A diet with large neutral amino acids supplementation as a combined treatment for difficult to control or late diagnosed patients with PKU – preliminary data

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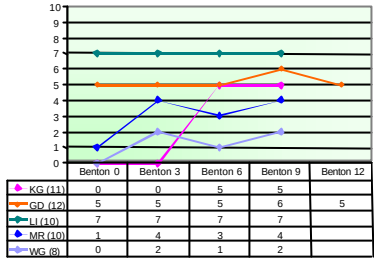
Introduction

The difficulties in maintaining a low-Phe diet, in some older children treated for PKU are well known. Untreated adults, especially mentally retarded consist another group of patients, for whom lowering the high Phe levels by dietary treatment, even if significantly beneficial in most of the cases, may not be easily accepted, producing many everyday difficulties.

On the other hand it was already proven that CSF concentrations of tyrosine and tryptophan, essential for dopamine, serotonine and norepinephrine synthesis, are reduced in hyperphenylalaninemia (HPA). Although great progress is being made on the field of understanding the molecular basis of PKU, the exact mechanism underlying impaired brain function remain still unclear. Direct neurotoxicity of high Phe levels and imbalance in large neutral amino acids (LNAAs), including beside of tyrosine and tryptophan also phenylalanine, leucine, isoleucine, valine and the others, seem to be the major cause. LNAAs compete with each other for entry into the brain by a common transport mechanism. Those, competition for the same carrier in brain-blood-barrier (BBB) might be the possibility to lower Phe influx, in the situation when the plasma levels of remain LNAAs are increased. This phenomena was a basis for developing a new strategy for PKU treatment, in which supplementation with LNAAs could permit the use of less restricted, low protein diet.

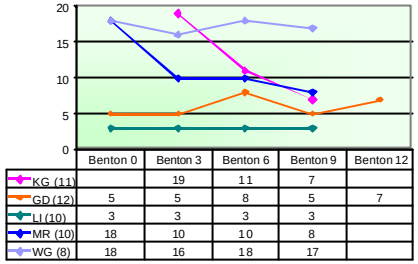
PreKUnil tablets (Nilab, Denmark) contain a large amount of LNAAs, mainly tyrosine and tryptophan, which by the competition for the same carrier, lower Phe transport into the brain and additionally restore the storages of neurotransmitters which are diminished in patients with high Phe levels. PKU patients on PreKUnil treatment are allowed to eat 80% of their daily protein requirement in the form of normal food, including products like ordinary bread, cereal, pasta, low-protein milk, fat meat, etc, which are avoided in the conventional low-Phe diet. High-protein foods, like low- fat meat, fish and eggs are not recommended. The remaining 20% of daily protein is given with the tablets. They should be taken three times a day, together with one of the main meals in the number calculated by multiplying body weight with a factor of 0.388. The maximum dose is 30 tablets per day. The Phe plasma concentration should be kept below 1500µmol/l.

This alternative method of PKU treatment has been used in Denmark, under the supervision of John F. Kennedy Institute since 1985 for more than 80 adults with PKU.



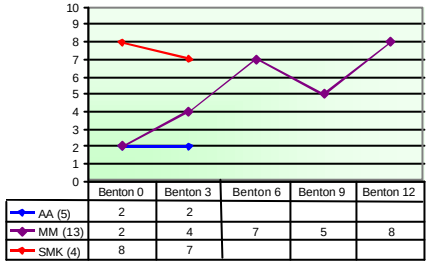
	Benton 0	Benton 3	Benton 6	Benton 9	Benton 12
KG (11)	0	0	5	5	
GD (12)	5	5	5	6	5
LI (10)	7	7	7	7	
MR (10)	1	4	3	4	
WG (8)	0	2	1	2	

Fig. 7. Group I Benton Visual Memory Test - number of correct items after 0, 3, 6, 9, 12 months of treatment with PreKUnil.



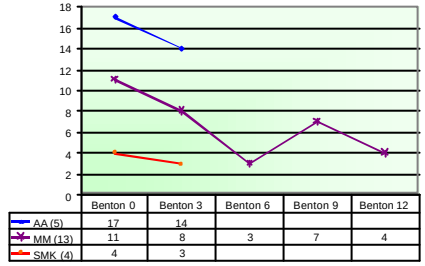
	Benton 0	Benton 3	Benton 6	Benton 9	Benton 12
KG (11)		19	11	7	
GD (12)	5	5	8	5	7
LI (10)	3	3	3	3	
MR (10)	18	10	10	8	
WG (8)	18	16	18	17	

Fig. 8. Group I Benton Visual Memory Test - number of mistakes after 0, 3, 6, 9, 12 months of treatment with PreKUnil.



	Benton 0	Benton 3	Benton 6	Benton 9	Benton 12
AA (5)	2	2			
MM (13)	2	4	7	5	8
SMK (4)	8	7			

Fig. 9. Group II Benton Visual Memory Test - number of correct items after 0, 3, 6, 9, 12 months of treatment with PreKUnil. * Patient SMN was not tested.



	Benton 0	Benton 3	Benton 6	Benton 9	Benton 12
AA (5)	17	14			
MM (13)	11	8	3	7	4
SMK (4)	4	3			

Fig. 10. Group II Benton Visual Memory Test - number of mistakes after 0, 3, 6, 9, 12 months of treatment with PreKUnil. * Patient SMN was not tested.

Methods

In present study , with the permission of Ethic Commission of Pomeranian Medical Academy in Szczecin, 9 patients with classical PKU were introduced to a low-protein diet, supplemented with LNAAs (PreKUnil tablets). Among them, there were 5 early-treated patients, with moderate or poor diet control, with special difficulties in compliance to dietary regiment and unsatisfactory intellectual development (Group I), and 4 late- detected patients (Group II), who were never on a diet (2 patients) or abandoned the diet 4 years ago (2 patients). The intellectual ability of the patients from Group II differed significantly between individuals. The main characteristics of patients present tab. 1.

Tab. 1. The main characteristics of patients from group I and group II.

	Group I	Group II
number of patients	5	4
age (years)	13 – 19 (mean 15.2)	22 – 39.5 (mean 27.8)
time of PKU diagnosis (years)	newborn period	1 – 38 (mean 15.5)
Phe levels at the beginning of PreKUnil treatment (µmol/l)	450 – 1350 (mean 860)	1240 – 2100 (mean 1650)
Phe levels during 1 year prior to the treatment with PreKunil (µmol/l)	360 – 1650 (mean 920)	720 – 2490 (mean 1830)
Phe levels during 5 years prior to the treatment with PreKunil (µmol/l)	420 – 1470 (mean 840)	no data
IQ at the beginning of PreKUnil treatment	40 –74 (mean 56)	below 25; 58; 74; 97;
duration of treatment with PreKUnil (months)	8 – 11 (mean 10.2)	4 – 13 (mean 6.5)

The low-protein diet supplemented with PreKUnil tablets was introduced gradually, during a short stay at the hospital. Patients and their relatives were trained about the dietary restrictions and principles of PreKUnil treatment. Patients were recommended to maintain the Phe levels below 1500µmol/l. Phe levels were controlled every 1-2 weeks.

Tab. 2. Changes in patients' behavior during PreKUnil treatment noticed by their parents (patient from Group I/patients from Group II).

		Number of patients with distortions presented prior to the treatment with PreKunil	Noticed changes	Comments:
Emotional changes	Improvement of psychological state	9	9	
	Decreased number of destructive behaviours	7 (4/3)	5(4/3)	2 patients: only temporal improvement[1]
	Better mood, patients more often feel happy	9	9	
Changes in the area of social contacts	Improvement of relationships with family members	9	8 (4/4)	1 patient: only temporal improvement[2]
	Improvement of contacts outside the family	9	9	
Intellectual abilities	Improvement of the ability to concentrate	9	9	
	Improvement of the approach to the everyday duties	9	9	

[1] The number of destructive behaviours in two patients (age of 18 and 23 years) varied from lower at the beginning of treatment with PreKunil, if compared to pre-treatment period, to higher at the end of observation. However, the social background of these patients is very problematic. It is possible, that the increase of the total number of aggressive behaviours was caused by pathology inside the family. Both patients were involved in conflict with their mothers. Parents from both families reported the same type of aggressiveness demonstrated by their children. It is possible that these behaviours disturbances were acting-out behaviours.

[2] Younger patient mentioned in point[1].

Tab. 3. Changes in patients' perception of illness on PreKUnil treatment.

Conclusions of questionnaire for patients		Changes in the perception of illness as a problem	Changes in abilities to cope with PKU	Changes in necessity of third persons' care	Attribution of PKU as a reason for being unhappy
Patient and duration of PreKUnil treatment (months)		PKU is for me: 1. a very difficult problem 2. a big problem 3. a little problem 4. not a problem at all	With my illness: 1. I cannot cope at all 2. sometimes it is very hard but I can manage 3. I can cope but it is not easy for me 4. I can cope easily without problems	According to my illness: 1. I need someone's care permanently 2. I need someone's care from time to time 3. I rather do not need anybody's care, I feel independent	PKU makes, that 1. I feel very unhappy all the time 2. I feel unhappy from time to time 3. I am sometimes worried 4. it has no influence on my feelings
MR (10)	Group I	Improvement 2 → 4	Improvement 1 ® 2	Improvement 1 → 2	Improvement 2 → 4
KG (11)	Group I	Improvement 2 → 4	Improvement 1 -® 2	Improvement 1 → 3	Improvement 2 → 4
GD (12)	Group I	Improvement 1 → 3	Improvement 2 → 3	Improvement 2 → 3	Improvement 1 → 3
WG (8)	Group I	Improvement 1 → 3	Improvement 1 → 3	Improvement 1 → 3	Improvement 1 → 3
LI (10)	Group II	Improvement 2 → 4	Improvement 2 → 4	Improvement 2 → 3	Improvement 2 → 4
MM (12)	Group II	Improvement 2 → 3	Improvement 1 → 3	Improvement 1 → 2	Improvement 1 → 3
AA (5)	Group II	Improvement 1 → 3	Improvement 1 → 3	Improvement 2 → 3	Improvement 1 → 3
SMK (4)	Group II	Improvement 2 → 3	No change 3 = 3	Improvement 2 → 3	No change 3 = 3

The duration of treatment (in months) is shown in the brackets.

* Patient SMN was not able to complete the questionnaire due to low IQ.

Methods of psychological studies:

Intelligence:

Wechsler Adults Intelligence Scale – Revised – after 0, 6 and 12 months of treatment.

Wechsler Intelligence Scale for Children – Revised – for patients younger than 17 years – after 0, 6 and 12 months of treatment.

Brunet-Lezine Scale for one, the most mentally-retarded patient (SMN).

Visual memory:

Benton Visual Memory Test (version C,D,E; method A – exposition trough 10 sec. and immediate reproduction from memory) – after 0, 3, 6, 9 and 12 months of treatment. For each examination a different version of test was used.

Questionnaires:

Every month, both patients and their parents were asked about the several issues connected with everyday life.

Questionnaire for patients concerned: changes in the perception of illness as a problem, abilities to cope with PKU, necessity of third persons' care and attribution of PKU, as a reason for being unhappy.

Parents were asked to fill in a questionnaire describing their children's behaviour and changes in emotional functioning, as well as changes in social contacts, both inside and outside the family.

Magnetic Resonance Imagines studies:

MRI examinations were performed at the beginning of the treatment, and every next 6 months.

MR scans were obtained with a 1.5T unit (Eclipse by Picker), using a head coil, and FSE and FLAIR sequences in T1- and T2- weighted images. Scans were performed in three planes: axial, sagittal and coronal.

In some patients, both T2- weighted and FLAIR sequences revealed extended areas of hyperintensive lesions corresponding to dysmyelination changes. To evaluate extension of white matter abnormalities, the grading according to Cleary[1] was used. Scores 0 – 5 were given for each from 12 anatomical regions. A score of 0 represented a normal scan; a score of 60 was the most severely affected possible.

[1] Cleary MA, Walter JH, Wraith JE, Jenkins JPR, Alani SM, Tyler K, Whittle D: Magnetic resonance imaging of the brain in phenylketonuria. Lancet 1994, 344: 87 – 90.

Results of MRI studies

MRI studies detected abnormalities of the brain in 2 patients from Group I (grade 8 in both) and in all 4 from Group II (grade 8 – 37). The mean grade of the initial MRI studies in Group I was 3.2, in Group II was 18.75. Follow-up MRI examinations were performed in all 5 patients from Group I and in 1 patient from Group II. No changes of grading were observed after 6 – 12 months.

Patient		FL	FR	PL	PR	OL	OR	TL	TR	BSL	BSR	OL	OR	Total
K.G.	MRI 0	0	0	0	0	0	0	0	0	0	0	0	0	**0**
K.G.	MRI 6	0	0	0	0	0	0	0	0	0	0	0	0	**0**
G.D.	MRI 0	0	0	0	0	0	0	0	0	0	0	0	0	**0**
G.D.	MRI 6	0	0	0	0	0	0	0	0	0	0	0	0	**0**
G.D.	MRI 12	0	0	0	0	0	0	0	0	0	0	0	0	**0**
L.I.	MRI 0	0	0	0	0	4	4	0	0	0	0	0	0	**8**
L.I.	MRI 6	0	0	0	0	4	4	0	0	0	0	0	0	**8**
M.R.	MRI 0	1	1	0	0	3	3	0	0	0	0	0	0	**8**
M.R.	MRI 6	1	1	0	0	3	3	0	0	0	0	0	0	**8**
W.G.	MRI 0	0	0	0	0	0	0	0	0	0	0	0	0	**0**
W.G.	MRI 6	0	0	0	0	0	0	0	0	0	0	0	0	**0**
A.A.	MRI 0	1	1	0	0	3	3	0	0	0	0	0	0	**8**
M.M.	MRI 0	4	2	2	2	3	3	0	0	0	0	0	0	**16**
M.M.	MRI 6	4	2	2	2	3	3	0	0	0	0	0	0	**16**
M.M.	MRI 12	4	2	2	2	3	3	0	0	0	0	0	0	**16**
S.MK.	MRI 0	1	1	2	2	4	4	0	0	0	0	0	0	**14**
S.MN.	MRI 0	5	5	4	4	5	5	5	4	0	0	0	0	**37**

FL/FR	– frontal left/right	grade 0	→	normal
PL/PR	– parietal left/right	grade 1	→	less than 10% white matter involved
OL/OR	– occipital left/right	grade 2	→	10 – 30% white matter involved
TL/TR	– temporal left/right	grade 3	→	30 – 50% white matter involved
BSL/BSR	– brainstem left/right	grade 4	→	50 – 75% white matter involved
OL/OR	– other areas left/right	grade 5	→	more than 75% white matter involved

Results

The chosen aspects of the effects of low-protein diet supplemented with PreKUnil tablets as an alternative method of treatment for patients with classical PKU are shown on the figures and in the tables below.

Beside transient headache in the oldest patient from Group II (possible insufficient compliance to the dietary regiments), no side effects of the treatment were observed.

All patients from Group I were generally able to follow the recommendation to maintain the Phe levels below 1500 µmol/l. However, except one patient with constantly lower Phe levels (39 - 728 µmol/l, mean 327 µmol/l), the remaining four had higher Phe levels, compared to the period when they were treated conventionally with a low-Phe diet. These four patients' Phe levels varied from 799 to 2084 µmol/l (mean 1346 µmol/l). The whole Group I Phe levels varied from 39 µmol/l to 2084 µmol/l (mean 1143 µmol/l).

Only one patient from Group II had most of the time Phe levels below 1500µmol/l (mean 1238 µmol/l). Phe levels of the remaining three patients were constantly higher than 1500 µmol/l and varied from 1960 to 2260 µmol/l (mean 2090 µmol/l). The whole Group II Phe levels varied from 941 µmol/l to 2260 µmol/l (mean 1806 µmol/l).

Results of psychological studies:

The duration of treatment (in months) is shown in the brackets.

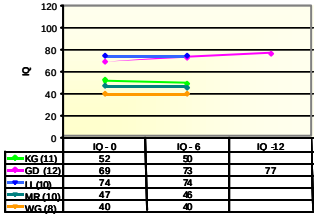


Fig. 1. Group I Wechsler Intelligence Scale - IQ after 0, 6, 12 months of treatment with PreKUnil.

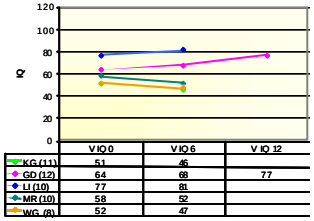


Fig. 2. Group I Wechsler Intelligence Scale - Verbal IQ after 0, 6, 12 months of treatment with PreKUnil.

Discussion

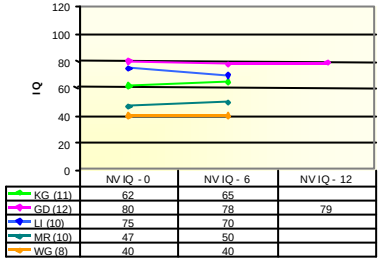
Due to a small number of patients and short time of observation it is to early to establish the final conclusions. Never the less, at least similar trend in both groups may be noticed. Neither deterioration nor evident improvement in intellectual abilities measured by IQ values was noticed in Group I. However, in three patients from this group we observed an increased number of correct answers with a decreased number of mistakes, according to the Benton Visual Memory Test. At the beginning of treatment these patients had the results, which may indicate the possibility of organic abnormalities in CNS. The results were below the norms adequate for their age and IQ. During the treatment they achieved results normal for their age and IQ.

In one patient (MM) from Group II we noticed changes in all examined areas, including IQ values, however these findings reliability needs a longer period of observation. The results in the Benton Visual Memory Test of this patient increased successively from 2 correct answers at the beginning, to 8 after one year of treatment with PreKUnil. The number of mistakes decreased from 11 to 4 respectively. Patients SMK and AA are treated too short for looking for differences in IQ, but in patient AA, changes in handiwork of the Benton Visual Memory Test (decrease of number of mistakes from 17 to 14) were noticed.

The improvement of the quality of life was noticed in all patients. Their perception of illness changed (it seems to be a less hard problem for them), they believe they are now more able to cope with PKU, they seem to feel more independent - the necessity of third persons' care became lower and the attribution of PKU as a reason for being unhappy varied – patients feel less negative emotions connected with being ill. The questionnaire filled in by parents showed the same tendency. Parents reported that their children's emotional functioning was better, there was a lower number of destructive behaviors. The improvement of relationships, higher level of concentration and more responsible approach to everyday duties were although observed.

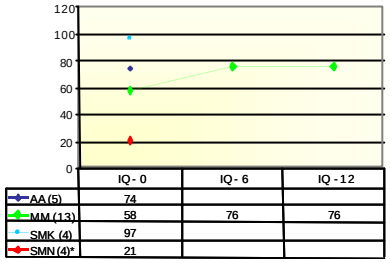
According to the Cleary's scale, the mean grade of the initial MRI changes in group I was 3.2, in group II - 18.75. This difference can be easily explained with a difference of mean Phe levels in both groups and with a lack of the dietary treatment or abandoning a diet in Group II. Despite the small number of patients in both groups, a trend towards a correlation between MRI findings and both Phe levels and dietary treatment can be observed. Follow-up MRI findings were the same like the initial ones. There was neither improvement and no aggravation of cerebral involvement. It may suggest that LNAAs supplementation can prevent a progress of the disease, even if patients are allowed to stay on the higher Phe levels.

In PKU patients for whom Phe is not adequately controlled because of noncompliance to the diet or other reasons (including economic) and for late-detected individuals with PKU, who were never on a diet, low-protein diet supplemented with LNAAs seem to be a promising alternative method of PKU treatment. This method is easy to use, needs less restricted regiment and brings to the patients more independence.



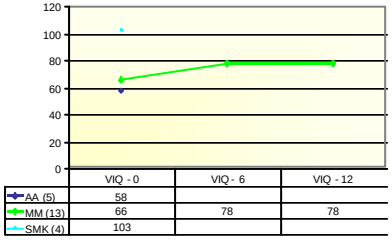
	NV IQ - 0	NV IQ - 6	NV IQ - 12
KG (11)	62	65	
GD (12)	80	78	79
LI (10)	75	70	
MR (10)	47	50	
WG (8)	40	40	

Fig. 3. Group I Wechsler Intelligence Scale - Nonverbal IQ after 0, 6, 12 months of treatment with PreKUnil.
* Patient SMN was tested with Brunet - Lezine scale.



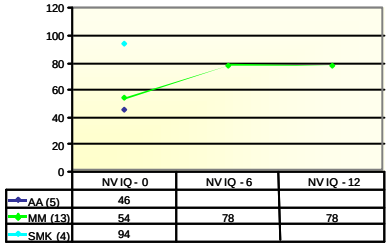
	IQ - 0	IQ - 6	IQ - 12
AA (5)	74		
MM (13)	58	76	76
SMK (4)	97		
SMN (4)*	21		

Fig. 4. Group II Wechsler Intelligence Scale - IQ after 0, 6, 12 months of treatment with PreKUnil.



	VIQ - 0	VIQ - 6	VIQ - 12
AA (5)	58		
MM (13)	66	78	78
SMK (4)	103		

Fig. 5. Group II Wechsler Intelligence Scale - Verbal IQ after 0, 6, 12 months of treatment with PreKUnil.
* Patient SMN was not tested.



	NV IQ - 0	NV IQ - 6	NV IQ - 12
AA (5)	46		
MM (13)	54	78	78
SMK (4)	94		

Fig. 6. Group II Wechsler Intelligence Scale - Nonverbal IQ after 0, 6, 12 months of treatment with PreKUnil. * Patient SMN was not tested.

20-year old PKU mother with her 1-year old twins. She started low-protein diet supplemented with LNAAs when the children were 3 moths old. Photo presented with the kind permission of a patient.