MR Microscopy of Multipotent Astrocytic Stem Cells Labeled with Multimodal Qdots Applied to a Neonatal Murine Model of Hypoxic Ischemic Encephalopathy

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Introduction Hypoxic Ischemic Encephalopathy (HIE) resulting from perinatal asphyxia is a major cause of neuronal injury and developmental deficits in neonates. Beyond supportive intensive care, there is currently no treatment available for HIE, although recent efforts have indicated the possibility of hypothermia as a plausible intervention in cases of moderate damage (1). For more severe insult, a potential treatment may be the implantation of neural stem cells in the cortex of HIE neonates in hope of restoring neuronal connections and development. Any evaluation of stem cell treatment in HIE must begin first by tracking the distribution of cells following injection. MR microscopy is well suited to this task, not only because it has the ability to interrogate the *in vivo* proliferation of stem cells longitudinally, but also can provide valuable information about the distribution and potential recovery of infarcted areas. In this study, unilateral HIE has been instituted in a neonatal murine model, and multipotent asctrocytic stem cells (MASC) labeled with a unique nanocrystalline contrast agent that provides both MR and optical enhancement have been injected into the effected cortex. High-resolution MR images acquired both *in vivo* and *ex vivo* display an integration of the MASCs along the major white matter tracts of the effected hemisphere as well as bilateral proliferation in the ventricles shortly following injection.

Methods *Gd-Qdot*: Using a water/oil microemulsion technique (2,3), Gd-functionalized, silica-coated quantum dots (Gd-Qdots) consisting of a photoactive component (CdS:Mn/ZnS) and a magnetic component (Gd(III)) were fabricated. The hybrid silica layer also improves aqueous dispersibility, reduces toxicity, and increases brightness. As assessed by TEM, the size of individual nanoparticles was 7-10 nm; hydrated Gd(III) ion content was estimated to be 107 per core-shell Qdot.
MASCs: Stem cells were derived from transgenic mice (Jackson Laboratory, Tg(GFPU)5Nagy/J, Stock#003115). In brief, subependymal zones (SEZs) of neonatal (P4-P9) green fluorescence protein (GFP) transgenic mice were obtained following decapitation. Tissue chunks were minced, incubated in 0.25% trypsin/EDTA and dissociated into single cell suspensions. Cells were pelleted and washed several times in media before being plated in culture flasks of N2 media supplemented with 5% FBS. Cells were passaged 1-2 times prior to labeling (3). Prior to injection, MASCs were incubated with Gd-Qdots coated with poly-L-lysine to facilitate uptake.
HIE: Hypoxia-ischemia was induced in C57/BL mice pups on day 7 (day 0 = birth) by the combined ligation of the right carotid artery and an exposure to hypoxic gas (4). The right common carotid artery was isolated and electrocauterized through a small incision in the neck. Pups were allowed to recover for 30 min. on a thermoregulated gel pad and 2 hrs with their dam. Pups then were placed in a temperature-controlled chamber perfused with a humidified gas mixture (8% O_2, 92% N_2) for 20 min. Using a glass, ~50,000 labeled MASCs in 1 µL of DMEM/F-12 were injected into the injured cortex 24 hrs following the induction of hypoxia-ischemia.
MR and Optical imaging: *In vivo* imaging was performed using an 11.1-T, 40-cm Magnex system interfaced with a Bruker Avance console. Anesthetized and maintained using a continuous flow of 2% isoflurane in oxygen, mouse pups (3-5-hrs post MASC injection) were place within a customized 2-cm quadrature half-saddle coil. High-resolution images (100x100x400 or 50x50x400 µm) were acquired with diffusion, T2 and T2* weighting using multislice 2D fast spin echo (FSE) and gradient recall echo (GRE) sequences. Following *in vivo* imaging, pup brains were excised and fixed. Higher resolution imaging was performed on intact brains using a 17.6-T Bruker magnet. Employing a customized 13-mm loopgap resonator, microimages were acquired using multislice 2D SE (40x40x200 µm) and true 3D GRE (30x30x30 µm) sequences. Following *ex vivo* MRI, optical analysis of Qdot accumulation was performed using epifluorescent and immunohistochemistry techniques.

Results and Discussion *In vivo* images (Fig 1) clearly demonstrate the ischemic region associated with HIE as a hyperintensity (red arrows) on both T2- and diffusion-weighted images, indicative of unilateral edema in the left cortical region as well as the hippocampus. The needle tract (yellow arrows) is clearly evident on all images. The MASCs (blue arrows) also are evident as a distinct hypointensity resulting from the bulk susceptibility effect of the Gd-Qdot on T2/T2* relaxation. T2W and DW images display an increased contrast along the left corpus callosum away from the injection site and toward the infarcted area while T2*W GRE images more clearly highlight this pathway and display bilateral infiltration of MASCs in the anterior ventricles. Although contrast from HIE insult has been reduced significantly by fixation, *ex vivo* analysis excellently delineates the distribution of MASCs along the major white matter tracts of the left hemisphere in T2, T2* and diffusion-weighted images (Fig 2). Further, 3D GRE images (Fig 3) reveal the full extent of MASC presence, particularly in the ventricular areas of both the left and right hemispheres. Optical assessments corroborate both the infarcted region and MASC distribution that is apparent in these MR findings. These images demonstrate the utility of MR microscopy and multi-modal contrast agents in evaluating the integration of MASCs shortly following injection in HIE subjects. Future studies will utilize these techniques to judge the efficacy of stem cell treatment in the restoration of neuronal development in HIE neonates.

Acknowledgments and References Data was acquired at the Advanced Magnetic Resonance Imaging and Spectroscopy (AMRIS) facility of the University of Florida McKnight Brain Institute. Funding was provided by pilot grants from the National High Magnetic Field Laboratory (SCG) and the NIH (NS02198, AHA 0255435B) (MDW). The authors also wish to thank the UF Molecular Imaging study group for useful discussions. (1) Shankaran S, et al. N. Engl. J. Med. 353(15):1574. 2005. (2) Yang H & Holloway PH. Appl. Phys. Lett. 82:1965. 2003. (3) Santra S, et al. J. Am. Chem. Soc. 127(6):1656. 2005. (4) Laywell ED, et al. Proc. Natl. Acad. Sci. USA. 97(25):13883. 2000. (5) Ten VS, et al. Behav. Brain. Res. 145(1-2):209. 2003.

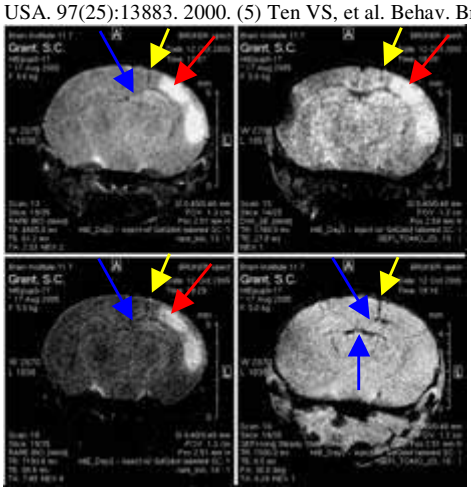

Fig 1. *in vivo* images of NSC-injected HIE pup:
Upper left: low T2W, effective TE=61 ms
Lower left: high T2W, effective TE=90 ms
Upper right: DW, b = 750 s/mm^2
Lower right: T2*W, TE = 6.5 ms

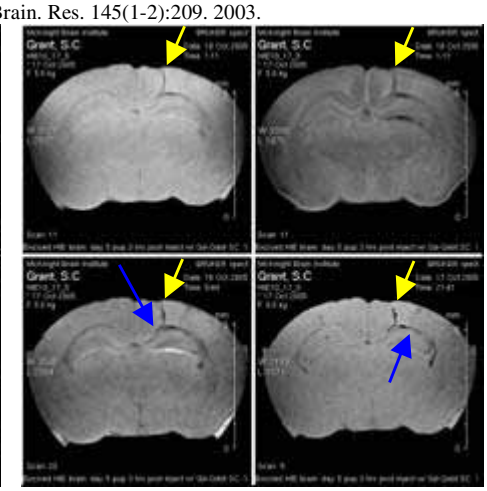

Fig 2. *ex vivo* images of NSC-injected HIE pup:
Upper left: low T2W, TE= 21.5 ms
Lower left: high T2W, TE= 43.5 ms
Upper right: DW, b = 1000 s/mm^2
Lower right: T2*W, TE = 15 ms

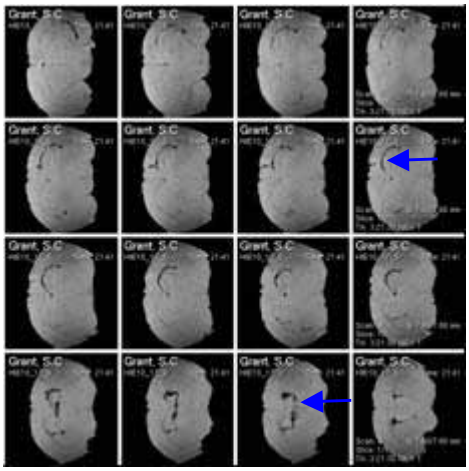

Fig 3. Slices from a true 3D GRE dataset acquired at an isotropic resolution of 30 microns.
Note the continuous unilateral hypointensity along the left hemisphere's corpus callosum with bilateral contrast in the ventricles.