Quantitative MR Tracking of Magnetically Labeled Mesenchymal Stem Cells in a Mouse Glioma Model at 3T

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INTRODUCTION

The poor survival of patients with malignant tumors partly relates to the inability to deliver therapeutic agents to the target. There is accumulating evidence that circulating bone marrow-derived stem cells have a tropism for tumors and could be used as delivery vehicles for tumor therapy. Anderson et al and Arbab et al have demonstrated the migration of SPIO labeled Scal+ cells, a subpopulation of CD34+ human hematopoietic stem cells, into the neovasculature of implanted glioma tumors in the mouse brain and flank, respectively [1,2]. Of the various multipotential stem cells, human mesenchymal stem cells (MSCs) are particularly attractive because they can be easily isolated, expanded in culture, and genetically manipulated using currently available molecular techniques. Quantitative tracking of MSCs within the tumor will allow further investigation of the extracellular and stromal components required for the incorporation of these cells into the tumor [3].

It has been shown that the T2* relaxation time is the most sensitive parameter to detect SPIO labeled cells [4]. In this study, different numbers of SPIO labeled human MSCs were injected intravenously and T2* maps of the implanted glioma tumors were used to assess the recruitment of SPIO labeled MSCs in a mouse model.

METHODS

Tumor Inoculation: 1×10^6 C6 glioma cells were implanted subcutaneously into the flanks of 36 nude mice. ***Cell Labeling:*** Human MSCs were labeled with Ferumoxides-protamine sulfate (FEPro) complexes by incubating cells overnight [3]. ***MSC Injection:*** Twelve mice were injected with unlabeled 1×10^6 MSCs (Control) intravenously through the tail vein on day 3 (3 days after tumor implantation). Twelve mice were injected with 1×10^6 SPIO labeled MSCs (Single) on day 3. The other twelve mice were injected three times with 1×10^6 SPIO labeled MSCs every other day starting from day 3 (Serial). ***MRI:*** MRI was performed approximately two weeks after the tumor implantation on a 3T Intera whole-body scanner with a 7 cm rat solenoid RF coil (Philips Medical System and Research Laboratories). T2* maps were acquired with a multiple gradient echo sequence: TR/TE = 1540/16ms; Flip Angle = 30; 13 echoes; matrix= 256 × 256; 15-19 slices; slice thickness = 1.0mm; FOV = 70mm; NEX = 4. ***Data Analysis:*** The T2* values were calculated by mono-exponential fitting with a custom designed IDL software. Tumor T2* was measured as the average over the tumor volume. ***Histology:*** After MRI, mice were euthanized and tumors were removed, sliced and stained with Prussian blue to verify the recruiting of MSCs.

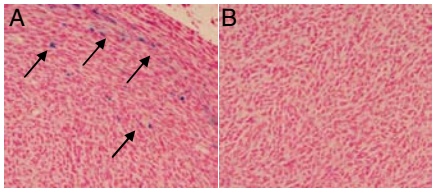

Figure 1. A. Prussian blue stain revealed blue spots (arrows) in tumors injected with SPIO labeled cells indicating migration of the SPIO labeled MSCs. **B.** Tissue slice from a control tumor free of Prussian blue positive cells.

RESULTS

One mouse in the Serial group was euthanized before MRI scan. Seven mice were excluded from the study because their tumor size was too big and necrosis started to develop at the time of the MRI scan. MRI data from a total of 28 mice were analyzed resulting in 9 mice for both Control and Single groups and 10 mice for Serial group. As shown in Figure 1, Prussian blue positive cells were identified in tumors of the mice injected with SPIO labeled MSCs indicating the migration of MSCs to these tumors.

Figure 2A and 2C are T2* weighted axial images of two mice from Control and Serial groups. The tumor from Serial group demonstrated reduced T2* due to the migration of SPIO labeled MSCs (Figure 2B, 2D). Statistically, tumor T2* of the mice from Single and Serial groups was significantly lower than that from the Control group (36.8 ± 3.2 ms for Control group, 26.3 ± 5.9 ms for Single group and 23.3 ± 4.4 ms for Serial group, Figure 3A, $p<0.01$). The recruitment of SPIO labeled MSCs also induced a shift in the T2* histogram of the tumor towards smaller T2* values in both Single and Serial groups (Figure 3B).

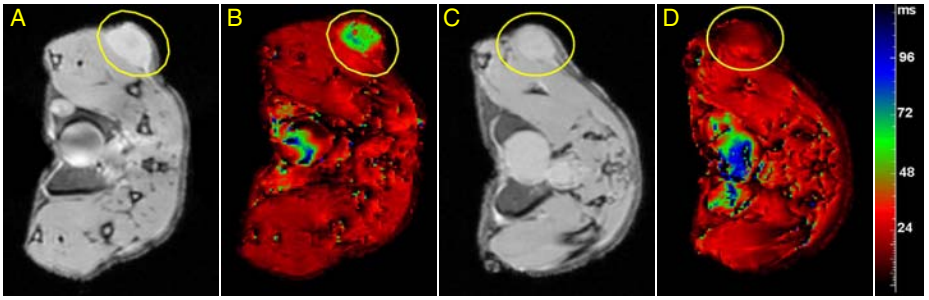

Figure 2. Axial T2* weighted images of two mice from Control group (**A**) and Serial group (**C**). Visually, there was no significant difference between the two groups on T2* weighted images. However, the T2* map of the mouse with serial injection (**D**) was much lower than that of the control mouse (**B**).

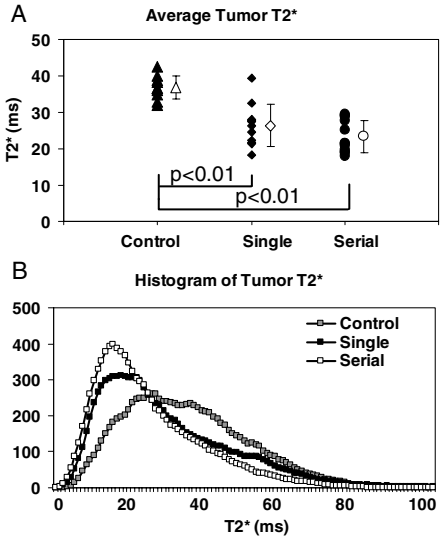

Figure 3. A. Scattered plot of tumor T2* in three groups. Tumor T2* of the mice from both Single and Serial groups was significantly lower than that of the controls. **B**. Histograms of tumor T2*. The migration of SPIO labeled MSCs from the blood stream induced a significant shift towards smaller T2* in both Single and Serial groups.

DISCUSSION

Migration of SPIO labeled MSCs to implanted glioma tumors was evaluated with MRI T2* relaxometry. T2* values of the tumors with both single and serial injections were significantly lower than the T2* vaules of the controls. According to the quantitative relationship between SPIO labeled cells and tissue T2* relaxation developed in [5] using a SPIO labeled tumor model, the number of SPIO labeled MSCs necessary to produce such a difference was 20,000 –30,000 cells in the tumor, which was about 2-3% of the injected MSCs. Although there is no statistical difference, T2* of the Single group demonstrated a bigger variation than that of the Serial group. Prussian blue positive cells were also observed in the tumors of Control group presumed to be macrophage phagocytosed red blood cells. However, the contribution of these cells to the tumor T2* value is relatively small considering the variance of the means and unlikely to play a significant role in the quantification. In conclusion, MRI T2* relaxometry could serve as a method for quantitative tracking of magnetically labeled stem cells in vivo.

REFERENCE: 1.Anderson et al. Blood 2005;105:420-425. **2**. Arbab et al. Stem Cells 2006;24:671-678. **3**. Arbab et al. Transplantation 2003; 76:1217-1223. **4**. Bowen et al. MRM 2002;48:51-61. **5**. Liu et al. ISMRM 2006; 680.