Neurochemical Modulation in a Rat Model of Birth Asphyxia Detected by In Vivo Proton MRS

T. Atwood[1,2], A. Ronca[3,4], G. Kleven[3], and J-M. Zhu[1,2]

[1]Biomedical Engineering, Wake Forest University School of Medicine, Winston-Salem, NC, United States, [2]Radiation Oncology, Wake Forest University School of Medicine, Winston-Salem, NC, United States, [3]Obstetrics and Gynecology, Wake Forest University School of Medicine, Winston-Salem, NC, United States, [4]Neurobiology and Anatomy, Wake Forest University School of Medicine, Winston-Salem, NC, United States

INTRODUCTION

Perinatal asphyxia is a primary determinant of neurological morbidity and mortality in human fetuses which may be caused by irreversible damage to the brain leading to neuron loss in certain brain regions [1]. More specifically, birth asphyxia is known to often lead to hypoxic-ischemic encephalopathy which may produce several long term adverse effects on neurological development and behavior changes, such as learning disabilities, mental retardation, spasticity and epilepsy [2, 3]. Previous studies have demonstrated the ability of *in vivo* magnetic resonance spectroscopy (MRS) to identify metabolic changes in infants exposed to various uncontrolled asphyxic events in clinical cases [4], but no studies have been performed to identify metabolic markers as they relate to a specific and controlled birth asphyxia event. The goal of this study was to identify changing neurometabolites in fetal rats, at several postnatal stages, as a result of controlled birth asphyxia.

MATERIALS AND METHODS

Animal Model with Birth Asphyxia Conditions (BAC): Gestational day 22 pregnant dams were administered via spinal anesthesia and the uterus externalized into a heated (37.5° C) saline bath. The blood supply feeding one uterine horn was occluded for 12 min (birth asphyxia condition) while the other uterine horn remained undisturbed (control condition). Fetuses were delivered by cesarean section, and were later cross-fostered to newly parturient dams.

MRI/S Data Acquisition: All experiments were conducted on 5 control rats and 5 BAC rats, in three time intervals, when the animals were 7, 35 and 60 days in age. MR experiments were performed on a 7T small animal MRI system (Bruker BioSpin, Ettlingen, Germany), equipped with an actively-shielded gradient set, with a maximum gradient strength of 400 mT/m. Signal excitation and reception were accomplished with a Litz RF coil (Doty Scientific, Columbia, SC). Conventional T_2-weighted RARE images (TE = 41 ms, TR = 3500 ms, slice thickness = 1 mm, matrix = 256 x 192) were acquired in coronal and axial orientations (FOV = 2.5 x 2.5 cm^2) to assess differences in anatomy and to assist in positioning of the MRS voxels. Spectra were acquired with a double spin-echo (PRESS) sequence (TE = 20 ms, TR = 2500 ms) with and without VAPOR water suppressions. Each individual water-suppressed spectrum was acquired with a spectral width of 4 KHz, with 512 repetitions. Localized voxel shimming was adjusted by using the FASTMAP technique [5]. Identical voxels (3 x 3 x 3 mm^3) were prescribed in a central location of the forebrain in the left hemisphere, below the cortex, to include tissue from both the striatum and hippocampus. The individual spectra were then processed and quantified using LCModel [6].

RESULTS

High quality MR spectra were acquired from both groups of rats, at each of the three time points. From the T_2-weighted anatomic images, neither visible lesions nor any obvious changes of image intensities were seen at any of the three time points. Before acquiring the unsuppressed water spectrum as the reference scan, each localized voxel was shimmed until the water peak had a linewidth (full width at half maximum) of < 10 Hz. All metabolites were reliably detected for quantitative analysis, with mean %SDs (Cramer-Rao lower bounds) less than the previously determined reliability cutoff of 20% [6]. Using LCModel, each detectable metabolite's concentration was compared as a ratio to the metabolite Creatine + Phosphocreatine (tCr), at each time point, in order to reduce systematic variations and to obtain the most accurate set of measurements possible. Table 1 lists the results of quantified metabolite peak ratios (proportional to the metabolite concentrations) for Choline-containing compounds (tCho), N-Acetyl Aspartate + N-Acetyl Aspartylglutamate (NAA), Glutamate + Glutamine (Glx), *myo*-Inositol (mI) and Taurine (Tau) to tCr. No significant changes were seen in the ratios of mI/tCr or Tau/tCr, for any of the three time points, between the control and BAC rats. However, the ratios of tCho/tCr, NAA/tCr and Glx/tCr all showed significant decreases in the BAC Rats. The ratio tCho was lower in the BAC rats at both the 7 and 60 day ($P \leq 0.05$) evaluation periods. A significant decrease was seen in the NAA/tCr ratio of the BAC rats at only the first time point ($P \leq 0.05$), and the ratio of Glx/tCr was significantly lower in the BAC rats at the 7 day ($P \leq 0.05$), 35 day and 60 day ($P \leq 0.1$) evaluation periods.

Metabolite Ratios	Control (mean ± SD)			Birth Asphyxia Condition (mean ± SD)		
	7 (days)	35 (days)	60 (days)	7 (days)	35 (days)	60 (days)
tCho / tCr	0.54 ± 0.05	0.24 ± 0.05	0.25 ± 0.02	0.39 ± 0.17	0.21 ± 0.03	0.19 ± 0.04
NAA / tCr	0.73 ± 0.11	0.97 ± 0.05	1.02 ± 0.15	0.58 ± 0.13	1.01 ± 0.06	1.07 ± 0.16
Glx / tCr	2.74 ± 0.4	1.95 ± 0.2	2.09 ± 0.04	1.79 ± 0.85	1.70 ± 0.24	1.74 ± 0.34
mI / tCr	0.77 ± 0.18	0.62 ± 0.06	0.76 ± 0.13	1.29 ± 0.85	0.69 ± 0.42	0.82 ± 0.12
Tau / tCr	2.02 ± 1.28	0.54 ± 0.19	0.38 ± 0.06	2.18 ± 1.22	0.46 ± 0.07	0.49 ± 0.05

Table 1: Summary of metabolite concentration ratio measurements

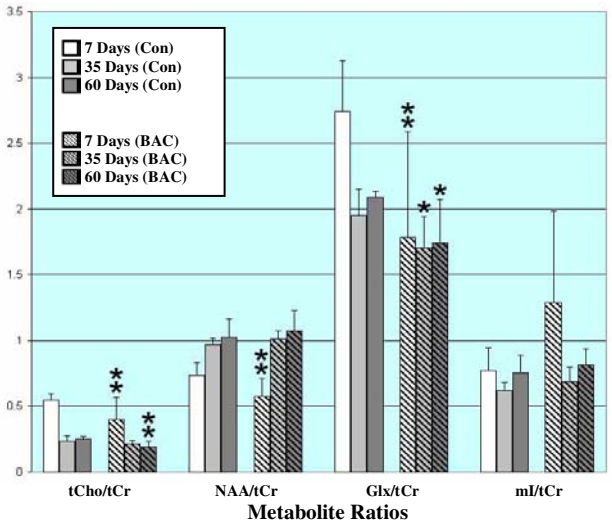

Figure 1: MRS metabolite ratio quantitation of control and birth asphyxia condition (BAC) rats (* $P \leq 0.1$, ** $P \leq 0.05$)

DISCUSSION

The significant decreases in the metabolite concentration ratios shown in figure 1 indicate that the metabolites tCho, NAA and Glx all might serve as sensitive neurochemical markers of both the effects and progression related to birth asphyxia. The age-dependent decreases seen in these metabolites also illustrate that the most prominent and significant changes are present in the early stages (approximately 7 days after birth), and decrease as the rats progress in age. These findings are currently being correlated with behavioral studies to help provide a more complete understanding of the adverse effects of birth asphyxia on neurological development and behavior.

REFERENCES

[1] Van de Berg WD *et al*., J Chem Neuroanat; 20(1):71-82, 2000, [2] Berger R *et al.*, J Soc Gynecol Investig; 9(6):319-328, 2002, [3] Perlman JM, Pediatrics; 117(3):S28-S33, 2006, [4] Garcias da Silva LF *et al.*, Pediatr Neurol; 34(5):360-366, 2006, [5] Gruetter R, Mag Reson Med; 29:804-811, 1993, [6] Provencher SW, Mag Reson Med; 30:672-679, 1993