Research

Randomised investigation to evaluate Phe fluctuation after overnight fasting in PKU patients treated with PKU GOLIKE versus standard amino acid protein substitute

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RANDOMISED INVESTIGATION TO EVALUATE PHE FLUCTUATION AFTER OVERNIGHT FASTING IN PKU PATIENTS TREATED WITH PKU GOLIKE VERSUS STANDARD AMINO ACID PROTEIN SUBSTITUTE

1 INTRODUCTION

Fluctuations in blood phenylalanine (Phe) concentrations may be an important determinant of intellectual outcome in patients with early and continuously treated phenylketonuria (PKU) *(Cleary et al, 2013)*. Evidence suggests that physiological fluctuations in Phe concentrations in patients with PKU within a day are different to those seen in healthy individuals on a normal diet with an inverse diurnal variation (Phe concentrations higher in the morning) *(MacDonald et al 1998)*. This suggests that protein catabolism predominates over protein anabolism during fasting periods *(Cleary et al, 2013)*. In line with this, prolonged fasting results in a small rise in Phe concentrations with a peak in the early morning before breakfast *(MacDonald et al 2003)*. The timing of protein substitutes also affects diurnal variation: distribution of protein substitute intake throughout 24 h stabilizes Phe concentrations *(MacDonald et al 1998, MacDonald et al 2003)*. The median variation in plasma Phe concentrations was 155 µmol/L per day, with a minimum of 80 µmol/L and a maximum of 280 µmol/L, among 16 patients with classic PKU aged 1 to 18 years *(MacDonald et al 1998)*.

The relationship between IQ and blood Phe levels is clear. However, only a few studies have specifically addressed Phe fluctuation and intellectual outcome and Phe concentrations and Phe fluctuations could not be separated with regard to their influence on IQ (*Cleary et al 2013*). Two studies have failed to determine this relationship between Phe fluctuations and cognitive impairment (*Anastasoaie et al 2008*). Phe concentrations build up in blood and brain and the ability to intrinsically produce the dopamine precursor tyrosine is lost. These changes do not solely affect the metabolism of dopamine. Also, reduced concentrations of noradrenaline and serotonin are found in PKU patients (on diet) and in the PKU mouse model (*Berguig et al 2019*).

PKU patients are a population at risk for sleep disorders due to deficits in neurotransmitter synthesis. Noradrenaline and serotonin neurotransmitters are known to be important regulators of sleep, wakefulness, and they move between these states. Analyses done on PKU mice and on PKU patients versus healthy people with 4 validated questionnaires confirmed sleep problems in PKU patients

(Brunenberg et al. 2017). On the contrary, sleep disorders (using Bruni's Sleep Disturbance Scale for Children) were similar in both groups, 15.6% in control group and 12.5% in PKU group and no correlations were found with peripheral biomarkers of neurotransmitter synthesis melatonin and serotonin (*Rosa Gassió, et al. 2019).*

The aim of this randomised controlled study is to examine if by giving a slow-release protein substitute if it will reduce overnight catabolism in blood Phe levels and if this in turn will affect sleep patterns.

2 STUDY OBJECTIVES

2.1 Primary study objectives

- Reduce catabolic episodes during the night (measured by analysis of urine markers)
- Reduce peak Phe levels in the early morning (measured with 3 blood spots at 5:00, 6:00 and 7:00am before breakfast).
- Improved quality of sleep, associated with improved blood Phe level.

3 STUDY PARAMETERS

3.1 Primary study parameters

- Blood Phe after administration of PKU GOLIKE and after amino acid protein substitute as mean value of day 6 and 7 and day 27 and 28 of treatment based on 3 blood spots collected at 5:00, 6:00 and 7:00am before breakfast.

3.2 Secondary study parameters

- Tyr blood concentration with dried blood spots at 5:00, 6:00 and 7:00am before breakfast as mean value of days 6 and 7, and days 27 and 28 of treatment.
- Blood concentration of branch chain amino acids (BCAA) from dried blood spots at 5:00, 6:00 and 7:00am before breakfast as mean value of days 6 and 7, and days 27 and 28 of treatment.
- **Urine sample** to measure: urea and creatinine
- Quality of sleep (sleep questionnaire)
- Palatability of protein substitute Bars and Krunches (Palatability questionnaire)

- Adherence to the prescribed diet (use of product vs prescribed, as reported by the patient)
- Any side-effects (gastro-intestinal in particular—AE collection)

4 STUDY DESIGN

4.1 Study design

This is a 2 arm, randomised, controlled, cross-over study in 16 children with PKU. Subjects who are currently taking a Phe free/low Phe protein substitute will be recruited for a 31-day trial.

Patients will be randomised to receive:

- The study product for 7 days as their last dose of protein substitute for the day (at least 15g PE) in an amount equivalent to their usual protein substitute PE; or
- An amino acid protein substitute for all daily doses for 7 days;

followed by a 2-week washout period on their usual protein substitute, and then 7 days of the other study arm.

During this time, patients/caregivers will be asked to:

- Collect 3 finger prick blood spots on days -1, 0, 6, 7, 20, 21, 27 and 28.
- Collect urine sample, second void of the day on days 0, 7, 21 and 28.
- Complete a questionnaire on sleep quality on day 0, 7, 21 and 28.
- Complete a 24 hour food diary on days -1, 0, 6, 7, 20, 21, 27 and 28
- Palatability questionnaire on days 7 or 28 (at the end of the period with PKU GOLIKE, if Bars or Krunches are used)

APR will supply the study product for participants free of charge.

5 SUBJECTS

5.1 Study population

16 children, diagnosed with PKU requiring a low phenylalanine diet will be recruited from one specialist PKU centre.

5.2 Inclusion criteria

1. Male and female PKU patients ≥5 years and ≤16 years of age.
2. Patients diagnosed with PKU via new born screening.
3. Taking a Phe free/low Phe protein substitute
4. On a low phenylalanine diet .
5. Absence of neurological deficiencies.
6. Adherence with dietary management and protein substitute.
7. Able to understand and comply with the requirements of the investigation and sign the Informed Consent Form/Assent form.

5.3 Exclusion criteria

1. Age <5 years old and >16 years old.
2. Patients with mild PKU or HPA.
3. On sapropterin therapy.
4. Patients with late diagnosis of PKU and neurological problems.
5. History of hypersensitivity to any excipients/components of the investigational product.
6. Pregnancy or breastfeeding during the study.
7. Any moderate to severe acute illness which in the opinion of the Investigator would interfere with the study procedures or study outcome.
8. History of poor co-operation, non-adherence with dietary management, or poor adherence to investigation procedures.
9. Participation in any other studies involving investigational or marketed products concomitantly or within two weeks prior to entry into the study.

5.4 Subject recruitment

16 children with PKU will be recruited. When an appropriate subject has been identified, they and their parents/caregivers will be sent a study information sheet via the post. They will be invited to request further information about the study if they wish by contacting the Dietitian. The Dietitian will explain the study in more detail either by telephone or via a face-to-face consultation in a

convenient location/preferred venue for the participant and their parent/caregiver. Recruitment of each patient will be by written informed consent, which will be completed by the parents/primary caregivers and taken by the Dietitian. Children will also complete an assent/consent form. Reimbursement will be offered for any travel expenses incurred for any visits to the hospital that are over and above normal/routine visits.

REPLACEMENT/NON-ADHERENT PATIENTS

Patients that are not compliant or need to be excluded for other clinical reasons to the treatment schedule will be replaced in order to obtain 16 complete subjects per treatment group. This procedure is according to cross-over design in order to have a complete matrix of data.

URINE COLLECTION

For the 16 children recruited, a second void of the day urine sample will be collected on days 0, 7, 21 and 28 to analyse for urea and creatinine. These will be frozen at the patients home (containers will be provided) and collected at the end of the study.

6 STUDY DESCRIPTION

6.1 Study description

Subjects who currently take a Phe-free/low Phe protein substitute (3 or 4 doses/day) will be recruited. Subjects will replace the last daily dose of their usual protein substitute with the study product for 7 days of the 28 day trial (either days 1-7 or days 22-28 based on random allocation). On the remaining study days, subjects will take an amino acid based protein substitute for all daily doses. There will be a 2 week washout period between study arms where subjects will take their usual protein substitute. The amount of study product prescribed will be calculated to provide the same amount of protein as their usual protein substitute. The last protein substitute (PS) dose of the day (amino acids or study product) will need to be taken between 7-9pm to allow an 8-10 hour fasting period overnight. Three finger prick blood spots will be collected and analysed for phenylalanine, tyrosine and BCAA at 5am, 6am and 7am on days -1, 0, 6, 7, 20, 21, 27 and 28. For the 16 subjects, a second void urine sample will be collected on days 0, 7, 21 and 28 for analysis of urea and creatinine. A quality of sleep questionnaire will be completed by subjects or their carers on days 0, 7, 21 and 28 and a 24 hour food diary on days -1, 0, 6, 7, 20, 21, 27 and 28. A palatability questionnaire will be completed by subjects or their carers on days 7 or 28 (at the

end of the period with PKU GOLIKE, if Bars or Krunches are used). Subject visits will be on days -2 (enrolment), 0, 7, 21 and 28 where the research dietitian will collect urine samples, blood spots, questionnaires and diaries.

7 STUDY PROCEDURES AND ASSESSMENTS

7.1 Study procedures

Protein substitute administration

Patients will continue to take the same prescribed protein equivalent divided into 3 or 4 daily doses according to standard practice. The last daily dose of protein substitute (Test or Reference) will need to be taken between 7-9pm to allow an 8-10 hour fasting period before morning finger prick blood spots are taken. The study product will be taken as the last dose on either days 1-7 or days 22-28 (based on random allocation), with an amino acid protein substitute taken for the other doses on the same days. There will be a 2-week washout period on days 8-21 where subjects will take their usual protein substitute.

Finger prick blood samples

Routine weekly finger prick blood samples will continue to be collected by the subject or parent/caregiver and sent to the hospital laboratory for analysis of phenylalanine, tyrosine and BCAA as is usual clinical practice for these subjects. Additional blood spots will be required in week 1 and 4 (3 blood spots will need to be taken at 5am, 6am and 7am on days -1, 0, 6, 7, 20, 21, 27 and 28 of the study period).

Urine Samples

Subjects will be asked to record dietary intake on day -1 and use this same menu for all days before urine collection to ensure consistency of intake on urine collection days. Urine samples will be collected on each day of sampling. For 16 recruits a second void of the day urine sample will be collected. This will be frozen at home until collected by a research dietitian at the end of the study.

Sleep quality questionnaire:

A questionnaire on the quality of subject sleep will be completed by subjects/carers on days 0, 7, 21 and 28.

Palatability questionnaire:

A questionnaire on the palatability will be completed by subjects/carers on days 7 or 28 (at the end of the period of PKU GOLIKE administration, if Bars of Krunches have been used).

Diet and Activity Diary

A 24 hour food/fluid diary will be recorded on days -1, 0, 6, 7, 20, 21, 27 and 28.

Adherence:

Prescription details, amendments and intakes will also be recorded by the dietitian.

Concomitant medication:

All concomitant medications taken during the study will be recorded in the CRF with indication, dose information, and dates of administration. Permitted study medications will be those that in the opinion of the Investigator do not affect the subject's safety or interfere with the study procedures or outcomes.

STUDY FLOW CHART

Visit day	Day -2	Day -1	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 8-21 Washout	Day 19	Day 20	Day 21	Day 22	Day 23	Day 24	Day 25	Day 26	Day 27	Day 28	
Informed Consent signature	■											2 week washout period on usual protein substitute											
Selection Criteria	■																						
Demographic data	■																						
Randomisation	■																						
Administration of the last dose of PS for the day (PKU GOLIKE or AA) 19.00-21.00				■	■	■	■	■	■	■						■	■	■	■	■	■	■	
Blood spot (for Phe, Tyr & BCAA) 5am, 6am & 7am		■	■						■	■				■	■						■	■	
Urine sample (2nd void of the day)			■							■					■							■	
Validated questionnaire on sleep quality			■							■					■							■	
24 hour diary food/fluid		■	■						■	■				■	■						■	■	
Palatability questionnaire (applicable only for Krunches and Bars)										■												■	
Study visit	■		■							■					■							■	