Investigation of Angiogenesis After Cell Therapy of Embolic Stroke in Rat Using MRI

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INTRODUCTION: Cell therapy improves functional recovery in experimental stroke[1,2], which may be related to cell therapy induced angiogenesis[3]. However, the relationships between cell migration, distribution and regional tissue remodeling have not been investigated. We have successfully monitored the temporal profiles of labeled cell migration and distribution after cell therapy in ischemic brain[1]. In this study, we report for the first time that quantitative MRI can dynamically monitor cell mediated induction of angiogenesis.

MATERIALS AND METHODS: Subventricular zone (SVZ) cells isolated from young adult rats were labeled by superparamagnetic particles using a biolistic device "gene gun" [2]. Eight male Wistar rats (3-4 months 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 before, one day, and weekly for 6 consecutive weeks after cell transplantation. Rats were sacrificed after the last MRI measurements. MRI 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 32x32x16 mm^3 field of view (FOV) with 0.125x0.125x0.25 mm^3 voxel size. T_{1sat} (T_1 in the presence of an off-resonance irradiation of the macromolecules of brain), T_2, cerebral blood floor (CBF), cerebral blood volume (CBV), and blood-to-brain transfer constant (K_i) of Gd-DTPA were used to characterize biophysical changes of angiogenesis after cell therapy. T_{1sat} was measured using TOMROP Look-Locker (L-L) sequence with saturation pulse[4]. CBF and CBV were measured using arterial spin labeling[5] and dynamic contrast enhancement[6] measurements, respectively. Gd-DTPA blood-to-brain transfer constant, K_i, was measured using dynamic L-L T_1 and constructed using the Patlak Plot[7] methodology. To detect superparamagnetic labeled-SVZ cells in the host brain and angiogenesis, brain sections were stained for iron using Prussian blue reaction, and for angiogenesis using vWF immunostaining. The relative changes of MR measurements in ischemic ROI (ischemia/contralateral ischemia) and vWF positive ROI (vWF/contralateral vWF) determined by vWF immunostaining were used to detect the regional differences.

RESULTS: The labeled SVZ cells selectively migrated towards ischemic boundary regions as detected by MRI from 1 to 6 weeks after cell transplantation (Fig 1, 3D). Although all the regions with angiogenesis contain labeled cells, not all the regions with labeled cells exhibit angiogenesis. Different temporal changes in biophysical parameters were detected between ischemic regions with and without angiogenesis. MRI revealed an increase in Ki in the angiogenic region (Fig1, K_i), which maximized at 2 weeks and returned to normal at 6 weeks. The vWF immunoreactive images of coronal sections which matched MRI sections from the same animal sacrificed at 6 weeks showed an increase in numbers of vWF immunoreactive vessels (Fig 1, left image in vWF staining A, black line area; left image in the high magnification image B) in vWF immunostained images, indicating that newly formed vessels leak maximally at 2 weeks. The angiogenic region exhibited increased ($p<0.05$) CBF and CBV compared to that in the non-angiogenic ischemic region at 6 weeks after cell therapy. The relative T_{1sat} and T_2 values in angiogenic region were also significantly lower (T_{1sat}, $p<0.05$ at 1, 3, 5, and 6 weeks and T_2, $p<0.05$ at 3, and 6 weeks) than that in the non-angiogenic ischemic region after cell therapy.

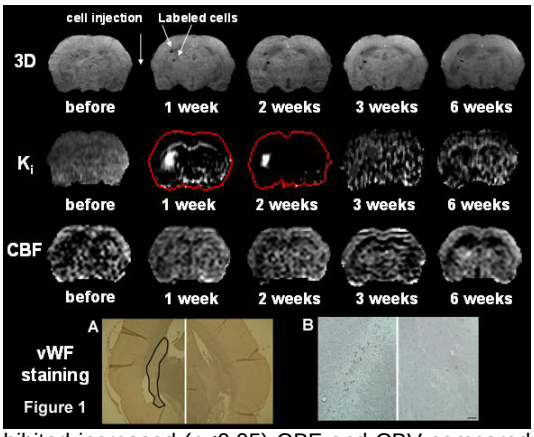

Figure 1

DISCUSSION: These studies show that MRI can dynamically monitor labeled cell migration, distribution and angiogenic impact on ischemic tissue. Angiogenesis after cell therapy in ischemic brain colocalizes with the distribution of implanted cells. These cells may promote angiogenesis by expressing or inducing the expression of angiogenic factors like VEGF in parenchymal tissue[8]. Our data also suggest that Ki, CBF, CBV, T_{1sat} and T_2 are sensitive MRI parameters to predict and identify cell induced angiogenic tissue.

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