Chapter 8

Brain dysfunction in phenylketonuria (PKU): Is phenylalanine toxicity the only possible cause?

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ABSTRACT

In phenylketonuria, mental retardation is prevented by a diet that severely restricts natural protein and is supplemented with a phenylalanine-free amino acid mixture. The result is an almost normal outcome, although some neuropsychological disturbances remain. The pathology underlying cognitive dysfunction in phenylketonuria is unknown, although it is clear that the high plasma concentrations of phenylalanine influence the blood-brain barrier transport of large neutral amino acids. The high plasma phenylalanine concentrations increase phenylalanine entry into brain and restrict the entry of other large neutral amino acids. In the literature, emphasis has been on high brain phenylalanine as the pathological substrate that causes mental retardation. Phenylalanine was found to interfere with different cerebral enzyme systems. However, apart from the neurotoxicity of phenylalanine, a deficiency of the large neutral amino acids in brain may also be an important factor affecting cognitive function in phenylketonuria. Cerebral protein synthesis was found to be disturbed in a mouse model of phenylketonuria and could be caused by shortage of large neutral amino acids instead of high levels of phenylalanine. Therefore, in this review we emphasize the possibility of a different idea about the pathogenesis of mental dysfunction in phenylketonuria patients and the aim of treatment strategies. The aim of treatment in phenylketonuria might be to normalize cerebral concentrations of all large neutral amino acids rather than prevent high cerebral phenylalanine concentrations alone. In-depth studies are necessary to investigate the role of large neutral amino acid deficiencies in brain.
INTRODUCTION

The pathogenesis of brain dysfunction in phenylketonuria (PKU; OMIM 261600) is still unknown.\textsuperscript{1,2} PKU is a monogenetic disease, in which mutations in the gene encoding the enzyme phenylalanine hydroxylase (PAH) cause a deficient activity resulting in increased phenylalanine and low to normal tyrosine concentrations in blood and tissues.\textsuperscript{1} Plasma phenylalanine, rather than tyrosine, concentrations are related to cognitive outcome in PKU. Furthermore, dietary phenylalanine restriction rather than tyrosine supplementation as the only treatment has been successful as treatment strategy.\textsuperscript{2} Thus, phenylalanine affects brain function, in one way or another.

In their review on the neurochemistry of PKU, Surtees and Blau addressed the effects of blood phenylalanine on cerebral free amino acid concentrations, and the effect of high blood and brain phenylalanine on neurotransmitters, cerebral protein synthesis, and myelin metabolism.\textsuperscript{3} However, some of the data used in their review were based on the rat model of hyperphenylalaninemia, which is now considered an inadequate animal model of PAH deficiency. From the time of publication in 2000, data on the PAH\textsuperscript{enu2} mice in particular have added new information about the pathogenesis of brain dysfunction in PKU.

In the present review we try to define different cascades, shown in Figure 1, and how they might interact and result in brain dysfunction in PKU. In this way, we aim to build further the concept of pathogenesis of brain dysfunction in PKU. Starting from the high plasma phenylalanine concentration, we discuss the importance of the blood-brain barrier (BBB), effects of high cerebral concentrations of phenylalanine, and the effects of low cerebral concentrations of other large neutral amino acids (LNAA).

Figure 1. The pathophysiology of brain dysfunction in phenylketonuria
The importance of the blood-brain barrier

On the basis of two important observations, we think that transport of LNAA across the BBB and its impairment in PKU is essential to the pathogenesis of mental dysfunction in PKU patients. First, the clinical symptoms and signs of PKU almost exclusively concern brain. Second, some untreated PKU patients with consequently high plasma phenylalanine concentrations escape from severe brain dysfunction. Studies with magnetic resonance spectroscopy (MRS) by Weglage and co-workers revealed that these patients have almost normal instead of very high phenylalanine concentrations in brain as observed in others. The studies of Weglage and co-workers substantiated the finding of untreated PKU women who did not show severe mental retardation but gave birth to non-PKU children with severe intrauterine growth restriction, including microcephaly, mental retardation, congenital heart defects and facial abnormalities. From the MRS observations it was concluded that in PKU patients transport of LNAA across the BBB is essential in the pathophysiology of brain dysfunction in PKU.

Phenylalanine enters the brain using the L-type amino acid transporter (LAT1, SLC7A5) in competition with eight other LNAA (i.e. tyrosine, tryptophan, valine, isoleucine, leucine, threonine, methionine, and histidine). The affinity of the LAT1 transporter for phenylalanine is high, resulting in a high transport velocity of phenylalanine even at only slightly increased blood phenylalanine concentrations. When blood phenylalanine concentrations are elevated, phenylalanine is preferentially transported across the BBB at the expense of other LNAA, resulting in high cerebral concentrations of phenylalanine and low cerebral concentrations of other LNAA. In this respect, we have to mention that rates of amino acid transport across BBB have not been estimated yet in PKU patients. The effects of high cerebral phenylalanine concentrations and low cerebral concentrations of other LNAA will be discussed in the next paragraphs.

Effects of increased cerebral phenylalanine concentrations

With the development of MRS it became possible to measure in vivo cerebral phenylalanine concentrations, and to investigate the relation with all possible outcome variables. So far, while the relation between plasma phenylalanine and outcome is beyond any doubt, the relationship between brain phenylalanine concentration and outcome is less clear. The group of Möller and Weglage showed a relation between brain phenylalanine concentrations and outcome. However, although the patients clearly escaped from severe mental retardation, at least some of them do not have a complete normal outcome either, even though brain phenylalanine concentrations are almost normal. The study of Schindeler et al. showed that a short period of supplementation of LNAA resulted in improvement of neuropsychological functions without a decrease of cerebral phenylalanine concentrations.

Studies in brains of untreated PAH mice showed decreased concentrations of neurotransmitters. It was suggested that high brain phenylalanine concentrations inhibited the enzyme activity of tyrosine hydroxylase and tryptophan hydroxylase. Phenylalanine was also
shown to inhibit the activity of both human tyrosine and tryptophan hydroxylase as expressed in E-coli.\textsuperscript{14} Still, the influence of high cerebral phenylalanine concentrations on this neurotransmitter synthesis is an issue of ongoing debate.\textsuperscript{15} Early \textit{in vitro} studies indicated that high cerebral phenylalanine concentrations may compete with cerebral tyrosine to be hydroxylated by cerebral tyrosine hydroxylase, so that tyrosine is formed in brain and the conversion of tyrosine to dopa is blocked.\textsuperscript{16-18} When brain cells in culture were exposed to high phenylalanine concentrations in the medium, net protein synthesis was decreased.\textsuperscript{19} High phenylalanine concentrations were also found to decrease the activity of HMG-CoA reductase, resulting in impaired cholesterol synthesis.\textsuperscript{20} As protein and cholesterol are essential parts of myelin, this finding is in line with observations of hypomyelination and gliosis in brain cells of PAH\textsuperscript{enu2} and wild type mice\textsuperscript{21} and in brains of deceased PKU patients (Bauman and Kemper 1982). Acute application of high concentrations of phenylalanine to cell cultures of neurones reduced glutaminergic synaptic transmission\textsuperscript{22,23}, synaptogenesis, and activity of pyruvate kinase.\textsuperscript{24} A decreased activity of pyruvate kinase may relate to the \textit{in vivo} finding of decreased energy metabolism in PKU patients using positron emission tomography.\textsuperscript{25}

**Effects of decreased cerebral concentrations of large neutral amino acids**

In our view, high plasma phenylalanine concentrations are intrinsically related to low cerebral concentrations of LNAA. Decreased cerebral concentrations of tyrosine and tryptophan may result in insufficient synthesis of dopamine and serotonin, respectively. In cerebrospinal fluid and brain of untreated and treated PKU patients, the concentrations of neurotransmitters are clearly decreased.\textsuperscript{26-31} Similar observations were made in brain tissue of PKU mice.\textsuperscript{31,12} Bonafé and colleagues showed that in a patient with mild PAH deficiency, resulting in moderate elevation of plasma phenylalanine concentrations, low concentrations of neurotransmitters can be found, and that supplementation of a combination of L-Dopa, tyrosine, and 5-hydroxytryptophan resulted in improvement of ataxia, and rigidity.\textsuperscript{32}

Dopamine deficiency is suggested to result in prefrontal cortex deficiency and is thought to be of significant importance in brain dysfunction in PKU.\textsuperscript{33-42} However, such a clearly defined neuropsychological deficit was neither found in PAH\textsuperscript{enu2} mice\textsuperscript{43,44,45}, which best resemble human PKU, nor in more recent studies on PKU patients.\textsuperscript{46-49} Moreover, dopamine deficiency, which underlies Parkinson and attention deficit hyperactivity disorder\textsuperscript{50}, is not specifically related to PAH deficiency. Furthermore, supplementation with L-dopa in PKU patients showed no positive effect on neuropsychological functions and visual evoked potentials.\textsuperscript{51}

Deficiencies in serotonin are thought to have a more general effect than dopamine deficiency\textsuperscript{42}, and may result in psychosocial dysfunction rather than impairment of cognitive function.\textsuperscript{33,52} Therefore, deficiency of tryptophan does not present a full explanation of the brain dysfunction in PKU.
Second, decreased availability of essential amino acids results in a decreased synthesis of protein in general. Owing to the PAH deficiency, tyrosine has become an essential amino acid as well. As a consequence, in PKU all LNAA are essential amino acids. Cerebral protein synthesis was decreased in PAH<sup>enu2</sup> mice. Besides this study, no published data are available on cerebral protein synthesis in PKU.

From the field of neurobiology we know that cerebral protein synthesis is essential for brain development and function. One of the best-known specific examples of cerebral proteins is myelin, which is often found to be abnormal in PKU. Myelin basic protein is essential for synthesis of myelin. The synthesis of myelin basic protein was impaired if one of the essential amino acids was deficient. Theoretically, the low concentration of any LNAA results not only in reduced amounts of myelin but also in low amounts of other proteins, including cerebral enzymes such as tyrosine and tryptophan hydroxylase, HMG-CoA reductase, pyruvate kinase as well as cerebral receptor proteins such as the glutamate receptor. These reductions in various proteins may result in decreased synaptic plasticity as well as decreased axonal outgrowth. Although the relationships between these processes and mental dysfunction have not been investigated intensively in PKU, the processes are known to be disturbed in mental dysfunction. This, in turn, may contribute to the brain dysfunction as seen in untreated and treated PKU patients.

**Cerebral phenylalanine toxicity and large neutral amino acid deficiency**

Combined, the above-mentioned studies indicate that it is difficult to distinguish between the effects of high cerebral concentrations of phenylalanine and low cerebral concentrations of the other LNAA. For example, the paper by Pietz and colleagues showed that supplying large amounts of LNAA decreases brain phenylalanine and improves cerebral electrophysiology. These data underlined the involvement of LNAA in the brain dysfunction of PKU patients but did not answer whether the effects were due to a decrease in brain phenylalanine or to increased availability of other LNAA. The data again show that explaining the cascade from the gene defect to the clinical phenotype, even in monogenetic traits, is not a simple task.

The data of Weglage and colleagues and Pietz and colleagues suggest the importance of normal cerebral phenylalanine concentrations, but the cerebral concentrations of other LNAA were not measured. Consequently, cerebral deficiency of LNAA rather than cerebral phenylalanine toxicity may prove to be the main cause of brain dysfunction of PKU. Therefore, preventing low cerebral LNAA concentrations might be of equal importance in preventing high cerebral phenylalanine concentrations. Following this line of reasoning, the aim of treatment in PKU would be to normalize cerebral concentrations of all LNAA rather than prevent high cerebral phenylalanine concentrations alone.

Such a theory could also apply to other diseases in which one of the LNAA is increased in plasma and brain, e.g. maple syrup urine disease with increased leucine concentrations, and tyrosinaemia types I to III with increased concentrations of tyrosine. Future animal and human studies are needed.
to confirm or refute this theory.

CONCLUSION

The cascade of the pathogenesis of brain dysfunction in PKU starts with the effect of the high blood phenylalanine concentration on BBB transport of LNAA into brain. In this review we emphasize that not only direct cerebral phenylalanine toxicity but also cerebral shortage of LNAA might cause brain dysfunction in PKU patients. Further in vitro and in vivo studies are necessary to confirm or refute this concept of brain dysfunction in PKU with possible consequences for treatment. As long as these data are not available, phenylalanine restriction remains the treatment of choice, especially during childhood and in maternal PKU.

REFERENCES


