Probing the Blood/Brain Barrier in Neonates: ^{1}H-MR Spectroscopy shows Low Protection against High Phenylalanine

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Introduction

Phenylketonuria (PKU) is the most frequent inborn disorder of amino acid metabolism, caused by a deficiency of phenylalanine (Phe) hydroxylase. This leads to accumulation of Phe in plasma and brain. Clinical features of PKU include severe impairment of brain development in untreated infants, but also acute reversible neurotoxic effects on brain function. The pathophysiology of both clinical manifestations is still unclear. Treatment consists of dietary restriction for Phe and supplementation with the other amino acids. ^{1}H-MRS has been used in a number of studies to determine the blood/brain ratio for Phe and the kinetics of Phe influx into the brain in adult PKU patients ((1-3) and refs therein) and healthy subjects (4). While the exact value for the blood/brain ratio is still disputed, and while it is also not settled whether there is major individual variance for this ratio in typical PKU patients (5), there is consensus that the Phe concentration is considerably (2-4 times) lower in brain than blood for adult patients. It is also undisputed that the most crucial time determining intellectual outcome is early childhood where the brain is particularly sensitive to toxins. Hence, dietary treatment is aimed at achieving very low blood Phe values in childhood. However, very little is known about the age-dependence of the blood brain barrier protection against high Phe values. There is no data for humans while animal and cell models suggest higher amino acid transport into brain in the neonatal period (6;7). Here, we report on ^{1}H-MRS studies to determine the blood/brain ratio for Phe in 2 neonates with PKU in comparison to values obtained for adult PKU patients.

Methods and Subjects

All spectra were recorded on a clinical 1.5T MR scanner (Signa, GE) using a quadrature head coil. Data acquisition and processing was reported earlier (3). In brief: PRESS localization (20ms TE, 2.0 and 2.5 s TR for adult and neonates, respectively, 128-256 acquisitions/spectrum, 1-4 spectra per subject, supraventricular ROI of 17-70 cm^3 for neonates and adults) including non-water-suppressed scans for referencing (eddy current correction, compartmentation information, quantitation standard). Spectral fitting was performed using prior knowledge of the Phe spectrum, a parameterized background signal and the lineshape obtained from the water scan (3). *Neonates*: Two PKU patients, investigated soon after diagnosis and before dietary treatment (Pt A, 43 weeks gestational age [GA], 9 days after birth, Pt B. 36 weeks GA, 9 days after birth). Pt. B was rescanned under treatment 5 days later. Two healthy neonates served as controls (43 and 44 weeks GA). All examinations performed during postprandial sleep without sedation. Individual acquisitions affected by subject motion were discarded. *Adults*: 5 PKU patients (23±6 years old), 6 healthy subjects (23±3 years old). Blood Phe values were determined on an automatic amino-acid analyzer (Biochrome, UK).

Results

^{1}H-MR spectra for patients and controls are plotted in Fig. 1. demonstrating the spectral quality and the qualitative findings. The neonatal spectra were of excellent quality allowing quantitative evaluations for Phe, in spite of the smaller ROI size and sometimes lower number of averages. The contribution of Phe at 7.35 ppm (superimposed on larger background signal) is particularly striking for Patient A with a fairly high blood Phe content. The main features in the downfield region of neonates compared to spectra from adults are the smaller contribution from the amide proton of NAA at 7.8 ppm and of the peak at 7.0 ppm. The background signals for Phe at 7.3 ppm do not show much age-dependence. Numerical results are listed in Table 1. The brain/blood ratio is strikingly higher for neonates than for adults. After treatment brain Phe values drop to normal levels in parallel with blood levels in patient B, such that their ratio is ill-defined.

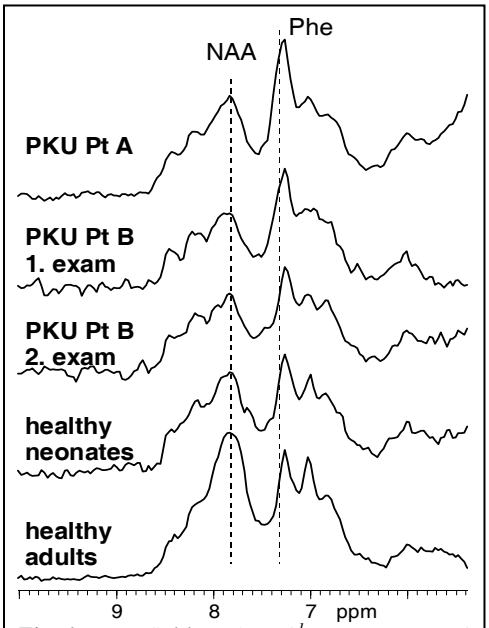

Fig. 1: *Downfield portion of ^{1}H-MR spectra of newborn PKU patients, healthy newborns, and adult controls*

	Blood Phe [mM]	Brain Phe [mmol/kg]	ratio
Neonate A	0.75	0.39	0.52
Neonate B	0.29	0.22	0.77
Neonate B	0.05	0.07	(1.2)
6 Adult Pts	0.72±0.3	0.21±0.07	0.29±0.04

Table 1*: Blood and brain Phe values*

Discussion & Conclusion

The present study shows that the human blood/brain barrier does not provide the same protection against high blood Phe values for the newborn as it does for the adult. At identical blood Phe levels the newborn PKU patients brain is exposed to much higher Phe levels than the adult. This underlines the importance of strictest dietary control for infants with PKU.

References

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