that elevated brain Phe concentrations are considered to be neurotoxic. Because all LNAAs share a common transport system in order to enter the brain, and high plasma concentrations of LNAAs may block the transport of Phe into the brain (13), increasing blood LNAA concentrations may reduce uptake of Phe into the brain. This hypothesis was first studied in rats with experimental hyperphenylalaninemia. LNAA-treated hyperphenylalaninemic rats were shown to have significantly reduced brain Phe concentrations compared to untreated hyperphenylalaninemic control rats, at similar blood Phe concentrations (12). Later studies in PKU patients showed that supplementation with valine, isoleucine and leucine resulted in slightly improved neuropsychological function in patients with PKU (3,14). More recent studies included the administration of all LNAAs. Pietz et al. (4) and Moats et al. (15) showed that oral LNAA supplementation reduced brain Phe concentrations, and improved

neurophysiological and neuropsychological functioning (4,15,16). Differences in outcome may be related to composition, dosing, route of administration and duration of the supplementation period.

Rationale B: tyrosine and/or tryptophan supplementation to increase cerebral neurotransmitter synthesis

The rationale for this therapeutic strategy is that high plasma Phe concentrations result in decreased brain neurotransmitter concentrations. This is reflected by reduced brain neurotransmitter concentrations in the PKU mouse brain (17-20), and by reduced concentrations of dopamine and serotonin and their metabolites in PKU patients, both in cerebrospinal fluid (CSF) (6,21,22), and in autopsied brain tissue (23). In PKU patients with an unrestricted natural protein intake, tyrosine and tryptophan supplementation have been shown to improve neurotransmitter metabolism, reaction time and vigilance (7-9), suggesting that dietary treatment could be replaced by a combination of tyrosine and tryptophan. Further support for such a treatment strategy was based on the theory of prefrontal lobe dysfunction (24). This theory presumes that the prefrontal cortex is most affected by dopamine depletion, because dopaminergic neurons innervating the prefrontal cortex have relatively high levels of activity and higher dopamine turnover, inducing dopamine dependency. Following these lines of consideration, large doses of tyrosine or L-dopa were expected to have a positive effect. However, later studies with large doses of tyrosine or L-dopa did not show positive results (25-29).

Rationale C: LNAA supplementation to decrease blood Phe concentrations

This therapeutic strategy of supplementing LNAA is based on the rationale that LNAA transport not only occurs at the blood–brain barrier (BBB), but also at the gut–blood barrier. After performing studies in mice (30), Matalon et al. performed an open and a double-blinded trial in patients, who received LNAAs three times daily with meals, while patients' regular diet was unchanged (5,31). A decrease of blood Phe concentrations up to 50% of initial values was found on LNAA supplementation of 0.5–1.0 g/kg/day (5,31). This suggests that LNAA supplementation superimposed on regular dietary treatment may reduce Phe absorption in the gastro-intestinal tract. If reduction in blood Phe concentrations indeed results from reduced Phe absorption, supplementation of all LNAAs at this dosage may not be required, as supplementation with threonine up to 50 mg/kg is reported to be sufficient to reduce blood Phe concentration by 20-50% (32). In addition, the reduction of blood Phe concentrations following LNAA supplementation may at least partly result from increased protein synthesis resulting from increased essential amino acid

availability, in case of essential amino acid deficiency. However, it is largely unknown whether the patients studied may have had essential amino acid deficiencies (due to insufficient intake of natural protein and/or protein substitute), as so far, none of the clinical studies on the effects of LNAA supplementation quantified the amount of natural protein and protein substitute intake patients used. Finally, reduced blood Phe concentrations on LNAA supplementation may just be a matter of timing of blood sampling, as suggested by prof. Bachmann (personal communication).

Rationale D: LNAA supplementation to increase cerebral amino acid concentrations

Reduced brain LNAA concentrations have been reported in PKU mice (18,19,33). In autopsied brain tissue of PKU patients, McKean reported reduced tyrosine concentrations (23). Restoring reduced brain LNAA concentrations in PKU may improve cognitive outcome. The rationale for LNAA supplementation to increase cerebral LNAA concentrations is, again, that LNAAs share the same transport system as Phe for entering the brain. Where rationale A hypothesizes that it is important to decrease the brain Phe concentration, rationale D hypothesizes that a deficiency in any essential amino acid in the brain may affect brain (protein) metabolism. Consequently, rationale D focuses on the effect of LNAA treatment on brain LNAA concentrations, rather than on (a decrease in) brain Phe concentrations. Hoeksma et al. (34) showed that a significant negative relationship exists between plasma Phe concentration and cerebral protein synthesis in patients with PKU. However, this finding does not differentiate between the influence of elevated brain Phe concentrations and reduced brain non-Phe LNAA concentrations on cerebral protein synthesis.

Rationale E: comparison of LNAA intake in LNAA treatment and present dietary treatment

Recent trials with LNAA supplements in PKU patients include LNAA alone (4), LNAA with extra lysine and/or arginine (5,15,16,31,35), or only threonine (32). Whatever combination of amino acids is given, LNAA treatment strategies start with a more or less normal (i.e. unrestricted) intake of natural protein. The response of blood Phe concentrations to LNAA treatment (4,5,15,16,31,35) is comparable to the response of blood Phe concentrations to the protein substitutes in the traditional dietary treatment (36,37).

To illustrate treatment-dependent differences in LNAA intake, we calculated prescribed LNAA intake on several treatment regimes, based on an adult PKU patient with a body weight of 70 kg (**Figure 1**). In **Figure 1**, prescribed LNAA intake

is compared among four different diets: 1. conventional dietary PKU treatment consisting of Phe-restriction combined with synthetic amino acid mixtures; 2. LNAA supplementation (0.5 g/kg/day) with natural protein intake at 0.8 g/kg/day, the recommended daily allowance (RDA) (38); 3. LNAA supplementation combined with conventional dietary treatment; 4. RDA of protein of healthy adults. For calculation of

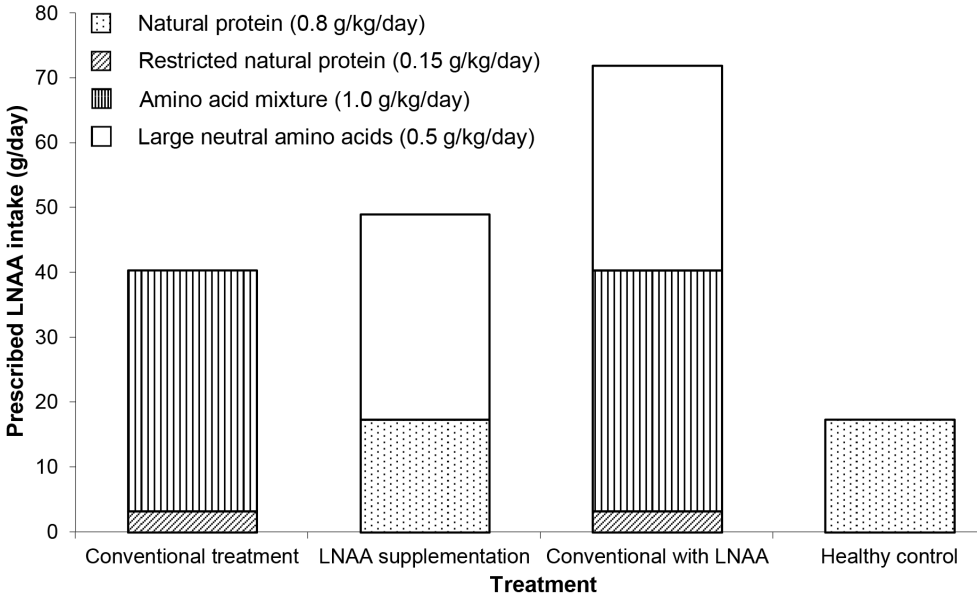

Figure 1 Prescribed large neutral amino acid intake (g/day) on different treatment regimes.

LNAA content of synthetic amino acid mixtures (treatment 1), the mean of the following 14 amino acid mixtures was used: PKU3® (Milupa); PKU3advanta® (Milupa); XP Maxamum® (SHS); Lowphlex Powder® (SHS); Phlexy-10 drinkmix® (SHS); Phlexy-10 tablets® (SHS); PK Aid-4® (SHS); Aminogran® (UCB Pharma); PKU Express Powder® (Vitaflo); Phenex-2® (Ross); Xphe advance® (Metax); Easiphen® (SHS); PKU Express Liquid® (Vitaflo); Lophlex Liquid® (SHS). Regarding LNAA supplementation (treatments 2 and 3), a dose of 0.5 g/kg/day is used, based on several recent trials following the work of Moats et al. and Koch et al. (15,35). Prescribed LNAA intake of these treatments was calculated as the mean of Lanaflex® (SHS international) and NeoPhe® (Solace Nutrition). The dietary regime of LNAA supplementation combined with conventional dietary treatment (treatment 3) reflects the regime studied by Matalon et al. (5,31).

Several assumptions underlie the calculated values. First, the dose of amino acid mixture in conventional treatment is set at 1.0 g/kg/day rather than the RDA

(0.8 g/kg/day), correcting for the assumed 20% of the mixture that is not absorbed in the gastro-intestinal tract. Second, in conventional treatment, LNAA intake resulting from natural protein intake is not taken into account, as the natural protein in this treatment is assumed to be of relatively low nutritional value. However, the distinction between protein of relatively low and high nutritional value has not been applied to the prescribed LNAA intake at RDA of healthy controls, resulting in some inconsistencies.

Based on these calculated prescribed LNAA intakes, **Figure 1** demonstrates that LNAA intake in PKU is higher than in healthy controls, regardless of the specific treatment given. Moreover, LNAA intake on LNAA supplementation is higher than LNAA intake on conventional treatment. However, the difference in LNAA intake between these two treatment forms is small relative to the difference between conventional treatment and the dietary regimen studied by Matalon et al. Finally, LNAA intake on the treatment strategy studied by Matalon et al. clearly results in a higher LNAA intake than either conventional therapy or LNAA supplementation, and markedly exceeds LNAA intake of a healthy control.

When LNAA treatment is discussed, not only the dose but also the composition is important. It is remarkable that LNAA treatment would need the addition of the non-LNAA lysine and arginine. When the primary aim is to block the entrance of Phe into brain, this would favour LNAA supplements consisting of specific amino acid such as leucine, isoleucine and methionine, which are considered to be highly effective at decreasing the cerebral Phe concentration (39). Following that hypothesis, giving 0.5 g/kg/day of all LNAA with also lysine and arginine might be changed into 0.5 g/kg/day of leucine, isoleucine and/or methionine (39). When the primary aim is to increase the influx of tyrosine and tryptophan, this would favour the supplementation of only tyrosine and tryptophan to increase the synthesis of the neurotransmitters dopamine and serotonin in brain. Following that hypothesis, giving 0.5 g/kg/day of all LNAA (with or without lysine and/or arginine) might be changed into 0.5 g/kg/day of tyrosine and tryptophan. This is clearly above the doses ever given to PKU patients so far (6-9). When, however, increasing the cerebral concentrations of all essential amino acids is the primary aim, all essential amino acids should be given in such a way that normal concentrations of all essential amino acids are achieved, taking the K_m of each amino acid into account.

Summary of current treatment strategies in LNAA supplementation

LNAA supplementation may have several treatments aims: reducing brain Phe concentrations, reducing plasma Phe concentrations, increasing cerebral neurotransmitter concentrations, and increasing cerebral essential amino acid

concentrations. Although LNAA treatment in PKU was initially intended to be unrestricted natural protein intake combined with LNAA supplementation, it should be noted that not all authors studied LNAA supplementation in this manner. This deserves attention when interpreting the findings below.

First, LNAA supplementation has been found to reduce brain Phe concentrations in patients, even at mean plasma Phe concentrations >1000 µmol/L (4,35). Second, LNAA supplementation may reduce plasma Phe concentrations (5,31,32), although not all authors report this effect (4,15,35). Third, data suggest that supplementation of tyrosine and tryptophan only does not sufficiently correct biochemical and neuropsychological abnormalities. However, recent LNAA trials supplement LNAA at 0.5 g/kg/day, a dose much higher than studied for tyrosine and tryptophan supplementation. At this dose, the effect of LNAA supplementation on cerebral neurotransmitter concentrations is currently unknown. Fourth, LNAA supplementation combined with unrestricted natural protein intake has been suggested to increase cerebral essential amino acid concentrations. This hypothesis remains to be studied in future trials.

In conclusion, LNAA treatment is seen as an alternative to conventional dietary PKU treatment. Although usually considered to refer to a single specific treatment modality, this paper shows that LNAA treatment may refer to at least two different LNAA treatment strategies, i.e. natural protein intake at RDA with LNAA supplementation, and conventional dietary therapy combined with LNAA supplementation. In addition, LNAA treatment may refer to supplementation of single amino acids, such as tyrosine, tryptophan, and threonine. These differences in treatment strategies are based on clearly different theories regarding mechanism of action. Consequently, more in-depth studies are necessary to investigate the potential role, dose and composition of LNAA in PKU treatment.

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