Effect of Standard Oral Aminoacid Mixtures on Cerebral Phenylalanine Content and the Dynamics of Blood-Brain-Barrier Dynamics in PKU Patients

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Introduction

Phenylketonuria (PKU) is the most frequent inborn disorder of amino acid (AA) metabolism, caused by a deficiency of phenylalanine (Phe) hydroxylase leading 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. Treatment consists of dietary protein restriction (Phe) and supplementation with the other AAs in order to prevent general AA deficiencies. Since all Large Neutral AAs (LNAA) are co-transported by the same carrier at the blood-brain-barrier (BBB), the high vascular Phe content can also lead to reduced cerebral levels of these LNAA. On the other hand, it has been shown [1] that continued oral substitution with high loads of LNAA can be used to block the entry of additional Phe (administered as an oral load) into brain for several hours – presumably because of a saturation of the inward-bound BBB carrier through the LNAA. In the current study, we investigated whether the same short-term effect is achieved by oral supplements with standard commercial AA powder in clinically realistic dosage and secondly whether the standard AA mixture, as recommended in normal dietary treatment of PKU patients, not only prevents deficiency in AA, but also leads to lower cerebral steady state Phe levels because of its modulating influence on the BBB transport.

Methods and Subjects

Short term effects: Five adult male PKU patients were given an oral L-PHE load (100 mg/kg_{BW}) in each of 2 test series. In one run, termed 'PHE_{AA}', PHE intake was supplemented with commercial AA mixture (corresponding to 0.8 g protein/kg_{BW}) distributed into 5 portions, given preload and up to 7.5 h postload. A baseline series was performed without AA intake ('PHE_{only}'). Plasma Phe, valine, methionine, leucine, isoleucine, tyrosine, histidine, and tryptophan were determined pre- and up to 24 h post-load. Brain Phe was measured by ^{1}H-MRS pre-, 11h and 22 h post-load from a large volume above the ventricular system.

Long term effects: Six adult male PKU patients were investigated two times. First, after a period of two weeks eating their usual diet but without AA mixture intake, and second, after two weeks on their usual diet, but supplemented with commercial AA mixture (corresponding to 0.8 g protein/kg_{BW} per day in 4 to 5 portions).

MR: All spectra were recorded on a clinical 1.5T MR scanner (Signa, GE). Data acquisition, processing, and prior knowledge fitting was reported earlier (1).

Modeling: Kinetic modeling of BBB dynamics was performed in analogy to Ref (2), but including asymmetric Michaelis Menton kinetics. Carrier affinities for LNAA needed to calculate the apparent Michaelis constant K_m^{app} were taken from (3).

Results

Fig. 1 a) and b) show blood (dashed line) and brain (solid lines) Phe levels obtained in the short term study (individual data in color, group mean in black). While in the baseline series, both blood and brain Phe levels increase as expected, the brain levels do not rise significantly during supplementation with commercial AA powder (0 to 11h). On average, they increase only afterwards and remain at lower levels compared to the baseline series. Fig. 1 c) presents the main results of the long term supplementation in the form of the brain/blood Phe concentration ratios. They show a decrease for all 6 subjects. Furthermore, K_m^{app} values, calculated from blood LNAA concentrations for each subject and time point, were found to correlate inversely with the brain/blood Phe ratio. Using basic assumptions, it turned out that symmetric MM modeling cannot explain the main features of both experiments (i.e. blocked entry, reduced ratio), but that an asymmetric model can. The details of this model (incl. asymmetry at the BBB introduced through the difference in vascular vs. brain LNAA concentrations only, and constant brain LNAA concentrations) are heuristic first approximations only.

Discussion & Conclusion

The short term study showed that cerebral inflow of Phe from an oral load can be blocked by standard dietary AA supplementation distributed over several daily doses. Furthermore, the long term data suggests that strict use of the standard AA supplementation with commercial general AA powder does not only prevent deficits in AA levels, which is the prime target effect, but that it additionally leads to the beneficent effect of lowering the cerebral Phe levels through LNAA competition for the common carrier at the BBB. Detailed kinetic modeling is hampered by lack of information on the cerebral LNAA content and unknown influence of other AA carrier systems.

References

1. Pietz J et al. J Clin Invest. 1999 ;103:1169. **2.** Moeller H et al JCBFM 1998;18:1184. **3**. Smith QR et al. J Neurochem. 1987;49:1651.

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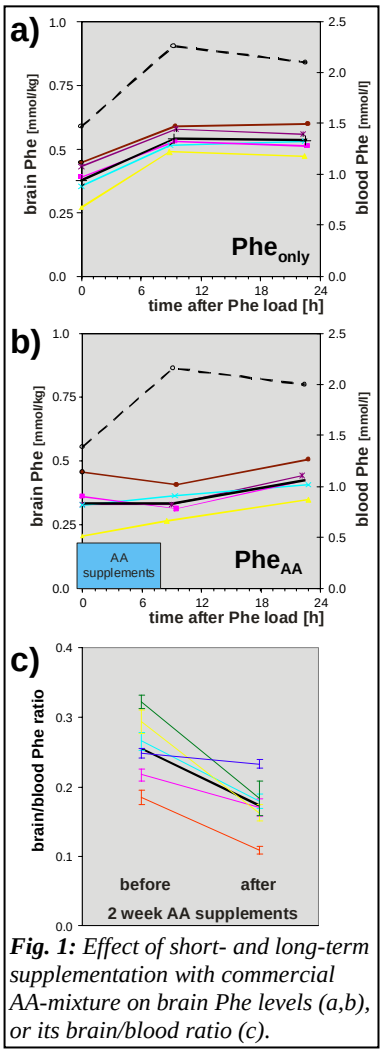

Fig. 1: *Effect of short- and long-term supplementation with commercial AA-mixture on brain Phe levels (a,b), or its brain/blood ratio (c).*