

Novel treatment strategy in mouse model of maple syrup urine disease

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Introduction: MSUD is an autosomal recessive disorder of BCAA metabolism presenting with life-threatening cerebral edema and dysmyelination in affected individuals. Treatment requires life-long dietary restriction and monitoring of BCAAs to avoid brain injury. Despite careful management, children commonly suffer metabolic decompensation in the context of catabolic stress associated with non-specific illness. The mechanisms underlying this decompensation and brain injury are poorly understood. Using a recently developed mouse model of intermediate MSUD (1), we show that rapid brain leucine accumulation displaces other essential amino acids resulting in neurotransmitter depletion and disruption of normal brain growth and development. A novel approach of administering norleucine substantially delayed encephalopathy in intermediate MSUD mice placed on a high-protein diet that mimics the catabolic stress shown to cause encephalopathy in human MSUD. Current findings suggest that norleucine administration is a potential treatment to prevent encephalopathy in children with MSUD.

Methods: Intermediate MSUD (iMSUD) mice were placed on a low-BCAA diet allowing them to survive. These mice were then placed on a high-protein diet at 6-weeks of age to induce encephalopathy. A second group of iMSUD mice were given 5% norleucine with the high-protein diet (n=6 per group). All mice were evaluated for behavior and MRI changes at 48 and 72 hours after high-protein diet exposure (N=6 for each group). Behavioral changes were scored using a scoring system as previously described (2). Amino acids were separated and measured from HPLC of brain extracts by UV detection after phenyl-isothiocyanate derivatization. MRI was performed on a 7.0 T Bruker system using a 2 mm birdcage coil. Mice were anesthetized with isoflurane (1-1.5%). A T2-weighted multi-echo spin echo sequence was used (twelve 1.0 mm thick slices, TR/TE=3000/8.7-121.8 ms, 14 echoes, 117X117 μm^2 resolution, 2 averages). Transverse relaxation time constants (T2) were calculated on a pixel-by-pixel basis from the corresponding exponential fits.

Results: BCAA (leucine) was shown to accumulate substantially in the brain of iMSUD mice placed on a high-protein diet (Fig. 1A and B). Other large neutral amino acids including tyrosine and tryptophan were depleted in the brain during this time and correlated with reduced brain dopamine levels. At 72 hours of high-protein diet exposure, iMSUD mice showed behavioral changes including ataxia and dystonia that correlated with abnormal MRI findings (Fig. 1A,C and D). Providing 5% norleucine reduced brain leucine levels and delayed onset of encephalopathy in iMSUD mice placed on the high-protein diet (Fig. 1A and C).

Conclusions: A high-protein diet raised brain leucine levels and induced encephalopathy in iMSUD mice. Accumulation of leucine likely competes with other large neutral amino acids using the same transporter and results in diminished tyrosine and subsequent dopamine levels. MRI changes in iMSUD mice correlated with behavioral changes. Administration of norleucine delayed encephalopathy and showed neuroprotection in iMSUD mice. Norleucine uses the same large neutral amino acid transporter as leucine and likely competes with leucine for brain access. Current finding indicate that norleucine may provide an effective treatment to delay acute encephalopathy in children with MSUD.

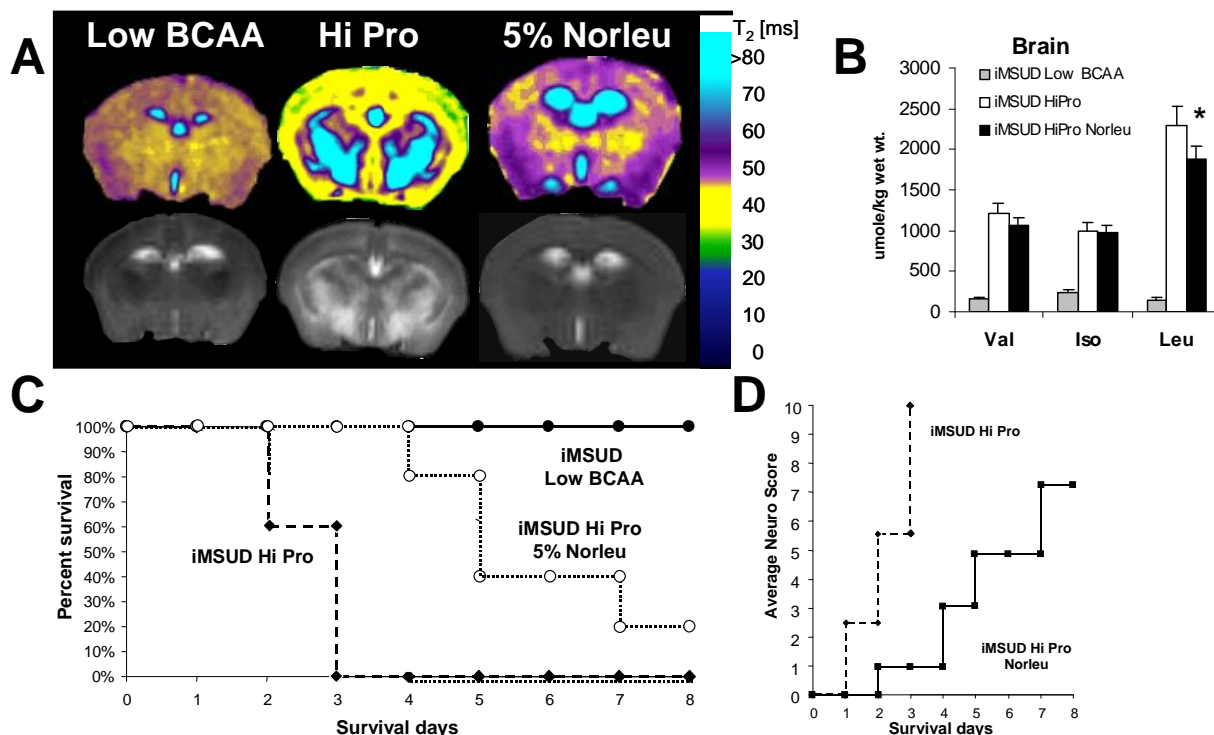


Figure 1. A) MRI images (bottom) and T2 maps (above) with associated colorimetric scale. **B)** BCAA levels in brain of iMSUD mice on a low BCAA diet or high-protein diet with or without 5% norleucine (valine-Val, isoleucine-Iso, leucine-Leu, Mean \pm s.e.m, * p < 0.01, n=6 per group), **C)** Survival of iMSUD mice on different diets, **D)** Average daily neurologic scores after starting high-protein diet in iMSUD mice with and without norleucine.

1. Homanics, G.E. et al. 2006. *BMC Med Genet* 7:33.
2. Zinnanti, W.J. et al. 2006. *Brain* 129:899-910.