MRI Detection of the Migrating Neuronal Precursors in Normal and Hypoxic-ischemic Neonatal Rat Brain by In vivo Cell Label with MPIO

J. Yang[1,2], J. X. Liu[3], G. Niu[2], Y. Liu[3], and E. X. Wu[1]

[1]Laboratory of Biomedical Imaging and Signal Processing, Department of Electrical and Electronic Engineering, The University of Hong Kong, Hong Kong, China, People's Republic of, [2]Medical Imaging Center, The First Hospital of Xi'an Jiaotong University, Xi'an, Shannxi Province, China, People's Republic of, [3]Institute of neurobiology, The School of Medicine, Xi'an Jiaotong University, Xi'an, Shannxi Province, China, People's Republic of

INTRODUCTION

In the most of MRI-based neural cell tracking, cells were labeled with an MRI contrast agent in culture, and then injected into the animal for imaging. The process of *in vitro* labeling cell is tedious and difficult to maintaining the undifferentiated stem cell state in culture. In recently, Shapiro et al demonstrated that the neural progenitor cells (NPCs) can be labeled *in vivo*. By intraventricular injection of the micronsized iron oxide particles (MPIOs), MRI can detect migrating neural precursors carrying MPIOs along the rostral migratory stream (RMS) to the olfactory bulb (OB) in normal adult rats [1]. In this study, we want using this new approach to implement *in vivo* magnetic cell labeling and MRI monitoring of the endogenous NPCs migration in the normal and hypoxic-ischemic (H-I) neonatal rats.

MATERIALS and METHOD

Animal preparation: All Sprague-Dawley neonatal rats divided into two groups. Group 1 (n=6): 10-day-old normal rats were stereotactically injected with 10ul (1.25×10^7) of 0.9 um diameter MPIOs (Bangs Laboratories, Inc.) into the anterior left lateral ventricle. The chosen position was 1 mm caudal from bregma, left 1.5mm, down 2mm. Group 2 (n=6): 7-day-old H-I rats (12g-16g weight) were created by unilateral ligation of left common carotid artery plus exposure to hypoxia for 2 hours at ambient temperatures of 37°C [2]. After 3 days post H-I insult, the MPIOs intraventricular injections were performed with the same protocol as Group 1. ***MRI scanning:*** All rats were performed MRI using a 7T animal MRI scanner (70/16 Bruker BioSpin MRI PharmaScan, Germany) and mouse brain coil in hour 3, day 3, day 7 and day 14 after the MPIOs injection. 3D gradient echo MRI was acquired with the following image parameters: TR/TE = 50/8 ms, flip angle = 10°, FOV = 2.5×2.5×1.2 cm^3, spatial resolution = 106×106×100 um^3, NEX=1, acquisition time ≈ 23 min. 2D gradient echo MRI was performed in the transverse direction with TR/TE1/TE2/TE3 = 550/8/14/20 ms, NEX= 6, FOV = 2.5 cm, slice thickness = 0.3 mm, spatial resolution = 98×98 um^2, 20 slices, acquisition time ≈ 10 min. *Histology:* Three rats in each group were respectively sacrificed in day 7 and day 14. The brains were perfuse transcardially with 10% formaldehyde and embedded in paraffin. The sagittal and transverse sections (8μm) were respectively cut in Group 1 and Group 2 rats, and processed for Prussian blue iron staining plus immunohistochemical staining for mature neurons (anti-NeuN).

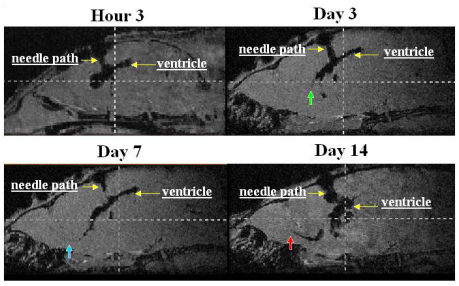

Fig. 1 3D gradient echo MR images in a normal neonatal rat show the migration of endogenous NPCs with MPIOs in a sagittal slice along the left RMS on 4 time-points.

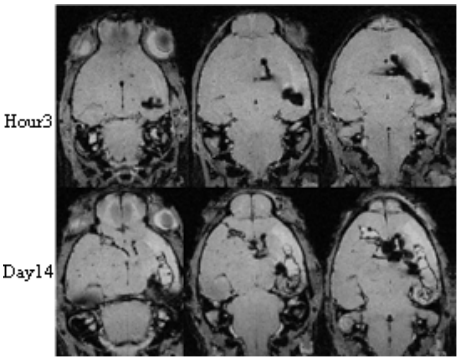

Fig. 2 2D gradient echo MR images (TE=14 ms) in an H-I neonatal rat show the migration of endogenous NPCs with MPIOs in 3 consecutive transverse slices on 2 time-points.

RESULTS and DISCUSSION

 All Group 1 rats were successfully injected MPIOs into left lateral ventricle. Fig. 1 showed 4 spatially close slices along the left RMS. MRI detected migration started in day 3 (green arrow), and clearly entered into the RMS pathway in day 7 (blue arrow). In day14 post-injection, MRI exhibited a dark contrast (red arrow) along the RMS and extending into the OB. This migrating time course in 10-day-old neonatal rats is similar to it in adult rats as Shapiro's report [1]. But the dark migrating lines in neonatal rats have more apparent contrast than them in adult rats, likely resulting from an enhanced new cell production for developmental requirement [1, 2]. All Group 2 rats were successfully created to hypoxic-ischemic (H-I) models and performed MPIOs intraventricular injections. MRI monitoring of NPCs migration in a severe H-I neonatal rat was showed in Fig.2. In day 14 post-injection, a dramatic migrating territory was presented in MRI, that the necrotic regions with high signal intensity were surrounded by irregular dark border. It may indicate that newly produced cells migrated to the infarct region and differentiated into NPCs in order to compensate the lost neural cells [3]. In this preliminary study, the double staining with iron and mature neurons was performed to spatial correlation with MRI findings. In the Fig.3A, a 2D gradient echo MR image (TE=8ms) in a mild H-I neonatal rat was acquired in day 7 post-injection, and showed the slight dark lines extend from posterior corner of left ventricle and needle hole (red arrows in Fig 3A). The migration of endogenous cells with MPIOs extending to the cortical injured boundary was demonstrated by Prussian blue iron staining, and presented as blue-black streams in Fig.3B (red arrow). Fig.3C and Fig.3D were respectively corresponding to blue and green box with a magnified view. Several cells, double staining with iron and $NeuN^+$, distributed sparsely in Fig. 3C (black arrow), and was close to the ischemic region, suggesting that migrating neuroblasts tagged with the iron particles can differentiate into mature granular interneurons[1,3].

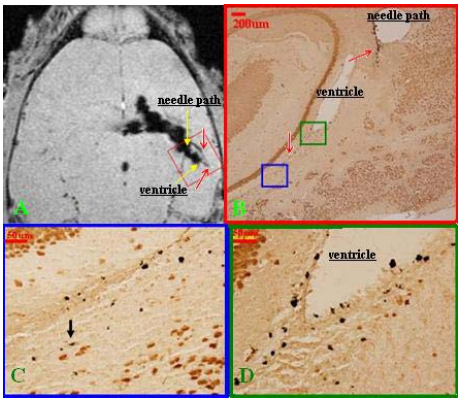

Fig. 3 The migration of endogenous NPCs with MPIOs in MRI spatially correlated with the histological findings.

CONCLUSION

In normal neonatal rat brain, the migrating pathway of endogenous NPCs with MPIO is mainly along the RMS to the OB. In H-I neonatal rat brain, the migration of endogenous NPCs with MPIO is mainly toward injured boundary. MRI can facilely detect the above migrations in 2 weeks. In vivo magnetic cell labeling of endogenous NPCs with MPIO and subsequently non-invasive, serial MRI monitoring should open up a new approach to probe into the mechanism of cell migration in the developmental brain under physiological and pathologic states.

REFREENCES

1, Shapiro EM, et al, EuroImage 2006; 32(3):1150-7. 2, Suzuki SO, et al., J Neurosci 2003; 23(10):4240–50. 3, Hayashi T, et al., Brain Research 2005;1038: 41–49.