13C MRS study of human ornithine transcarbamylase deficiency

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Aim: To investigate glutamine and glutamate turnover in ornithine transcarbamylase deficiency (OTCD).

Background: OTCD is a treatable, X linked disorder of ureagenesis leading to hyperammonemic encephalopathy. The etiology of brain injury in OTCD is not fully known. ^{1}H MRS studies have demonstrated elevations in glutamine and decreases in myoinositol and choline in patients who are clinically symptomatic as well as ostensibly asymptomatic subjects [1-3]. Depletion of myoinositol has proven to be a marker of the disease and its concentration inversely correlates with disease severity. While ^{1}H MRS is a sensitive tool to detect biochemical abnormalities in individual patients, the specificity suffers from the complex peak pattern due to J-coupling and signal from different compounds co-resonating at similar chemical shifts. In vivo ^{13}C MRS can reliably quantitate distinct signals from Glu and Gln. Unambiguous assignment of these metabolites can contribute to a better understanding of the pathogenesis and treatment of brain dysfunction in urea cycle disorders, categorize subjects as to their phenotype, and is an innovative approach that will advance knowledge in this field. ^{13}C metabolic flux measurements have proved useful in grading severity of a related brain disorder, hepatic encephalopathy (HE) [4].

Methods: Three subjects with partial OTCD were studied with quantitative ^{1}H and ^{13}C MRS and compared to control, unaffected subjects. Clinical information is summarized in Table 1. Informed consent was provided and the study was approved by the Internal Review Board of the Huntington Memorial Hospital, Pasadena, CA. ^{13}C MRS was performed on a 1.5 Signa GE Scanner equipped with a standalone broadband decoupling hardware. Axial T2 weighted MR images and localized quantitative proton spectra (PRESS TE/TR 35msec/1.5 msec) were acquired with the standard GE head coil from a 12.5 ml volume of mixed gray and white matter of the occipital region. Proton decoupled ^{13}C spectra were acquired with a half volume surface coil using a 4 kHZ excitation bandwidth and WALTZ 4 decoupling bandwidth 1000 Hz centered at 2.7 ppm in the proton spectrum. After natural abundance ^{13}C MRS was obtained in 5 minute blocks for 20-30 minutes, intravenous low dose infusion of 0.23g/kg body weight of 99% 1-13C glucose (Cambridge Isotopes Laboratory CIL) (20% W/V) over 10 minutes was commenced and unlocalized ^{13}C MRS acquisition continued over 120 minutes [5]. Myoinositol which does not become enriched, was used as an internal reference for quantification of other ^{13}C metabolites.

Results: All subjects tolerated the procedure. 1-^{13}C glucose appeared in the ^{13}C brain spectra of all subjects within minutes. Enrichment of C1 – thru C5 (not shown) of glutamate and glutamine occurred, as expected from normal operation of turns 1, 2 and 3 of the cerebral TCA cycle. Enriched ^{13}C HCO_3^-, observed in all patients and controls after 60 minutes, confirming complete glucose oxidation. While preliminary interpretation (flux rates to be derived) indicates essentially normal glucose uptake and further metabolism through the neuronal glial glutamate-glutamine cycle in treated or asymptomatic patients with OTCD, excess of enrichment in glutamine C4 and C2 (Figure 1) compared to control is consistent with the anticipated abnormality in glial glutamine synthesis in OTCD.

Table 1: Subjects characterirstics

Subjects	Age of onset	Symptoms	Disease control
54 yo, M, OTCD	38 yrs	coma	good
29 yo, M, OTCD	infancy	recurrent coma, hyperammomenia	poor
35 yo, F, OTCD	16 yrs	headaches, hyperammonemia	good
control	N/A	N/A	N/A

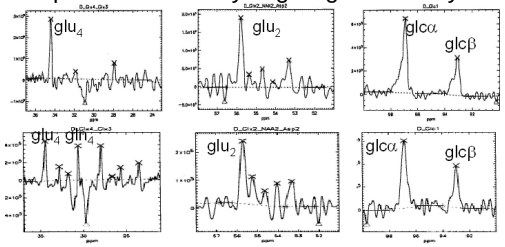

Figure 1: Difference spectra of ^{13}C enrichment of Glc, Glu Glu4, Gln 4 and Gln2, 45 mir after start of 1-^{13}C glucose infusion in normal (top) and OTCD subject (bottom).

Discussion: Urea cycle enzyme defects impact the brain in complex ways. Using ^{13}C MRS we confirm that OTCD subjects metabolize administered ^{13}C glucose through three turns of the neuronal TCA-cycle. As in an earlier study of patient with HE [5], excess of ^{13}C glutamine is consistent with the anticipated abnormalities in glial glutamate metabolism. Further elucidation of flux rates may identify subtle differences between symptomatic and asymptomatic OTCD carriers.

Conclusion: Combined ^{1}H and ^{13}C MRS in patients with a treatable inborn error is feasible and may contribute to better understanding of pathogenesis, diagnosis and preventive therapies for OTCD, the commonest genetic disorder of urea cycle.

References: [1] Gyato et al Ann Neurol 2004, 55, 80-86; [2] Gropman and Batshaw Mol. Genet. Metab. 2004, 81 suppl 1 S58-62; 3. [3] Gropman et al Mol. Genet. Metab. 2007, 90: 243. [4] Bluml et al. MRM 2001, 45, 981-993. [5] Moreno et al. MRM 2001, 46, 39-48.

Acknowledgements: Supported by K12RR17613, U54RR019453-04 and 1M01RR020359-010058 (AG), NIDA K2521112 (NS), NARSAD Mental Health Research Association (KH), NIH NS048589 (BDR) and travel grant to PSA from CIL..