Preliminary Study of Hypoxic Ischemic Rat Pup Model using Hyperpolarized 13C Spectroscopy

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Introduction: Hypoxic-ischemic injury occurs in approximately 25,000 live births in the US every year. Injuries evolve over days to weeks, with some neurons rapidly undergoing necrotic cell death while others suffer delayed impairment in energy metabolism and die more slowly. Animal studies involving rats [1,2] have shown that average diffusivity is reduced on diffusion weighted imaging, lactate is increased on proton MRS, and ratio of phosphocreatine (PCr) to inorganic phosphate (Pi) is increased during hypoxic-ischemic insult. While *in vivo* anatomic, diffusion, and proton spectroscopic MR imaging have provided biomarkers to assess the tissue conditions and with strong correlations with outcome [3,4], the exact timing and metabolic trajectories of these injuries remain unclear, and also its biological and molecular events central to hypoxic ischemia development and progression are still poorly understood. This preliminary study is designed to test the ability of using hyperpolarized ^{13}C metabolic imaging to assess tissue metabolism after ischemic insult.

Methods: Studies were done on a 14.1-T Varian 600WB microimaging system (Agilent Technologies, Santa Clara, CA, USA) equipped with 55-mm 100-G/cm gradients and 40mm animal ^{1}H and ^{13}C probes. A special animal cradle was used to hold the rat pup with heating bed and physiological monitoring wires. 99% [1-^{13}C]pyruvic acid was mixed with trityl radical and Dotarem and polarized for approximately 1 hour using the ^{13}C HYPERSENSE Polarizer (Oxford Instruments). 200 uL 80mM hyperpolarized pyruvate was injected over a course of 15 s through the jugular vein. To study hypoxic ischemic encephalopathy injury, we used middle cerebral artery occlusion (MCAO) model. All animal experiments were done 3 hours after MCAO and re-perfusion. Three rat pups were studied. All protocols were reviewed by our institutional review board.

A high-resolution 3D T1-weighted gradient echo image, TR/TE/Flip = 27ms/6ms/40^0 was used for localization. A spin-echo diffusion sequence (TE/TR 20ms/1.2s, FOV 30mm, 256x1218) with b value of 1000 s/mm^2 was used to confirm the extent of the ischemic injury. In-vivo rat pup anatomic images were acquired TE/TR 20ms/1.2s with FOV of 30mm and 256x128 matrix at 1mm thick slices.

Interleaved spectrally selective ^{13}C imaging of lactate after injection of hyperpolarized pyruvate was acquired using a 3D GRASE sequence with 3s repetition time. The sequence began when the injection started. The total acquisition time was 60s.

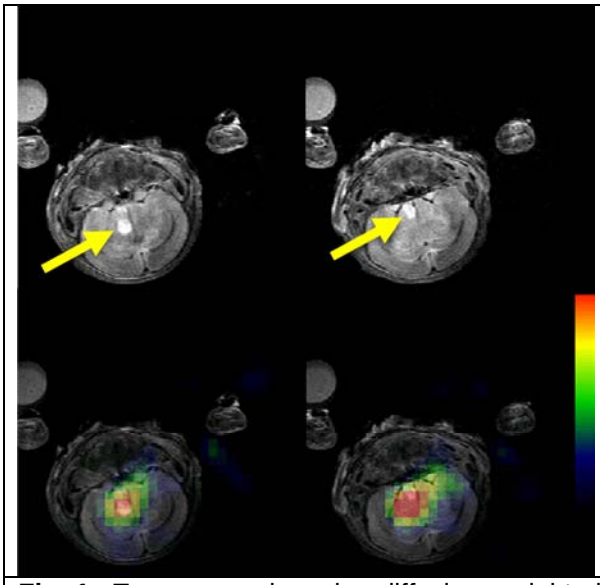

Fig 1. Top row: spin echo diffusion weighted image from a P7 rat pup that underwent MCAO for 3 hours. B-value of 1000 s/mm^2, TE/TR 20/1200ms, matrix 256x128, FOV 30x30 mm, 1mm thick slices with no gap. The arrows are pointing to a small ischemic region. Bottom row: color coded dynamic lactate images (acquired using 3D GRASE with 3 second repetitions) overlayed on top of anatomic T2 images. Increasing red intensity corresponds to higher levels of lactate.

Results: Fig 1 demonstrates the high levels of lactate conversion in the area of the ischemic injury, confirmed by high signal intensity in the diffusion weighted images.

Conclusion: We have demonstrated the feasibility of using metabolic imaging of hyperpolarized ^{13}C pyruvate to study metabolic changes in hypoxic ischemic injuries, which will help elucidate the mechanisms of these injuries and the subsequent recovery processes.

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