MRI Detection of Angiogenesis in Ischemic Brain Remodeling After Cell Therapy in Rats with Stroke

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INTRODUCTION: Cell therapy has been demonstrated to be beneficial for improved functional recovery in experimental stroke[1,2]. However, little is known about the effects of these cells on host cerebral tissue in leading to functional recovery. Recent studies have demonstrated that cell therapy of stroke in rats increased levels of rat vascular endothelial growth factor (VEGF), which consequently enhanced angiogenesis[3]. However, current methodologies used to detect angiogenesis after cell therapy are restricted to one time invasive measurements. In this study, we report that MRI can dynamically monitor not only the migration and localization of transplanted magnetically labeled cells but also the status of angiogenesis after cell therapy.

MATERIALS AND METHODS: subventricular zone (SVZ) cells isolated from young adult rats were cultured in Iscove′s modified Dulbecco′s medium in the presence of basic fibroblast growth factor (10ng/ml, R&D System, Minneapolis, MN) for eight days. Cultured SVZ cells were labeled by superparamagnetic particles using a biolistic device "gene gun" [2]. Male Wistar rats (3-4 month old) were placed in a stereotaxic frame. Six microliters of PBS, containing approximately 1×10^5 superparamagnetic labeled-SVZ cells, were injected via percutaneous injection into the cisterna magna 24 h after the induction of embolic right middle cerebral artery occlusion[2]. MRI measurements were performed immediately, and weekly for 5 consecutive weeks after transplantation and the rats were sacrificed after the last MRI measurements. NMR measurements were performed with a 7 T, 20 cm bore, Magnex superconducting magnet equipped with a 20 G/cm, 12 cm bore gradient insert. To measure migration and localization of labeled cells, three dimensional gradient echo MR images were obtained with TR=40 ms, TE=10 ms, 30^0 of flip angle, 32x32x16 mm^3 field of view (FOV). The 256x192x64 matrix was interpolated to 256x256x64 (0.125x0.125x0.25 mm^3) for analysis. To detect angiogenesis using permeability related parameters, five slice R_1 ($1/T_1$) maps were acquired using TOMROP Look-Locker (L-L) sequences 128x64, FOV 32 mm, 2 mm slice thickness, 24 pulses, TE/TR 4/50 ms, with a 3 sec dwell time between inversion. Each image set took approximately 3 min, and sets were obtained at 3 min. intervals for up to 30 min. after injection of Gd-DTPA. After one R_1 map was acquired, a bolus of 0.2 mmol/kg Gd-DTPA was manually injected following a 0.4 ml saline flush. R_1 values were converted to gadolinium concentration. Maps of Gd-DTPA blood-to-brain transfer constant, K_i, were constructed using the Patlak Plot[4] methodology. To detect superparamagnetic labeled-SVZ cells in the host brain and angiogenesis, brain sections were stained for either iron using Prussian blue reaction, or for angiogenesis using vWF immunostaining.

RESULTS: MRI signals were detected at the cisterna magna immediately after the injection of labeled cells. These MR signals were detected only in the ischemic hemisphere after two days of cell transplantation. The labeled SVZ cells selectively migrated towards ischemic boundary regions as detected by MRI from 2 days up to 5 weeks after cell transplantation. Gd-DTPA blood-to-brain transfer constant, K_i exhibited sensitivity to BBB leakage caused by neoangiogenesis after cell therapy. Figure 1 shows the temporal evolution of Ki maps at 1 (A), 2 (B), and 5 weeks (C) after SVZ transplantation, respectively. MRI revealed an increase in Ki in the subcortical region, which maximized at 2 weeks and returned to normal at 5 weeks. The vWF immunoreactive images of coronal sections which matched MRI sections from the same animal sacrificed at 5 weeks showed an increase in numbers of vWF immunoreactive vessels (left image in D, black line area; left image in E) in vWF immunostained images, indicating that newly formed vessels leak maximally at 2 weeks.

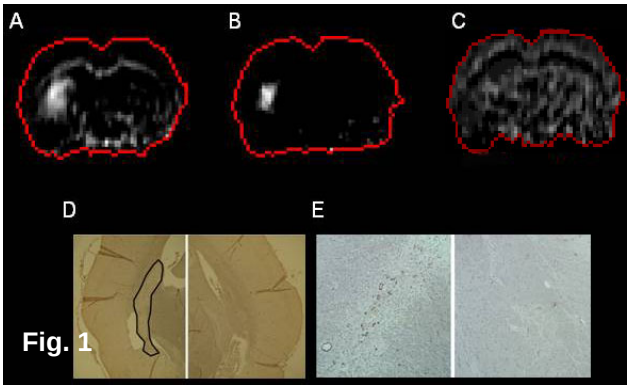

Fig. 1

DISCUSSIONS: These studies show that MRI can detect angiogenesis after SVZ treatment and that grafted adult SVZ cells selectively migrate towards ischemic boundary regions in the adult stroked rats when SVZ cells were intracisternally transplanted after stroke.

REFERENCES: 1. Zhang ZG, Jiang Q, Zhang R, et al. *Ann Neurol*. 2003;53:259-63. 2. Zhang RL, Zhang L, Zhang ZG, et al. *Neuroscience*. 2003;116:373-82. 3. Chen X, Li Y, Wang L, et al. *Neuropathology*. 2002;22:275-9. 4. Ewing JR, Knight RA, Nagaraja TN, et al. *Magn Reson Med*. 2003;50:283-92.