Tracking Enhanced Green Fluorescence Protein (EGFP) and Micrometer-Sized Particles of Iron Oxide (MPIO) Labeled Mesenchymal Stem Cells (MSCs) in a Myocardial Infarction Model with Granulocyte-Colony Stimulating Factor (GCSF) Modulation

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INTRODUCTION

Cellular MRI has undergone rapid development recently, having been applied to track cells (1). Iron oxide particles have been used as cellular imaging contrast agent to label and detect the cells of interest (2). The MPIO can cause pronounced signal attenuation in T_2*-weighted MRI (3), allowing for the detection of single cells with individual particles (4, 5). In this study, we labeled the MSCs with EGFP and MPIO. GCSF was injected into one group of animals to modulate the infiltration rate of MSCs. We hypothesize that MPIO-laden MSCs were able to infiltrate into the myocardial injury site and could be monitored using MRI. Furthermore, we also hypothesize GCSF would facilitate the MSCs infiltration and hence enhance signal attenuation in MRI.

MATERIALS AND METHODS

C57Bl/6 male mice (6-8 weeks old) were irradiated with an 8-Gy dose and received rescuing bone marrow transplantation 1 day post-irradiation. The labeled MSCs ($3\text{-}7x10^5$) were transplanted into the tibial medullary space 2 days post-irradiation. The mice were then divided into a sham-operated group (Sham, n=7), a MI group without GCSF injection (MI-GCSF, n=7) and a MI group with GCSF injection (MI+GCSF, n=3). At 14 days post-MSCs transplantation, the two MI groups underwent MI injury via permanent ligation of the left anterior descending (LAD) coronary artery while the Sham group underwent open-chest surgery without ligating the LAD. For the MI+GCSF group, GCSF was injected subcutaneously 1 day post-MI. MRI was performed at baseline, 3 days (D3), 7 days (D7) and 14 days (D14) post-surgery. Short-axis cardiac images were acquired using a T_2*-weighted MRI sequence implemented on a 7-T MRI spectrometer (Bruker Instruments, Billerica, MA, USA). An ECG-gated gradient echo sequence with flow compensation (GEFC) was used. To acquire images for contrast-to-noise ratio (CNR) analysis, the imaging parameters were as follows: TR/TE = 120/4 ms, FA = 30°, FOV = 30 mm, Matrix Size = 256 x 256 and 8 averages. The CNR, representing the signal difference between the infarcted zone (or corresponding site in the Sham group) and an unaffected area at the left ventricular wall, was calculated. A GEFC CINE sequence was used for cardiac function analysis with the following parameters: TR/TE = 16/4 ms, FA = 25°, FOV = 30 mm, Matrix Size = 128 x 128, 8 averages and 8 frames per cardiac cycle. The left ventricular ejection fraction (LVEF) was calculated for functional comparison. The MRI results were further confirmed by *ex vivo* fluorescent microscopy and histology.

RESULTS

Gradual signal attenuation at the MI site was observed post-MI (**Figure 1**) for both MI groups, potentially due to the accumulation of MPIO-labeled cells. The CNR was significantly different between the MI and Sham at D3, D7 and D14 (**Figure 2**). The signal was attenuated more in the MI+GCSF than the MI-GCSF group, due to the fact that GCSF could facilitate the mobilization of stem cells post-MI. Although the MI+GCSF group showed a trend of functional improvement in LVEF (**Figure 3**), it is not statistically significant due to the small number of animals. Dual-labeled cells, with both EGFP and MPIO, were detected in the infraction site and peri-infarction zone in fluorescence micrographs. Iron accumulation was also observed in the Prussian blue staining. Further histological validation is warranted in the ongoing study.

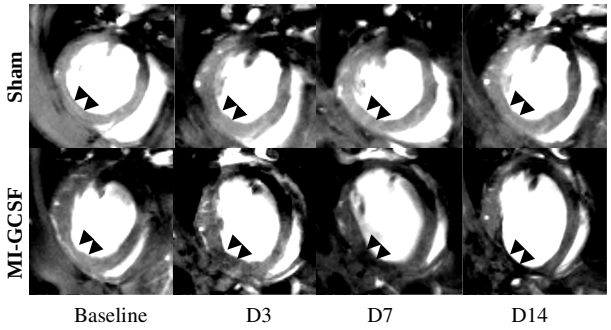

Figure 1. The temporal cardiac MRI. Arrows indicate the MI site in the MI-GCSF group or corresponding site in the Sham group.

CONCLUSIONS

Migration and infiltration of MPIO-labeled MSCs from bone marrow into the myocardial infarction and peri-infarction site was temporally monitored *in vivo* by MRI. Furthermore, the infiltration rate of MPIO-labeled stem cells can be modulated and enhanced by GCSF, which could be differentiated in MRI by the further signal attenuation. This current study provides a way to understand the nature of stem cell migration during myocardial injury and the healing process. Results of this study suggest a potential approach in cell therapy to noninvasively monitor migration of labeled cells in the MI related events.

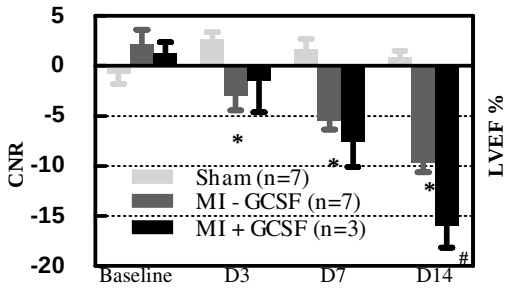

Figure 2. CNR comparison ($p^*<0.01$, MI-GCSF versus Sham; $p^\#<0.05$, MI+GCSF versus MI-GCSF).

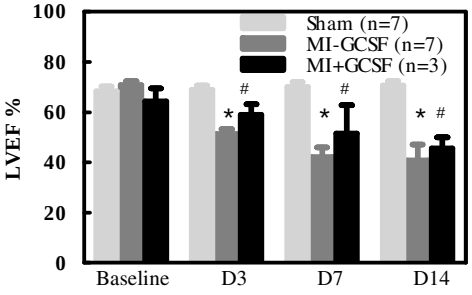

Figure 3. The LVEF ($p^*<0.01$, MI-GCSF versus Sham; $p^\#<0.05$, MI+GCSF versus Sham).

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