IRON imaging of magnetoelectroporated mesenchymal stem cells in a rabbit hindlimb ischemia model

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Introduction

Magnetoelectroporation (MEP)[1] offers a method for rapid magnetic labeling of cellular therapeutics. Inversion Recovery with ON (IRON) water suppression imaging[2] is a method to create positive signal enhancement of superparamagetic iron-oxide labeled cells to enhance the detection of small numbers of stem cells using MRI. Both methods provide valuable tools for translational cellular therapy, but the combination of these two techniques has not been tested in vivo. Because there are often no durable treatment options for patients with intermittent claudication (IC) or chronic limb ischemia due to infrainguinal peripheral arterial disease (PAD), cell-based therapies offer an exciting option for therapeutic arteriogenesis. The goal of the current study was to implement these two new methods in a relevant rabbit model of hindlimb ischemia for the delivery and tracking of MSCs as a bench to bedside model of MR-guided determination of cellular therapeutic efficacy.

Materials and Methods

Cell culture Bone marrow aspirates from the tibia were obtained from anesthetized male New Zealand white rabbits (< 6 mo old). Mesenchymal stem cells were isolated after suspending the bone marrow in heparinized saline, performing a density gradient (Histopaque-1.077g/ml, Sigma), and subsequently washing the mononuclear cells layer with PBS. The cell culture was then plated in culture medium (DMEM- low glucose (Gibco) with 1% antibiotics and 10% selected FBS). After 3 cell passages, the cells were frozen and then thawed prior to magnetic labeling by MEP. The adipogenic and osteogenic potential of bone-marrow expanded cells was examined to determine stem cell multipotency.

Instant magnetic cells labeling: The MSCs were trypsinized, washed, and resuspended in PBS at $2x10^6$ cells per 600 µl. Ferumoxides (Feridex, Berlex Laboratories, Inc.) were added to the MSC suspension at Fe 11.2 mg/ml and MEP[1] was performed at 130V for 17ms. The MSCs were rinsed and resuspended in PBS prior to transplantation.

Rabbit hindlimb ischemia model: A nonsurgical superficial femoral artery (SFA) occlusion was created in New Zealand White rabbits using a percutaneous carotid artery approach under x-ray angiographic. Multiple platinum thrombogenic coils were placed in the SFA to create left hindlimb ischemia. MSCs were delivered at 24-48 hours post-occlusion under MR guidance.

MR imaging: : Animals were sedated, intubated, and maintained on intravenous thiopental for the duration of the imaging study. The animals were placed supine with a 5 channel phased array coil over the pelvis in a 3T XMR scanner (Achieva, Philips). Three plane scout images were obtained followed by a T2-prepared 3D magnetic resonance angiogram (TR/TE=14/3.8 ms; 270x216 mm^2 FOV; 20º FA; 12 TFE factor; 0.35x0.35x1.5 mm voxel size) to determine the extent of SFA occlusion. Allogeneic ferumoxide-labeled MSCs were injected intramuscularly in the medial thigh based on the MRA. To test the sensitivity of detection of positive enhancement of MR-labeled MSC by IRON imaging[2], multiple injection were performed at concentrations ranging from $1\text{-}5x10^5$ cells per injection. IRON imaging parameters were: TR/TE=2000/11.6 ms; 180x135 mm^2 FOV; 24 TSE factor; 0.45x0.45x3 mm^3; and dual IRON pulse. Contrast-to-noise ratio on traditional spin-echo images were compared to IRON images of stem cells injection sites relative to normal skeletal muscle.

Results and Discussion

A uniform population of multipotent bone-marrow derived mesenchymal stem cells was demonstrated by the ability to differentiate the culture expanded cells into adipocytes and osteocytic lineages (Fig 1). The viability of cells after thawing was 90% and MEP of thawed cells reduced viability ~20%. T2-prepared MRAs demonstrated total occlusion of the SFA at 48 hours after coiling. MEP-labeled MSCs in concentrations of $<1x10^5$ were not readily visible as hypointense lesions on traditional FSE images (CNR=-1.93 ± 1.0, Fig 2A). However, MEP-labeled MSCs were easily visualized on IRON images as hyperintense dipole signals (CNR=14.48±1.9). Moreover, hyperintense signal from the magnetic susceptibility due to platinum coils was more complex and could be readily distinguished from the typical dipolar hyperintense signal of MEP-labeled cells (Fig 2B). Follow-up MRA at 1 week post-MSC injection demonstrated the development of collateral vasculature from the deep femoral artery (Fig 3).

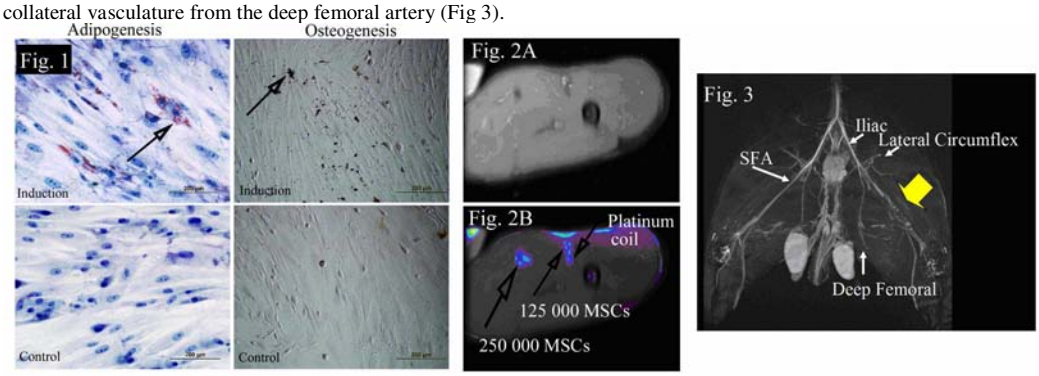

Fig. 1. Fig. 1. Left- the adipocytic lineage post in vitro differentiation, the arrows indicate the red oil stained vacuoles of lipid. Right - osteocytic lineages post in vitro differentiation, the arrows indicate calcium deposits, black stained with silver nitrogen. Fig. 2A FSA imaging of MEP-labeled MSCs in rabbit thigh muscle. Fig. 2B. IRON image of MEP-labeled MSCs in rabbit thigh muscles. Fig. 3. Angiography showing the development of collateral vasculature from the profundus femoral artery.

Conclusion

MEP-labeling of MSCs provides a fast, convenient method to MR-label cells. The present study indicates that the IRON method will be useful not only for noninvasive tracking of MEP-labeled cells but also discerning them from other image artifacts, such as platinum coils. In addition, small quantities of MEP-labeled MSCs were easily demonstrated using IRON imaging.

References

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