Effect of Large Neutral Amino Acids on Maternal Phenylketonuria Offspring

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1. Introduction

Phenylketonuria (PKU) is an inborn error in the metabolism of the amino acid phenylalanine (Phe) due to the deficiency of an enzyme phenylalanine hydroxylase (PAH). Current therapy consists of a Phe – restricted diet for life to ensure the healthiest development. It is particularly important for PKU women in the reproductive age group to comply with the diet, since elevated maternal blood Phe levels during pregnancy are teratogenic to the fetus.

Because the placental barrier favors influx of Phe into the fetal compartment, the fetus is exposed to h Phe concentrations approximately twice that in maternal blood, resulting in intrauterine growth retardation, microcephaly, psychomotor retardation and congenital heart defects. This is referred to as Maternal PKU Syndrome (MPKU) and was first reported by Dent in 1957 [1] and Mabry, et al, in 1963 [2]. In MPKU the damage caused to the fetus by the PKU mother's high blood Phe levels is irreversible.

The Maternal PKU Collaborative study, sponsored by the National Institute of Child Health and Human Development, started in United States in 1984, observed that the reproductive outcomes improved when strict control of maternal blood Phe level (2 -6 mg/dl) was instituted before or at the time of conception and maintained throughout pregnancy. This can be achieved if the PKU women adhere to a strict low Phe diet at least from the time of conception, but since such diets are not highly palatable, strict adherence to the diet is not typically achieved and the desired blood Phe levels are not maintained. Therefore, in order to achieve good patient compliance, alternative methods of treatment of PKU are being investigated.

The studies conducted earlier have shown that large neutral amino acids (LNAA) share a common transporter in the brain and that increasing other LNAAs competitively lowers the Phe transport into the brain. These LNAA transporters are present in the placental barrier as well as in the intestines [3]. The aim of the present study is to investigate whether LNAA supplementation of the diet will reduce maternal and fetal blood Phe levels through competitive inhibition with Phe at the transporter.

2. Materials and Methods

Materials

The PKU mouse model *BTBR-Pah^{enu2}* was used. All animal used experiments described in this paper were approved by the Institutional Animal Care and Use Committee. These mice simulate human classical PKU, including the maternal effect in which progeny gestated in mutant females are damaged during prenatal development. These animals have a normal menstrual cycle of 4 days and gestational period of 19½ days.

The basal diet was 5001 diet (a standard mouse diet). These are in the pellet form and are ground to a powder. The LNAA was obtained from Dr. Reuben Matalon's Lab, UTMB, Galveston. The composition of the LNAA mix is shown in Table 1.

Table 1: Composition of the LNAA powder

Amino Acid	Amount (mg)
L -Tyrosine	162.4
L - Tryptophan	42.46
L - Methionine	26.64
L - Isoleucine	24.19
L - Leucine	108.25
L - Threonine	26.64
L - Histidine	25.01
L - Lysine	25.01
L - Arginine	25.01
L - Valine	29.19

Experimental design.

The homozygous mutant females were paired with heterozygous mutant males. On the day of mating, females were randomly assigned to control or test groups and blood was collected from the tail (1st bleed) of the female under aseptic conditions. The control animals were started on powdered 5001 diet, about 6 g / mouse / day whereas the animals in the test group were started on the LNAA

supplemented diet, i.e., 1g LNAA + 5 g powdered 5001 diet / mouse / day. A second blood sample was collected on the 10th day of gestation and a third at the time of necropsy that was performed on the pregnant female a day prior to the natural termination of pregnancy (i.e., 18½ day).

On the 18½ day of gestation, the female was euthanized by CO_2 asphyxiation. Fetuses were removed quickly and blood collected from the common carotid artery using heparinized hematocrit tubes. After removing all the fetuses the maternal blood was collected from the descending aorta.

The blood collected during the three bleeds was saturated onto S&S 903 filter paper and stored in the refrigerator at 4°C before Phe analysis. The quantitative determination of Phe in the dried blood filter paper was done by using a modified fluorometric procedure published by McCaman and Robins in 1962 [4].

3. Results

The maternal blood Phe levels in the control group were higher than in the test group ($P < 0.01$). The lower levels of blood Phe in the fetal test group were also significant, the P value was 0.057.

Table: 2

LNAA supplemented maternal blood Phe levels (Average)		
Control	Test (n =7)	
(n = 6)	Pre LNAA	Post LNAA
23.4	24.9	18.5

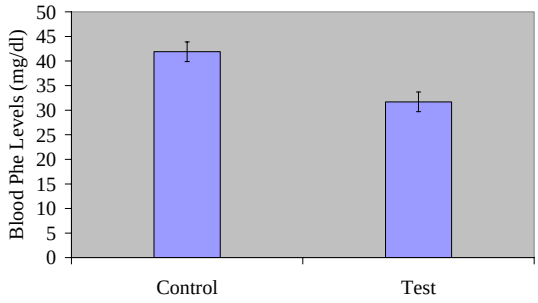

Fig. 1.Fetal blood levels in the control and the test groups.

Significance

The results suggest that LNAA effectively competes with the Phe transport across the intestinal and perhaps also across the placental barrier, thereby not only is the maternal blood Phe lowered but the fetus also has lower levels of blood Phe.

4. Conclusion

According to WHO, “Prevention is better than a cure”. This is very true in the case of MPKU, where maintaining blood Phe near normal during pregnancy prevents congenital birth defects. For this good patient compliance is required. But achieving good patient compliance is very difficult, because the Phe restricted diet is not only unpalatable but expensive. PKU women in the reproductive age group are often cognitively limited and emotionally distressed, and most often of low socioeconomic status. In addition, pregnant women due to numerous hormonal changes taking place in their body, have an increased craving for a variety of food. All these factors may impede compliance with treatment. Therefore there has been a continuous effort to find other modalities of treatment for PKU, where the patients can consume a normal diet and at the same time maintain blood Phe levels under control. The results of our experiment show significant decrease in both maternal and fetal blood Phe levels in the test group as compared to the control group. These encouraging results suggest that LNAAs can be used as the conventional treatment of MPKU allowing the patients to have a more liberal diet. As the brain is the most severely affected organ in the MPKU syndrome, we would like to see the effect of these large neutral amino acids on brain Phe levels of the PKU offspring

5. Acknowledgement

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6. References

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