Theoretical and Practical Aspects of Preventing Fetal Damage in Women with Phenylketonuria

Stephen Krywawych, M. Haseler, and D. P. Brenton

University College and Middlesex School of Medicine, Department of Medicine, The Rayne Institute, 5 University Street, London WC1E 6JJ, United Kingdom

The problem of mental retardation in children born to mothers with phenylketonuria (PKU) was first raised in 1956 at the 23rd Ross Pediatric Conference in Columbus, Ohio, by the late Charles E. Dent, and subsequently reported in the proceedings in 1957 (1). Six years later a high incidence of mental retardation was described among 14 children born to three mothers with PKU (2). These findings of mental retardation, microcephaly, congenital heart disease, and other organ defects were confirmed in other studies (3–5). Perry et al. (6) drew attention to the unrecognized PKU patients presenting at antenatal clinics. They described two sisters with plasma phenylalanine levels at around 1 mmol/liter who between them had nine non-PKU children with an impaired IQ. In 1980, Lenke and Levy (7) reviewed a survey of 155 mothers with PKU who had 423 offspring and 101 spontaneous abortions (including many of those reported earlier in the literature). Spontaneous abortion, mental retardation, microcephaly, congenital heart disease, and low birth weight became recognized as the major clinical problems associated with maternal PKU. With the introduction of screening of newborns for phenylketonuria in the 1970s, resulting in a population of young successfully treated adults with PKU by the late 1980s, a widespread problem of maternal phenylketonuria has emerged. Any attempts at treating this condition to prevent damage to the developing fetus requires an understanding of the placental transport of amino acids, embryonic development, and the mechanisms of fetal damage.

PLACENTAL TRANSPORT OF AMINO ACIDS

Transport of amino acids from the maternal circulation to the fetal side serves to provide for fetal metabolic (anabolic) requirements and to a much smaller extent to satisfy the metabolic requirements of the trophoblast (8). With the exception of cystine, taurine, glutamic, and aspartic acid, all other amino acids are actively trans-
ported from the maternal to the fetal side. Unlike the fetal surface, the maternal surface of the trophoblast has microvilli which structurally resemble the brush border of intestinal and renal epithelium and is in direct contact with maternal blood. Investigations on human placental brush border membrane vesicles have demonstrated three transport mechanisms: the A system, the L system, and the ASC system (9). The first system, the A system, transports proline, glycine, and α-amino isobutyric acid and is inhibited by methylalanine. The second system, the L system, demonstrates preference for the aromatic and the large neutral amino acids (e.g., leucine, phenylalanine, tyrosine, and methionine) and is inhibited by 2-aminonorbornane-2-carboxylic acid, but not significantly by methylalanine. Finally, the third system acts upon the basic and short chain neutral amino acids (e.g., alanine, serine, threonine, and glutamine) and is inhibited by both 2-aminonorbornane-2-carboxylic acid and methylalanine. In guinea pig placenta, membrane transporters have been shown to exist on both the maternal and fetal sides of the trophoblast but only unidirectional transport at the maternal interface is sodium dependent (10,11). The $K_m$ value for phenylalanine at the fetal side was threefold higher than on the maternal side. Transport studies in membrane vesicles from term human placenta indicate that saturation of placental phenylalanine transport is unlikely at phenylalanine concentrations seen in affected PKU mothers, but competitive inhibition of tyrosine and tryptophan may occur.

With the exception of threonine, all maternal plasma amino acid concentrations fall in pregnancy (12). On the other hand, transport of amino acids across the placenta produces higher fetal than maternal values, the ratio between the two differing for different amino acids. In our patients, for maternal plasma phenylalanine concentrations ranging from 112 to 657 μmol/liter the fetal/maternal phenylalanine ratio averaged 1.6:1. This gradient is still maintained at much higher maternal values than 657 μmol/liter. The higher fetal/maternal plasma phenylalanine ratio has been shown to occur from early pregnancy (13,14). To maintain fetal values below 500 μmol/liter, maternal phenylalanine values require to be kept below 300 μmol/liter. It is of course still debatable how low the fetal values need to be to ensure a normal child.

**EMBRYONIC DEVELOPMENT**

Rapid development of the fetus occurs during organogenesis, 3–8 weeks after fertilization. At 5 weeks postfertilization the 10-somite embryo develops a contracting heart, still a simple tube within a pericardium, and by 8 weeks the development to an adult heart form is virtually complete. Neural crest cells may also contribute to the cardiac development. The heart is therefore most vulnerable during the initial 8 weeks, and congenital heart disease has been shown to occur at an increased incidence in those babies born to mothers in whom the plasma phenylalanine concentration has remained above 900 μmol/liter. In our experience two of three infants resulting from pregnancies conceived before diet when mother's plasma phenylal-
amine concentration was high in the first trimester had severe congenital heart malformations and died. The third may also have more minor heart defects.

The neural folds meet anteriorly at 3 weeks, and complete posterior closure occurs at 4 weeks. However, neural tube defects appear to be uncommon in untreated maternal phenylketonuria (15,16). The very high incidence of microcephaly and mental retardation, however, probably has its origins during the first trimester since poor control then may still result in microcephaly even if later control is satisfactory (16, 17).

The formation of the trachea begins at 3 weeks and major malrotation of the midgut occurs later, between 6 and 12 weeks. Esophageal atresia, diaphragmatic hernia, and malrotation have been described in maternal PKU and certainly originate during the first trimester (7).

MECHANISMS OF FETAL DAMAGE

From the data on 53 offspring in untreated pregnancies (18), some authors argued that there is a graded effect of maternal plasma phenylalanine concentrations on the brain development of the offspring (19,20). However, for other defects it is possible that there is a threshold value of around 600 μmol/liter for maternal plasma phenylalanine concentration, below which congenital malformations may not occur more commonly than in the normal population.

From the postnatal treatment of homozygous patients with phenylketonuria it is evident that the neurological system is vulnerable to phenylalanine in the young child but less so in the older child and young adult. It is likely that similar mechanisms of damage to nervous tissue operate in the fetal state as in the postnatal period. The precise mechanism for the postnatal damage has not been elucidated, but an inadequate supply of some amino acids to the brain, deranging protein synthesis or neurotransmitter synthesis, is likely to be a factor contributing to brain damage (21). High plasma phenylalanine concentrations in rats have been shown to decrease the entry of related long chain neutral amino acids into brain (22–24). Similarly, the transport of methionine, leucine, tyrosine, valine, and glutamine but not arginine and lysine was inhibited at the ovine blood-brain barrier by increased concentrations of phenylalanine (25). This interference occurs as a result of competition by different amino acids for a common carrier across the blood-brain barrier. Three carrier systems for the transport of amino acids have been identified; one for the short chain neutral amino acids, the second for the long chain neutral amino acids, and the third for the basic amino acids (26).

Phenylalanine undergoes facilitated transport by a carrier that is also active on the branched-chain amino acids tryptophan, tyrosine, histidine, methionine, and glutamine. The carrier at the blood-brain barrier is similar to that of the L-system of Christensen (27) but with much lower $K_m$ values. At the normal plasma amino acid concentrations this carrier is almost saturated, and therefore not only is the flux of an amino acid across the blood-brain barrier sensitive to any changes in its concen-
Fetal damage in women with phenylketonuria

tration, but is also influenced by changes in the concentration of other competing amino acids.

There is limited experimental information in animals concerning the kinetic parameters of amino transport at blood-brain barrier in the developing fetus. The blood-brain barrier depends on the development of tight junctions between capillary endothelial cells forming an impermeable barrier to water-soluble substrates. As the development of such a barrier occurs in early gestation, it is reasonable to assume that the transport of amino acids may be important in the pathology of brain damage in the developing fetus in maternal phenylketonuria. During fetal development these amino acid imbalances are likely to be further exacerbated by the generation of the placental gradient for phenylalanine from maternal to fetal side. The exact mode of damage by the brain amino acid imbalance is unknown, but its effect may be directed on neuron replication, protein synthesis, axonal and dendritic development and growth, myelation, or neurotransmitter metabolism.

Low concentrations of dopamine and serotonin have been found in the cerebrospinal fluid (CSF) and in the brain tissue of homozygous subjects with PKU when plasma phenylalanine concentrations were elevated (28). Furthermore, a reciprocal relationship has been observed between the concentration of plasma phenylalanine and that of plasma and CSF serotonin and dopamine and their metabolites (29–32). Phenylalanine may inhibit dopamine and serotonin synthesis by restricting the entry of their precursors tyrosine and tryptophan into brain or even possibly by inhibiting the enzymes involved in the synthesis of these monoamines (33–37). Disturbed monoamine synthesis is more likely to be responsible for the reversible behavioral and psychological problems observed in treated patients with phenylketonuria on occasions when the plasma phenylalanine concentration is elevated, but may also be related to the permanent irreversible neurological damage seen in untreated patients with PKU.

Specific interventions through the administration of selected amino acids to correct the deficiency of such amino acids in the brain of patients with phenylketonuria has been proposed as a therapeutic approach (38). The supplementation with branched-chain amino acids of normal diets fed to young rats with induced hyperphenylalaninemia has markedly improved their maze-learning techniques (39). Similar branched-chain supplements fed to maternal rats with induced phenylketonuria have restored normal birth weights in the offspring (40). These branched-chain amino acid supplements may act by restricting phenylalanine entry into brain, thereby preventing it from exerting its toxic effects, or simply by correcting a deficiency of branched-chain amino acids caused by the inhibition of their transport across the placenta or into the CNS. Recently, the exodus of small neutral amino acids from brain tissues has been shown to be inhibited in rats with induced hyperphenylalaninemia (41). This is thought to occur because the normal exchange of large neutral amino acids in the L-system for proline, alanine, and glycine accumulated intracellularly by the sodium-dependent A system is inhibited by the elevated concentrations of phenylalanine; this also explains the lower concentrations of these three amino acids in the plasma from patients with phenylketonuria (42). In view of the uncer-
tainty as to whether low blood concentrations of glycine affect the supply of glycine as a neurotransmitter in brain it has been proposed that these nonessential amino acids should also receive attention (41).

Clearly, more work is required on designing the composition of diet for treatment of phenylketonuria and maternal phenylketonuria. Consideration must also be given to spreading the intake of the amino acid supplement over a longer duration so as to minimize wide fluctuations in plasma amino acid concentrations caused by swallowing of large quantities of rapidly absorbed amino acid mixtures.

TREATMENT AND MANAGEMENT

A phenylalanine-restricted diet is introduced preconceptionally as the means of treatment to prevent in utero fetal damage in maternal PKU. It is essential that all pregnancies are planned. This can be effective only if patients understand the harmful effects that a mother’s high phenylalanine may have on the brain and heart of the developing fetus and how this can be averted by dietary manipulation. Management therefore really starts with counseling given to teenage girls with PKU. To avoid unwanted pregnancies the importance of contraception should be discussed. Patients are encouraged to explain their condition to their future husbands and to bring them for similar counseling.

If the patient has been off dietary restriction for several years, the reintroduction of the diet is likely to require hospital admission to reacquaint the patient with using the diet and collecting Guthrie samples twice weekly for phenylalanine estimations. Full amino acid analyses are carried out monthly. It is not possible to define an absolute threshold value for maternal plasma phenylalanine concentration below which there is no risk to the fetus. Earlier attempts to keep the maternal values below 500 μmol/liter were not always successful in the first trimester and would have allowed fetal phenylalanine concentrations to rise above 800 μmol/liter, a value higher than acceptable in the treatment of young growing children with PKU. Maternal values <250 μmol/liter are needed to keep the fetal values <400 μmol/liter. The first trimester is a period of extensive organ development and lowest maternal tolerance to phenylalanine. Pregnancy nausea and vomiting may be a problem in dietary control. Tolerance gradually increases into the second and third trimesters, coinciding with the increased phenylalanine requirement for growth in the rapidly growing fetus and the development of the phenylalanine hydroxylase enzyme in fetal liver between 16 and 20 weeks. A very rapid rise in tolerance has been observed in our patient with a twin pregnancy. From about 18 weeks the diet administered to all our patients is further supplemented with 2–3 g of tyrosine daily to produce a total intake of 8 g daily.

OUTCOME OF TREATMENT

At University College Hospital we have now looked after 11 pregnancies in seven mothers with 12 children born (one twin pregnancy). None has inherited phenyl-
ketonuria. Of the three conceptions where diet began at 7, 12, and 7 weeks, two infants died of congenital heart disease and the third child was considered to have suffered possible supraventricular tachycardias in infancy. The crucial nature of dietary control in the first trimester as far as congenital malformations is concerned is self-evident from what has been said about embryonic development. The marked decline in congenital heart disease with preconception diet is clear from Lenke and Levy (7). Levy and Waibsren (18), and Drogari et al. (16). None of the nine children born at University College Hospital after preconception diet has a cardiac abnormality or other congenital defect (but see below). These results are consistent with the view that damage to the fetus occurs as a result of high maternal phenylalanine concentrations and not as a result of damage to the sperm or ovum preconceptionally or from unforeseen complications that may occur during dietary restriction during pregnancy.

The data of Drogari et al. (16), collected from many centers including our own, indicated that both birth weight and head circumference at birth are related to phenylalanine concentration at conception, which in turn is related to two factors. First, preconception diet obviously lowers the phenylalanine at conception, and this group presumably remains much better controlled in the first 8 weeks than those not on diet where pregnancy is not diagnosed and dietary control not established until 8 weeks or later. Second, the phenylalanine at conception also reflects the severity of the mother’s PKU, whether treated or untreated, since lower phenylalanine concentrations are likely to be more easily achieved by diet in mothers with milder forms of the disease. Phenylalanine concentrations at conception therefore are likely to correlate closely with first-trimester control. Indeed, from our own data we can show correlations between birth weight and head circumference with maternal plasma phenylalanine concentration in the first trimester. It is surprising perhaps that first-trimester control is so important to the overall growth and head size of the baby. To look independently at the effects of second- or third-trimester control is difficult. The practical difficulties of first-trimester control relate to the fact that it is the period of lowest phenylalanine tolerance and it produces the highest mean values for phenylalanine of any trimester.

The postnatal development of the children born to the PKU mothers is being closely followed at University College Hospital by Dr. Anne Stewart. Of the nine infants born after preconception diet, eight are progressing normally. Minor neurological signs have frequently been noticed in these on very careful neonatal examination, but all have disappeared with time. Intellectual assessments is also being carefully assessed and so far the children are in the normal range for tests appropriate to their age group. There remains one exception—one child out of the nine is grossly abnormal, with seizures and profound developmental delay. At the age of 18 months this child has minimal head control. Investigations at University College Hospital and at the Hospital for Sick Children, Great Ormond Street, have failed to find a cause. The child appears much more profoundly abnormal than usual for the offspring of mothers with untreated PKU. The mother’s first pregnancy was ectopic and terminated with emergency surgery. For that pregnancy and the second she started diet
preconception. Analysis of her amino acids in pregnancy has shown no differences from the findings in PKU mothers on diet where the pregnancy outcome has been normal. Although this baby’s grossly abnormal development does not seem to be due to the mother’s PKU or its management, it is a cautionary reminder to us that we may not have all the answers yet to maternal phenylketonuria, and follow-up of the offspring of PKU mothers for years to come is essential.

REFERENCES


11. Eaton BM, Mann GE, Yudilevich DL. Transport specificity for neutral and basic amino acids at maternal and fetal interfaces of the guinea pig placenta. J Physiol (Lond) 1982;328:245-58.


DISCUSSION

Dr. Wang: Is there any difference between the fetal outcome of maternal typical PKU and maternal atypical PKU?

Dr. Krywawych: The data presented here and other published work indicates that congenital abnormalities in the developing fetus are associated with raised maternal phenylalanine levels at conception and during pregnancy. Congenital heart damage occurs predominantly at maternal plasma phenylalanine concentration above 600 µmol/liter at conception and in pregnancy. For the intellectual development there appears to be a graded effect, whereby the abnormality occurs at lower maternal plasma phenylalanine concentrations but its frequency increases with increasing phenylalanine concentration. Plasma phenylalanine concentrations in this unsafe lower
range would be expected not only in treated typical classical phenylketonuric subjects but also in the milder atypical ones untreated.

Dr. Endres: I think there is a confusion of the nomenclature. Atypical PKU, as you, Dr. Krywawych, define it, is mild PKU or hyperphenylalaninemia called non-PKU HPA, and what you, Dr. Wang, mean is tetrahydrobiopterin (BH₄)-deficient hyperphenylalaninemia or "atypical PKU." Nobody can answer your question because up to now we don’t have a patient with such a disease old enough to become pregnant. I think the prognosis for such a pregnancy would be rather uncertain. There is no experience in treating a woman during pregnancy with neurotransmitter precursors and BH₄.

Dr. Schaub: For the clinician it is important to hear what you recommend: What blood level of phenylalanine do you recommend in maternal PKU?

Dr. Krywawych: Previously, we aimed at controlling the maternal blood phenylalanine level at a concentration of 500 μmol/liter. Currently, we have reduced this figure still further to a value of between 120 and 300 μmol/liter.

Dr. Casar: Dr. Krywawych, you mentioned that you were surprised to see that these infants have microcephaly at birth. I am not surprised, since if there is a negative effect of phenylalanine early in gestation, this might interfere with the neural crest cells. From this layer of cells other neurons divide and from here they will migrate. Thus a toxic effect that interferes with this division and migration can result in microcephaly.

Dr. Casar: Dr. Krywawych, you explained to us in your paper that phenylalanine had a negative effect on some of the neurotransmitters. It is important to realize that neurotransmitters early in gestation function more as trophic factors than as real neurotransmitters, and thus they can interfere with growth and development of the brain. In this respect did you see any differences in the effect of phenylalanine, according to the period of gestation in which you had difficulty in controlling the maternal blood level of phenylalanine?

Dr. Krywawych: We have not actually studied these differences related to control and gestational age. In all the cases where we have experienced poor control of the maternal blood phenylalanine concentration, this period of poor control was confined to conception and the first trimester.

Dr. Sauder: Everybody shares the opinion that it is very important to keep phenylalanine blood levels under 400–600 μmol/liter at the time of conception. But the problem from a practical point of view is that the time of conception does not depend only on the physician (!). So the real difficulty in day-to-day practice is maintaining the phenylalanine level under 500 μmol/liter sometimes for many months. I remember a paper on the use of oral phenylalanine ammonialyse in PKU which resulted in a lowering of the phenylalanine levels by a few milligrams per 100 ml. Do you have an opinion on this therapy in addition to the diet?

Dr. Krywawych: No. I do not have an answer to this. We have not tried this on our patients. We have attempted at conception and during pregnancy to maintain the plasma phenylalanine level at 300 μmol/liter by diet alone. This is extremely difficult but becomes easier into the second and third trimesters. As better control of maternal plasma phenylalanine results in a more favorable outcome for the pregnancy, maybe your proposal should be given more serious consideration.

Dr. Hobbs: Having worked on isoamylases I think there are some lessons to be learned. We chose by chance to use pig pancreatin. This is immunologically identical to the human enzyme, so most patients do not make antibodies to it. For almost any other animal you make antibodies to the enzyme, and if you choose bovine pancreatin it could be useless. It would generate antibodies that could prevent the enzyme from working. My conviction would be that if you try these enzymes they might work for 6 weeks and then fail. There was an attempt to treat
hyperammonemia in liver disease with a urease, and it does not work after a few weeks. The human being makes antibodies and stops the enzyme working.

**Dr. Van den Berge:** I would not be as negative as Dr. Hobbs with respect to enzyme replacement therapy with purified enzyme preparations. In two inborn errors of purine metabolism, adenosine deaminase deficiency (1) and purine nucleoside phosphorylase deficiency, good clinical results have been obtained with polyethylene glycol (PEG)–modified enzymes, given intramuscularly. PEG attachment to the enzymes markedly decreases their clearance from the circulation, their attack by degradative enzymes, and their antigenicity. PEG-modified enzymes are thus valuable in lowering the concentration of toxic metabolites in plasma.

**Dr. Endres:** I would like to underline what Professor Saudubray said. I think young women or girls with PKU never know when they will become pregnant. And you remember the figures Dr. Krywawych showed us: among the offsprings of those mothers who had phenylalanine levels between 3 and 10 mg/dl (170–570 μmol/liter), 21% had IQs below 75 and 24% had microcephaly. This 3–10 mg range is very great. The question arises as to whether these babies, the 21% or 24%, are to be found mainly in the upper part of this range or whether they are spread throughout the range. So what we don’t know at the moment is where the safe limit is. Is it sufficient enough to say that the diet has to be restricted in phenylalanine in order to keep the plasma level of phenylalanine below 10 (570 μmol/liter) or, as in our prospective study in Germany, below 5 mg/dl (285 μmol/liter). But I am not sure whether 5 mg is low enough.

**Dr. Krywawych:** I agree that we are not certain exactly what the safe limit is. We have decreased our earlier recommended limit still further from 500 μmol/liter. The phenylalanine concentration on the fetal side of the placenta, which is always higher than that on the maternal side, is probably the most important factor to consider.

**Dr. Holton:** I am not sure what significance one can attach to animal experiments on the competitive inhibition of amino acid transport into brain. For a number of years we look at the question of the effect of histidine on the transport of amino acids into brain, and found that it has exactly the same effect as phenylalanine on the transport of other amino acids. You said that the blood-brain barrier is established very early in the fetus. I wonder whether you could put a time to that? We have been looking at fetal cerebrospinal fluid amino acids and we have found high levels, a completely different pattern to those of infants.

**Dr. Krywawych:** I cannot put a precise time on the development of the blood-brain barrier. It is dependent on the formation of tight junctions that have been identified histologically early in gestation. I agree that it is very difficult to extrapolate from animal experiments to the human condition. However, from the $V_{max}$ and pK values for these amino acid carriers in humans it can be calculated that in histidinemia, tyrosine and tryptophan influx is decreased to only 50 to 60% of normal, whereas in phenylketonuria it is decreased to about 20%. To attain the same degree of inhibition for tyrosine and tryptophan in phenylketonuria as occurs in histidinemia, the plasma phenylalanine concentration would have to be maintained at approximately 450 μmol/liter.

**Dr. Vis:** Available information from the literature, as well as the data shown by Dr. Krywawych, point to the fact that one of the conditions for a mother with PKU to enable her to give birth to a normal newborn is very strict dietetic management. When a PKU patient informs me that she wants to become pregnant, I always prescribe a very strict dietetic treatment for a test period of 2 months, before conception, to evaluate the compliance of the mother-to-be. The dietetic prescription for a pregnant woman with PKU is not easy to establish, because besides phenylalanine restriction, a very precise balance needs to be maintained between the intakes of various other nutrients. We don’t know that much about the nutritional requirements of the fetus. For instance, diets low in phenylalanine are usually also low in taurine, which is
not synthesized by the fetus, although that nutrient is essential for the development of the fetal brain. Trace element intakes also need to be monitored. Most PKU diets, for example, are low in selenium. I would like to ask at what time of the day plasma phenylalanine levels need to be measured. The authors suggest that levels below 400 μmol/liter (6.5 mg/dl) or even 600 μmol/liter (10 mg/dl) are safe. However, phenylalanine levels in blood are sometimes extremely variable during the day (postprandial levels for instance). Further to this, I would like to emphasize Dr. Eggermont’s comment about the risk in case of fetal heterozygosity. Even in the normal fetus the enzymatic machinery responsible for the conversion of phenylalanine to tyrosine comes into action very slowly. We all know of transient hyperphenylalaninemia in premature newborns. This phenomenon is quite certainly even more important in the heterozygous fetus.

Dr. Krywawych: At the moment I do not think that we really know what the complete composition of the diet should be. Regarding the taurine requirement of the fetus, from investigations on placental transport there is some evidence suggesting that taurine does not cross the placenta. The timing of blood sampling complicates data, for it is not always possible to time the collection of samples at precisely the same point in relation to the ingestion of food. Fluctuations of plasma amino acid levels do occur in relation to feeds. Future diets may need to look toward a slower and more even release of amino acids into the blood. Consideration also needs to be given to a more balanced dietary amino acid composition so as to restore in the plasma concentration more normal ratios of the individual amino acids relative to phenylalanine.

REFERENCE