Magnetic Resonance Imaging of Endothelial Progenitor Cells Transplanted to Rat Hindlimb Ischemia Model Using Novel Water Soluble Contrast Agents

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Statement of Purpose: Endothelial progenitor cells (EPCs) are crucial in the regeneration of injured tissues and organs by a molecular mechanism known as angiogenesis[1,2]. EPCs have been therapeutically used to stimulate angiogenesis by transplantation into animal models of ischemic limbs. In these studies, cells were intravenously transfused into animals or transplanted directly into the muscle to see the function of EPCs in tissue revascularization. According to these studies, the therapeutic importance of EPCs in ischemic tissues regeneration processes was already demonstrated but the mechanisms are still unclear. Therefore, there is an imperative to advance in techniques capable of visualizing the EPCs recruitment, homing, and migration of cells, which were activated by cytokines to the site of injury; a process which has to be studied. The ability to track cells in vivo would provide insights into many basic and practical questions related to EPC therapy. We here tried to establish new MRI system for tracking EPCs in vivo. So far, superparamagneteic iron oxide (SPIO) particles have been used for labeling diffetent types of cells. However, some reports pointed out that MR signals in cells labeled with SPIO are sometimes attributed to the signal for macrophages which ingested free SPIO leaking out of the labeled cells. Therefore, we synthesize a water soluble gadolinium chelate contrast agent called Dex-DOTA-Gd3+. A DOTA-Gd was conjugated to biocompatible dextran carrier. Free Dex-DOTA-Gd3+ in the body is expected to have rapid clearance in the body. EPCs were labelled with the Dex-DOTA-Gd3+ to investigate the role of MRI tracking of EPCs.

Methods: Dex-DOTA-Gd3+ is synthesized by amination of dextran (MW 40kD) and reaction with Mono-N-succinimidyl 1,4,7,10 Tetraazacyclodecane-1,4,7,10 tetraacetate (DOTA). Subsequently, the reaction product was purified by dialysis in distilled water and analyzed by UMR dialysis membrane.

Bone marrow (BM) was flushed from femurs and tibias of F344 rats (4 weeks old, male) after previous cytokine inducted mobilization of bone marrow-derived EPCs by using G-CSF. CD34 and FLK-1 positive bone marrow cells were isolated by means of magnetic beads. Cells were placed in fibronectin coated dishes and cultured with endothelial cell basal medium (EBM-2) supplemented with EGM-2 singleQuots.

The Dex-DOTA-Gd3+ was delivered into EPC cells by mean of electroporation. Subsequently, 10 mM of Dex-DOTA-Gd3+ were added to the medium and an electrical pulses were applied to the cells using a CUY-21 electroporator (NEPPA GENE, Tokyo, Japan) under the following conditions: field strength 300 V/cm, number of pulses 10, pulse duration 5 ms. Cells were then cultured for one hour and washed several times with PBS.

The left femoral artery and vein of male F344 rats and their branches were ligated and totally excised through a skin incision. The femoral artery and vein were excised from their proximal origin as a branch of the external iliac artery to the distal point where it bifurcates into the saphenous and popliteal arteries. Rats (n=10) were injected in three places with a total of 150 µl containing Dex-DOTA-Gd+3 labeled EPCs

Results: MRI as a high-resolution technique for imaging endothelial progenitor cells (EPC) in vivo would provide important insights about the different phenomena germane to angiogenesis of ischemic limb model such as: recruitment, migration, and incorporation to the foci of vascularization. To study the function of MRI in the therapeutic angiogenesis, an MRI contrast agent based on a dextran derivative was synthesized by dextran amination and subsequently reacted with a gadolinium chelate complex, called Dex-DOTA-Gd3+. The contrast agent was introduced into the EPCs by electroporation and then the stability and biocompatibility of the contrast agent was analyzed in vitro and in vivo. Results show the ability of Dex-DOTA-Gd3+ to efficiently label EPCs and provide a sufficient MR signal to locate the cells inside the limb. Most important was the feasibility of monitoring the migration of labeled EPCs to the ischemic region due to chemoattraction and lastly, a different pattern of behaviour in the migration of labeled cells was observed

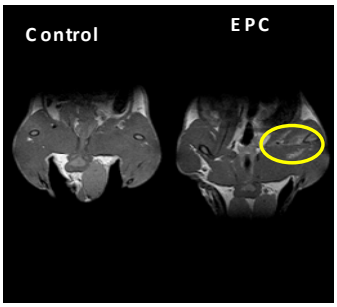

Figure 1. MRI images after intramuscular injection of 2×10^7 EPC into rat ischemic model. T1 weighted images were acquired at a geven period of time after transplantation. on a 1.5 T compact MR imaging system. Sequence: spin echo, coronal, slice 1 mm, TR=2000 ms, TE=9 ms.

when an anomaly in the MRI acquisition images appeared seven days postransplantation.

References:

1. Risau, W. Mechanism of angiogenesis. Nature 386, 671-674 (1997).
2. Folkman, J. & D'Amore P.A. Blood vessel formation: what is its molecular basis?. Cell 87, 1153-1155 (1996).