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 placenta favors influx of Phe into the fetal compartment, the fetus is exposed to high 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 to maintain maternal blood Phe level at 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 range of blood Phe levels is not maintained. Therefore, in order to achieve good patient compliance, alternative treatment methods for PKU are being investigated.

Studies conducted earlier have shown that large neutral amino acids (LNAA) share a common transporter in the brain and that increasing other LNAAs competitively lower 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

The PKU mouse model *BTBR-Pah^{enu2}* was used. All animal-based experiments described in this paper were approved by the Institutional Animal Care and Use Committee. These mice simulate classical human 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 standard mouse diet, 5001. 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

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 when necropsy that was performed on the pregnant female a day prior to the natural termination of pregnancy (i.e., day 18½).

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 absorbed 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 described by McCaman and Robins in 1962 [4].

Statistical analysis of the maternal blood Phe levels done by using ANCOVA, to control for initial variability in the pretest scores thereby eliminating selection bias. Fetal blood Phe levels were analyzed using ANOVA.

3. Results

Statistical analysis of the maternal blood Phe levels done using ANCOVA showed that there is a significant ($p < 0.05$) decrease with 16.7% LNAA and 33.4% LNAA supplementation (Table 2). This decrease was seen at the time of 3rd bleed. Fetal blood Phe levels showed a significant decrease ($p<0.05$) at all levels of supplementation (statistical method used, ANOVA).

Table 2: Maternal Blood Phe

Dependent Variable[d]	Control	16.7%	20%	33.4%
Phe T2[b]	32.6[a]	26.9[a]	29.4[a]	29[a]
Phe T3[c]	29.4[a]	19.2[a]	23.2[a]	14.1[a]

a. Covariates appearing in the model were evaluated at the following values: Phe level of mother = 35.83mg/dl

b and c : Phe level at second and third bleed respectively.

d = measure of dependent variable = mg/dl

Table 3: Fetal blood Phe

Group	Control	16.7%	20%	33.4%
Blood Phe (mg/dl)	49	34.3	40.4	25.3

The results suggest that LNAA (16.7% and 33.4% levels) competes with Phe transport across the intestinal and perhaps more effectively across the placental barrier. Therefore, not only is the maternal blood Phe lowered but the fetus also has even lower levels of Phe in the blood.

Adverse effects

Anencephalic fetuses, one in each of two litters, were observed at the 33.4% LNAA dosage. These could be due to LNAA, maternal factor or a combination of both factors. Such a birth defect is extremely rare in this mPKU mouse model. Since it was not observed in the control group, the possible role of LNAA for this adverse effect is very likely. We also saw more of embryonic and fetal death in the experimental group than in the control group, which may mean that, in the experimental group, the fetuses were affected not only by the maternal blood Phe levels but also by the presence of large amounts of LNAA which then crosses the placental barrier to supply the fetus. A similar trend was seen in studies conducted on pregnant women in New York. When their diet was supplemented with high protein content during pregnancy, a high incidence of low birth weight offspring and / or birth defects were observed in neonates born to these patients [5].

4. Conclusion

Based on the above observations we can conclude that though LNAA seemed to decrease the blood Phe levels it was not without adverse effects. Moreover, the adverse effects outweigh the therapeutic effects especially at the highest supplementation rate employed. The more important aspect of this therapy will be the effect of this LNAA supplementation on brain Phe levels and on the incidence of congenital heart defects. Another interesting study would be the effect of LNNA supplementation which has been modified in such a way that there is an increase in the dietary LNAA without increasing the total dietary amino acid level.

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