Tracking MEP-labeled Mesenchymal Stem Cells Relative to New Vessel Formation Using MRI in a Rabbit Model of Peripheral Arterial Disease

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Introduction:

The successful treatment of Peripheral Arterial Occlusive Disease (PAOD) relies on the restoration of a sufficient blood supply to the ischemic tissue. Amongst many experimental therapies, cell-based therapy offers an option to enhance arteriogenesis. Two possible mechanisms that explain the benefits of MSCs transplantation are: 1) MSCs differentiate into vascular elements (endothelium, pericytes) and provide the "building blocks" for new or enlarged vessels, and/or 2) the MSCs release cytokines that promote and facilitate endogenous arteriogenesis.

Magnetic resonance imaging can be used to monitor the cell delivery, as well as, to serially interrogate the degree of cellular retention and engraftment. In addition, MRI can be used to visualize the development of new collateral vessels as well as the effect on tissue perfusion to the downstream ischemic tissue bed.

In the present study, magnetically-labeled, allogeneic, bone marrow-derived MSCs were injected intramuscularly into an endovascular rabbit hindlimb ischemia model. High resolution, fast, gradient echo imaging was used for tracking the MSCs, and T2-prepared MR angiography (MRA) was used to monitor of neovascularization.

Methods:

Mesenchymal stem cells were isolated from the bone marrow of male New Zealand White rabbits. The MSCs were expanded (3 passages), trypsinized, mixed with ferrumoxides (Feridex, Berlex Laboratories, Inc.) at a concentration of 2 mg of Fe/ml, loaded into electroporation cuvettes and electroporated (20 pulses, 50 mV, pulse duration 1 ms) for magnetic labeling[1]. After labeling, the cells were incubated for half an hour in cold medium supplemented with 10% serum. Hindlimb ischemia was induced by a minimally invasive, non-surgical, endovascular method where thrombogenic platinum coils were deployed in the superficial femoral artery[2]. Twenty-four hours after ischemia induction, female rabbits were randomized to receive $13x10^6$ magnetoelectroporated (MEP)-labeled MSCs (n=6) or sham injections (0.3 mg of Fe/ml of PBS) in the ischemic medial thigh.

MRI was performed on clinical 3T Phillips Achieva scanner immediately after injection and 1 and 2 weeks post-injection. An adiabatic T2-prepared MRA was acquired with the following imaging parameters: TR/TE=14/3.8 ms; FOV=270x216 mm^2; spatial resolution = 0.34x0.35x1.5 mm^3; TFE factor of 12; and FA=20°. Fast gradient echo images in the axial plane were acquired to document the MEP-labeled MSC injections with imaging parameters of: TR/TE=18/11 ms, spatial resolution=0.5x0.63x3 mm^3, 15° flip angle; and 18-20 slices. At two weeks, X-ray angiography was performed to confirm MRA findings. Subsequently, the animals were humanely euthanized, and tissue was harvested for histological analysis.

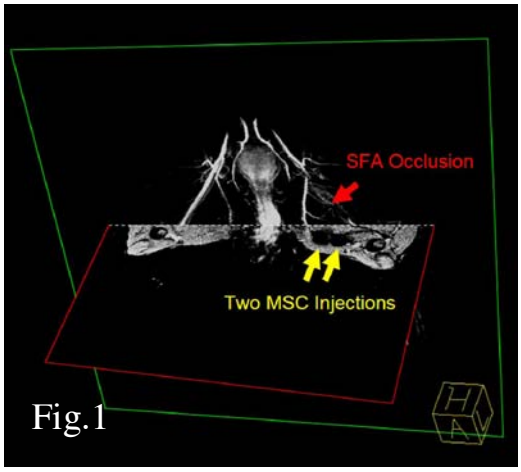

Fig.1

Results:

MEP resulted in efficient magnetic cell labeling with <5% decrease in cell viability. High resolution MRI demonstrated a close proximity of the MEP-labeled MSCs to the neovasculature based on T2-prep MRA (Fig.1). MRAs at 2 weeks demonstrated a high agreement with X-ray angiography (Fig.2). Incorporation of the contrast agent laden cells into the small vessel walls was confirmed with Prussian Blue (Fig.3a), anti-dextran (Fig.3b) and CD31 staining .

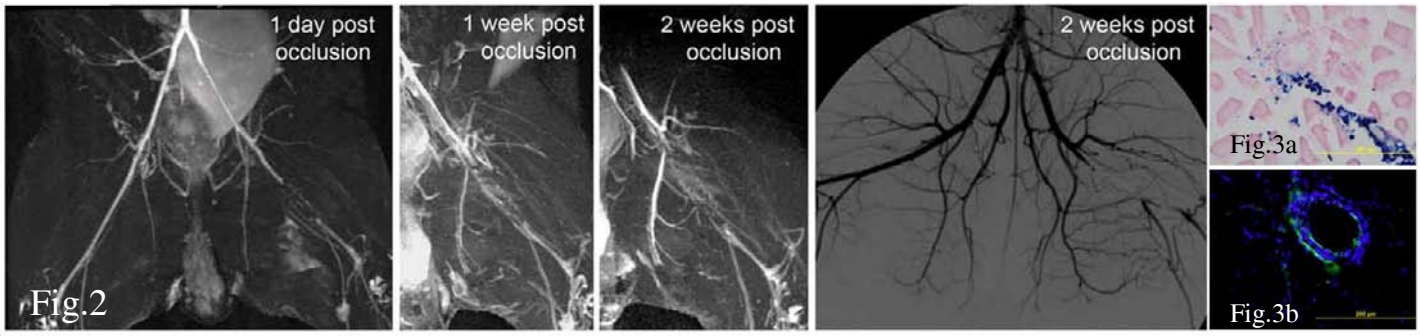

Fig.2

Fig.3a

Fig.3b

Conclusion:

High resolution imaging of MEP-labeled MSCs allows the assessment of cellular therapy delivery location relative to new vessel formation on MRA without the use of contrast agents or arterial access. MRA assessment of angiogenesis was confirmed on X-ray angiography. Moreover, histological confirmation of the incorporation into the vessel walls of the MSCs suggests an active role in the induction of new vasculature. Thus, MRI may provide a useful means of determining the fate of MSCs in future clinical trials of PAOD.

References:

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2. Liddell, R.P. et al. J Vasc Interv Radiol, 2005. 16(7): p. 991-998.