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Large neutral amino acid therapy and phenylketonuria: a promising approach to treatment☆

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Abstract

Six subjects with classical phenylketonuria (PKU) were treated with large neutral amino acid supplements (PreKUnil, Nilab, Dk) at 0.4 g/kg/day in equally divided doses three times each day on an increased natural protein diet. All six subjects had low or deficient blood concentrations of both tyrosine and tryptophan, which are precursors for dopamine and serotonin, respectively, at the beginning of the study and were increased substantially throughout the study. Blood phenylalanine concentrations remained essentially unchanged, while the brain phenylalanine concentrations gradually decreased toward the carrier range as seen in parents of children with PKU. Two subjects were diagnosed with clinical depression and were in counseling programs at initiation of the study. At the end of the study all patients reported increased energy and overall improvement in well-being.

Keywords: Magnetic resonance spectroscopy; Tyrosine; Tryptophan; PreKUnil; Phenylalanine hydroxylase; Dopamine; Serotonin

Introduction

The phenylalanine (Phe)-restricted diet has been the mainstay of treatment for phenylketonuria (PKU) since its development by Horst Bickel et al. [1]. Implementation of the Phe-restricted diet shortly after birth reduces blood Phe concentrations, avoiding the severe consequences of untreated PKU, such as mental retardation. However, the diet is very difficult to adhere to over time [2–4]. We estimated that at least 60% or more of adults and adolescents with PKU at our clinic are not following a Phe-restricted diet.

The diet regime restricts the intake of Phe in natural foods and requires a medical product, which is high in all the other amino acids necessary to synthesize protein along with vitamins, minerals, and essential fatty acids. Despite the attempts by the manufacturers to make it palatable, many individuals, especially adolescents and adults, reject the taste and the quantity that must be consumed. The diet regime usually is supplemented with low protein products such as breads, pastas, and baking products, which are extremely expensive and difficult to cook. Many states do not cover the cost of these products [5].

The role of large neutral amino acids (LNAA) and their transport to the brain in PKU has been a topic since 1953 when Christensen [6] proposed that the high level of Phe in the blood interferes with the transport of other LNAA into the brain. In 1976 Andersen and Avins [7] reported that brain Phe levels were lowered in rats by giving large doses of Phe and a single amino acid or a combination of other LNAA. This prompted researchers to seek alternatives to the treatment by adding supplements of other large neutral amino acids [8–12]. However, these approaches were not widely accepted. Since that time, there have been several studies using Magnetic Resonance Spectroscopy (MRS) to measure

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brain Phe concentrations and its significance in clinical outcome [13–15]. Recently, Pietz et al. [16] confirmed that the LNAA blocked the Phe transport into the brain by giving an oral ''Phe challenge'' with and without LNAA and measuring the influx of Phe into the brain.

The results of a pilot study conducted at Childrens Hospital Los Angeles using MRS to evaluate brain Phe concentrations compared to blood Phe concentrations using LNAA therapy is the subject of this report.

It should be noted that since 1985 The John F. Kennedy Institute in Denmark has pioneered the use of LNAA therapy on PKU adolescents and adults with difficulties adhering to the Phe-restricted diet regime. They report that despite elevated Phe concentrations no change in MRI or aggravation of cerebral involvement after a 2-year trial [17] and after 15 years of treatment no cognitive difference or adverse effects between those adolescents and adults on the standard diet compared to those on PreKUnil [18].

Methods

Six individuals (4 females and 2 males) ranging in age 20–34 years (Table 1) agreed to participate in this study which was approved by the investigational review board and informed consent was obtained from each participant according to hospital policy. At baseline 24 h, 48 h, one month, and six months, each subject received an MRS and plasma amino acids were obtained. Diet records were obtained and analyzed at each visit. Immediately after baseline, measurements were obtained and each subject was given PreKUnil tablets (Nilab, Dk) at 0.4 g/kg body weight. The composition of LNAA per PreKUnil tablet consists of L forms of tyrosine, tryptophan, methionine, isoleucine, threonine, valine, leucine, histidine, and arginine. The average dose was 10 tablets to be taken before each meal (3 times each day). They were instructed to consume a ''relaxed'' diet, which included unlimited fruits, vegetables, and grains, with small amounts of cheese and yogurt and up to two small servings per week of meat, if desired. Vitamin/mineral tablets were added as well as calcium supplements.

The MRS procedures have been well described [19–23]. Mutation analyses of the phenylalanine hydroxylase gene were performed at the John F. Kennedy Institute in Glostrup, Dk [24]. Plasma amino acids were obtained using a Hitachi 8800 amino acid analyzer. Diet records were analyzed using the Amino Acid Analyzer, version 3.3 produced by Ross Metabolics, Abbott Laboratories.

Results

Table 1 details the subjects' profile. All were classified as classical PKU with severe mutations. The diet records were collected and analyzed at each visit and indicate their average protein intake ranged from 0.6 to 1.0 g/kg each day.

The mean blood Phe value for all six subjects at baseline was 1.448 mM (24 mg/dl). Subsequent means at 14 and 48 h after starting on oral PreKUnil tablets at the recommended dose of 0.4 tablets/kg/day were 1.398 and 1.453 mM (23 and 24 mg/dl), respectively. The mean levels at one month and six months of therapy were slightly lower at 1.345 and 1.315 mM (22 mg/dl) (see Fig. 1). Blood tyrosine (Tyr) and tryptophan revealed a different pattern of response. Over the period of treatment a gradual increase of tyrosine from the baseline value of 0.033 (0.5 mg/dl) to 0.081 mM (1.3 mg/dl) (Fig. 2) and tryptophan from 0.030 (0.72 mg/dl) to 0.073 mM

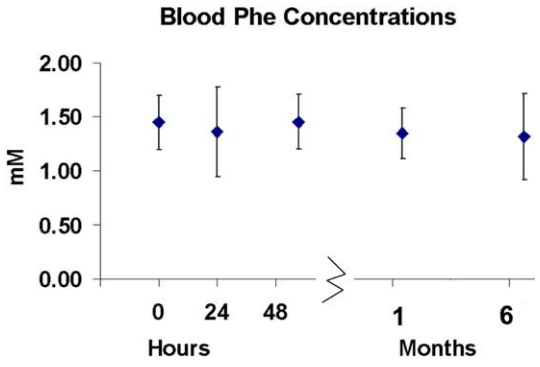

Fig. 1. Mean blood Phe over six months.

Table 1
Subject profile

Subject	Age (yr)	Sex	Diet	Mutation	Weight (kg)	Protein intake[a] (g/kg)	Phe intake (mg/kg)
1	29	F	Y	R408W/R408W	65	0.8	38
2	34	M	Y	Y387H/delT323	78	1.0	43
3	34	F	N	R158Q/IVS10nt-11	90	0.7	32
4	20	M	Y	IVS12nt1g → a/I65T	70	0.8	33
5[b]	33	F	N	L348V/L348V	48	1.0	43
6[b]	24	F	N	IVS12nt/I65T	84	0.6	23

[a] Includes protein equivalent from PreKUnil.
[b] Off diet > 10 years.

(1.8 mg/dl) (Fig. 3). In contrasting the blood data to the brain Phe concentrations, results over the six months of treatment resulted in a decrease from 0.452 mM at baseline to 0.265 mM (Fig. 4). All subjects reported having more energy while on the PreKUnil and felt better overall. One subject was lost to follow up after three months in the study due to unknown reasons. Two subjects who were depressed also clinically improved.

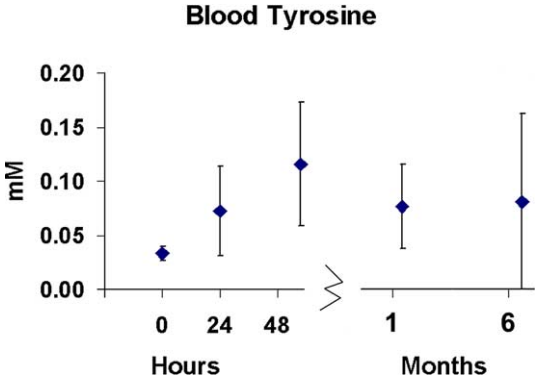

Fig. 2. Mean blood tyrosine over six months.

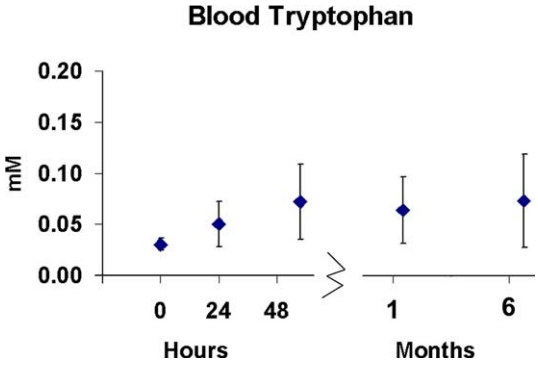

Fig. 3. Mean blood tryptophan over six months.

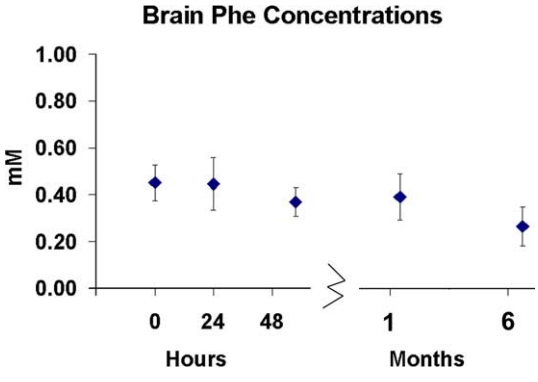

Fig. 4. Mean brain Phe over six months.

Discussion

For over 40 years the focus of treatment for persons with PKU has been on blood phenylalanine control. At this institution we have performed MRI/MRS studies on over a hundred PKU individuals to determine brain Phe concentrations compared to blood Phe concentrations. We have data on 5 normal controls and 4 carriers (parents of PKU individuals) [25]. Based on our observations, we believe that brain Phe concentration can be used as a measure to determine appropriate blood Phe concentration on an individual basis.

In this study three of the four females participating have been off diet for over 10 years with blood Phe concentrations well over 1.2 mM (20 mg/dl). Since PreKUnil is not recommended for pregnant females due to the increased blood Phe concentrations above the recommended range for pregnancy, all female participants were taking birth control measures.

Subjects 1 and 2 had always taken the prescribed medical product but blood Phe concentrations were typically above 1.2 mM (20 mg/dl). It is interesting to note that the blood Phe concentrations overall were reduced slightly despite an increased protein diet and the brain Phe concentrations showed a clear reduction over the six months with wide variability. This trend is consistent with Dr. Reuben Matalon's experiments in the PKU mice that were supplied PreKUnil tablets [26].

The two persons observed with mild depression were both involved in counseling programs. Both had made significant clinical improvement by the end of the study. In all six subjects blood concentrations of tyrosine and tryptophan were in the low range or deficient despite the increased protein intake at baseline. Also, the ratio of Phe/Tyr was decreased on average from 44.5 to 15.1.

In two subjects leucine and isoleucine were in the deficient ranges prior to PreKUnil therapy. Both were normalized after 48 h of treatment. It is highly suggestive that the depression that we see in PKU is related to diminished dopamine and serotonin levels in the brain caused by the low blood concentrations of tyrosine and tryptophan [27–29]. However, the brain Phe concentrations also decreased as well as the ratio of brain to blood Phe, which may be a contributing factor.

Based on the Danish experience and in these preliminary findings, we suggest that balancing the ratios of amino acids in the blood and the subsequent effect this has on the level of those amino acids in the brain is of relevance in maintaining intellectual achievement and neurological health. The impact of this novel approach to treatment also has significance for treating the depression, which often accompanies persons with PKU off dietary treatment. No adverse clinical symptoms occurred due to the approach to treatment. Clinically, all six subjects reported improvement of general demeanor and increased energy despite similar blood Phe

concentrations maintained throughout the treatment period. The two subjects who were mildly depressed at the initiation of treatment reported overall improvement as well. The occurrence of depression is a clinically serious disorder and was the basis for offering them the opportunity to participate in this study. Future studies are needed to evaluate and quantify these psychological findings on a larger scale.

In view of the fact that many adults and adolescents are either off diet or do not adhere to the Phe-restricted, it would be prudent to offer the LNAA therapy. The use of the LNAA therapy has been shown to improve amino acid profiles as well as increase tyrosine and tryptophan concentrations in the blood, which are precursors for dopamine and serotonin. Our findings indicate that the brain concentration of phenylalanine decreases toward the carrier range within six months of therapy with PreKUnil tablets despite increased natural protein intake. In addition, the product was well accepted by all six subjects.

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