Behavioural effects of phenylalanine-free amino acid tablet supplementation in intellectually disabled adults with untreated phenylketonuria

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Abbreviations

AA, amino acid

CSF, cerebrospinal fluid

LNAA, long neutral amino acid

PAH, phenylalanine hydroxylase

Phe, phenylalanine

PKU, phenylketonuria

Keywords: Untreated PKU, behaviour, amino acid tablets, diet supplementation

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Abstract

Aim: To evaluate the effects of phenylalanine (Phe)-free essential amino acid (AA) tablets enriched in tyrosine and tryptophan on the performance of intellectually disabled adult patients with untreated phenylketonuria (PKU).

Methods: Phe-free AA tablets and placebo tablets were administered to 19 untreated PKU subjects on a normal diet for six months in a prospective double blinded cross over study. The adaptive behaviour of the patients was tested prior to the study and at six and 12 months after the start, using a simplified version of the Vineland Adaptive Behaviour Scale. For each sub-domain, the patients were rated either "0" (for poor performance) or "1" (for good performance). Neurological signs and symptoms and specific behavioural characteristics were recorded monthly by caretakers. Every six months, neurological examination of the patients was performed, and the caretakers were interviewed. The statistical significance of the results was tested by means of the Fisher's exact and Wilcoxon tests.

Results: The most significant changes were an improved concentration and the development of a meaningful smile, which were observed in 44% and 43% of the patients on AA tablet treatment, respectively, but not patients on placebo. Other important, but less significant changes included increased awareness of external stimuli (63%) and less self-injury (43%), and 40% were smiling and laughing occasionally. The mean overall rating increased from an initial value of 6.3 to 10.1 in patients when on AA tablet treatment ($p=0.002$), and to 7.0 in patients when on placebo ($p=0.068$). The difference between active AA treatment and placebo was statistically significant ($p=0.027$).

Conclusions: This pilot study suggests that Phe-free AA tablets enriched in tyrosine and tryptophan may improve the quality of life in some intellectually disabled adults with untreated PKU.

Introduction

Phenylketonuria (PKU) is a common disorder of amino acid metabolism caused by a deficiency of the liver enzyme, phenylalanine hydroxylase (PAH). Untreated PKU usually results in progressive mental retardation with IQ scores <50, and this deterioration is related to blood phenylalanine (Phe) levels (1). The chronically high levels of Phe in untreated patients also cause emotional dysfunction (e.g. depression), behavioural changes, and neurological complications (e.g. epilepsy, ataxia, tremor, and spasticity) starting in early childhood and progressing during adolescence (2-4). Early diagnosis and treatment with a Phe-restricted diet prevent mental retardation and other complications (5), provided that blood Phe levels are maintained at near-normal levels during childhood, perhaps even for life (1, 6).

In countries with screening programs, virtually no new cases of untreated PKU are reported. However, in countries where an efficient screening program has not yet been implemented, it is

important to improve the quality of life for untreated PKU patients by reducing psychological, behavioural and health-related problems. Previous studies have suggested that untreated adults with PKU may benefit from a low-Phe diet (7-9). However, due to the difficulties associated with dietary therapy in intellectually disabled patients, there has been considerable interest in alternative treatment regimens, including treatment with large neutral amino acids (LNAAs).

In a recent study, we showed that treatment with Phe-free essential amino acid (AA) tablets enriched in tyrosine and tryptophan was sufficient to maintain intellectual achievement and neurological health in young PKU adults on a liberalized diet. No adverse clinical symptoms occurred during this approach to treatment (Ref. 10, and manuscript in preparation). Here, we have conducted a prospective double blinded placebo-controlled cross over study to examine the possible effects of these AA tablets on behaviour and neurological symptoms in patients with severe intellectual disability due to untreated PKU.

Patients and Methods

We approached all late-diagnosed PKU patients in Denmark (N=60) for possible participation in this project. The majority of these patients had severe or profound intellectual disability and were unable to give their consent. For 41 of the patients, caretakers or family members declined participation in the study. Consent was obtained for the remaining 19 patients, who were the subjects of this study. There were seven females and 12 males, age 32 to 82 years (Table 1).

PAH gene mutations were analysed as previously described (11). There were no changes in the patients' food, and their blood Phe levels showed only little variation during the project. The patients were divided into two groups and each group was treated with either Phe-free AA tablets (PreKUnil®, PreKULab, Denmark) or placebo tablets for a six-month period and the opposite treatment for the next six months. Caretakers were advised to divide the daily dosage of AA tablets into three doses and to give them along with a mixed meal in order to obtain optimal benefits of the amino acid composition.

To test the behaviour of the patients, we developed a scale that is based on the "Vineland Adaptive Behaviour Scale" and composed of simple and relatively concrete descriptive items. This scale allows an acceptable differentiation of sub-domains and contains all psychometric characteristics necessary for making a meaningful conclusion on the mental performance of the patients (Table 2). The adaptive behaviour of the patients was tested prior to the study and at six and 12 months after the start. In addition, the caretakers recorded neurological signs and symptoms and specific behavioural characteristics monthly in a questionnaire. Every six months, neurological examination of the patients was performed by one of us (HSK), and the caretakers were interviewed. Patients, caretakers and raters had no knowledge about the actual phase of treatment.

For each sub-domain, the patients were rated either “0” (for poor performance) or “1” (for good performance). The overall rating for each patient was calculated as the sum of the sub-domain ratings. The data were analysed using the 2-tailed Fisher's exact test and the Wilcoxon matched-pairs test (SPSS Version 10.0).

Results

Nineteen intellectually disabled adult patients with untreated PKU were included in this study. The overall level of functioning of these subjects at the start of the study is described in Table 1. In all subjects, *PAH* mutation genotype and serum Phe levels were consistent with classical PKU (12) (Table 1). Phe-free AA and placebo tablets were administered on a normal diet for six months in a cross over study. The most significant changes observed in patients on AA tablet supplementation were improved concentration (7 of 16 patients who rated “0” before treatment, 44%) (Table 3) and the development of a meaningful smile (6 of 14, 43%) (Table 4). Four of 10 (40%) were smiling and laughing occasionally (Table 3). None of these changes was observed in the patients when on placebo. In addition, 10 of 16 patients (63%) became more aware of external stimuli (54%), 3 of 7 (43%) had a less self-injurious behaviour, and 5 of 11 (46%) could maintain eye contact. However, these changes were also observed occasionally in patients on placebo. Even though our study included self-oriented patients reluctant to group work, two of the patients started participating in group activities during AA treatment (Table 4). One of the patients became more aggressive and anxious while on AA tablet supplementation (pt. 1), probably because he was not used to communicating with his surroundings.

Among the 19 patients, four had epileptic seizures and were treated with antiepileptic drugs. Supplementation with AA tablets did not decrease the number of epileptic seizures in these patients. Furthermore, there were no changes in gross or fine motor functions or in daily living skills in any of the 19 patients (data not shown). One of the patients, however, helped the caretakers in preparing the dinner table while taking AA tablets. Finally, no significant changes were observed in obeying rules or in politeness. Five of the 19 patients (26%) did not show any improvement while on treatment with AA tablets.

To evaluate more broadly the effects of Phe-free AA treatment on the patients’ performance, we calculated the overall rating for each patient. The mean overall rating for the 19 patients increased from an initial value of 6.3 (S.D. 6.1) to 10.1 (S.D. 5.4) in patients when on AA tablet treatment ($p=0.002$), and to 7.0 (S.D. 6.0) in patients when on placebo ($p=0.068$). The difference between active AA treatment and placebo was statistically significant ($p=0.027$).

Discussion

Despite the beneficial effects of dietary treatment in adults with PKU (7-9), this type of treatment has not gained widespread use, primarily due to difficulties in introducing and adhering to a Phe-restricted diet. The common problems associated with dietary compliance in adolescence and young adults are even greater in intellectually disabled PKU patients who for the first time are offered a Phe-restricted dietary regimen (13, 14), underlining the need for alternative treatment regimens.

Several studies have suggested that LNAA supplementation may be helpful for PKU individuals who are unable to maintain low serum Phe levels (10, 15-17). Here, we studied the behavioural effects of treating intellectually disabled PKU patients with Phe-free AA tablets enriched in tyrosine and tryptophan. A weakness of this study was that informed consent could be obtained from only 19 of the 60 living Danish patients with late-diagnosed PKU. Furthermore, the study would probably have been strengthened by an A-B-A design instead of the A-B design, and by the inclusion of a wash-out between each phase. Nevertheless, all examined parameters were likely to be readily reversible upon discontinuation of treatment, and the main examination of the patients was performed at the end of each phase, which would minimize on-off and carry-over effects. The most frequent changes associated with this treatment were improved concentration, development of a meaningful smile and increased awareness of external stimuli. Also, an important fraction of the patients had a less self-injurious behaviour, could maintain eye contact, and were smiling and laughing occasionally. These changes are similar to those observed in intellectually disabled PKU patients treated with a Phe-restricted diet (13, 14), suggesting that AA tablets may be a valid alternative to dietary therapy.

In 1982, Berry et al. (18) reported that oral administration of valine, isoleucine and leucine significantly reduced the cerebrospinal fluid (CSF) levels of Phe in PKU patients on an unrestricted diet. Later it was shown that supplementation of a relaxed diet with large doses of tyrosine and tryptophan normalized blood levels of these amino acids as well as the levels of dopamine and serotonin metabolites in CSF (19, 20). A recent study (17) showed that treatment of PKU patients on a normal diet with Phe-free AA tablets enriched in tyrosine and tryptophan increased blood concentrations of tyrosine and tryptophan to normal levels. Furthermore, the brain Phe concentrations showed a significant reduction over a six months period. These data suggest that the improvements observed in the present study of intellectually disabled PKU patients treated with AA tablets may be due to both a reduction of Phe concentrations and an increase in dopamine and serotonin synthesis in the brain.

In summary, Phe-free AA tablets enriched in tyrosine and tryptophan may be offered for treatment of intellectually disabled individuals with untreated PKU to improve their quality of life.

After this study was terminated, it was considered unethical to put patients who had shown clear improvements on AA treatment back on normal diet, and the Danish government has recently approved to cover the expenses for this treatment in the future. However, because AA treatment is only beneficial to some of the patients, and because it cannot be predicted in advance who would actually benefit, implementation of this treatment should be based on individual trials.

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Table 1 Clinical characteristics and *PAH* mutation genotypes of the patients included in the study

Patient No	Age	Sex	Genotype	Phe levels (µmol/l) during the study period	Self managing[b]	Self destructive	Walking	Talking
1	49	M	IVS10nt-11g>a/I174T	1383 (849-1684)	Yes	No	Yes	No
2	82	M	IVS12nt1g>a / n.d.	1270 (1020- 1684)	Yes	No	Yes	Yes
3	50	F	E280K/V245E	n.d.	Yes	Yes	Yes	No
4	72	M	R408W / R408W	1726 (1418-2120)	No	Yes	No	No
5	55	F	IVS12nt1g>a /P281L	1628 (1413- 1868)	No	Yes	Yes	No
6	48	M	R408W / R158Q	852 (537-1387)	No	Yes	Yes	No
7	38	M	IVS12nt1g>a / I174T	1656 (1320- 2100)	No	No	Yes	No
8	75	M	R408W / n.d.	1393 (1201- 1554)	No	Yes	Yes	Yes[c]
9	77	F	IVS12nt1g>a / IVS10nt-11g>a	1265 (1132- 1374)	No	Yes	Yes	No
10	53	M	IVS12nt1g>a / IVS12nt1g>a	1391 (1176- 1710)	Yes	No	Yes	No
11	49	M	IVS12nt1g>a / IVS12nt1g>a	1567 (1383- 1821)	Yes	No	Yes	Yes
12	40	F	IVS12nt1g>a / IVS12nt1g>a	1312 (1090- 1760)	No	No	Yes	No
13	43	F	IVS12nt1g>a / IVS12nt1g>a	1344 (1175- 1675)	No	Yes	Yes	No
14	50	F	R408W/P281L	1378 (1025- 1851)	Yes	No	Yes	Yes
15	45	F	IVS12nt1g>a / IVS12nt1g>a	1339[a]	No	No	No	No
16	61	M	IVS12nt1g>a/IVS12nt1g>a	1331 (1192- 1436)	No	No	Yes	No
17	53	M	IVS12nt1g>a / R408W	1545 (1202- 1884)	Yes	No	Yes	Yes[c]
18	51	M	IVS12nt1g>a / R408W	1530 (1286- 1792)	Yes	Yes	Yes	Yes[c]
19	51	M	IVS12nt1g>a / IVS12nt1g>a	1667 (1307- 2054)	No	Yes	Yes	No

n.d., not determined; [a]Only one value recorded; [b]Eating and using the bathroom without support; [c]Only single words

Table 2 Checklist for assessment of behavioural changes in severely retarded patients, adapted from the Vineland Adaptive Behaviour Scale

COMMUNICATION SKILLS		
Receptive	*Expressive*	
Awareness of external stimuli	Smiling	
Concentrating	Laughing	
Turning eyes and head towards sound	Humming of tunes	
	Talking	
Following instructions	Less self-injury	
Listening and attending		
SOCIAL SKILLS		
Interpersonal Relationship	*Play and Leisure Time*	*Coping Skills*
Looking other people in the eye	Hitting buttons and musical keyboards	Screaming
Meaningful smile		Shouting
Aggressiveness	Participating in group activities	Following rules
Anxiety	Playing with toys	Showing politeness
	Rubbing soft toys	
MOTOR SKILLS		
Gross	*Fine*	
Walking	Putting cubes into cups	
Running	Making tower with cubes	
Jumping	Playing with balls	
Sitting without support		
Climbing		
DAILY LIVING SKILLS		
Personal	*Domestic*	*Community*
Eating without support	Preparing food and table	Using telephone
Bathroom education	Stealing food from others' plate	Understanding hot and cold
Using diaper	Preparing bed	Understanding money

Table 3 Changes in patients' communication skills during treatment with amino acid (AA) tablets. Only patients who rated "0" before treatment are included.

	Number of patients presenting improvements when on		p value
	AA tablets	**placebo**	
Receptive			
Awareness of external stimuli	10/16	4/16	0.073
Concentration	7/16	0/16	**0.007**
Turning eyes and head towards sound	0/12	0/12	-
Following instructions	0/7	0/7	-
Listening and attending	2/13	0/13	0.48
Expressive			
Smiling	4/10	0/10	0.087
Laughing	4/10	0/10	0.087
Humming of tunes	1/16	1/16	-
Talking	0/16	0/16	-
Less self-injury	3/7	1/7	0.56

Table 4 Changes in patients' social skills during treatment with amino acid (AA) tablets. Only patients who rated "0" before treatment are included.

	Number of patients presenting improvements when on		p value
	AA tablets	**placebo**	
Interpersonal Relationship			
Looking other people in the eye	5/11	1/11	0.15
Meaningful smile	6/14	0/14	**0.02**
Less aggressive	6/12	3/12	0.40
Less anxious	4/14	2/14	0.65
Play and Leisure Time			
Hitting buttons and musical keyboards	3/12	0/12	0.22
Participating in group activities	2/12	0/12	0.48
Playing with toys	0/9	0/9	-
Rubbing soft toys	0/5	0/5	-
Coping Skills			
Less screaming	5/10	3/10	0.65
Less shouting	3/10	1/10	0.58
Following rules	0/17	0/17	-
Showing politeness	0/17	0/17	-