MAGNETIC RESONANCE IMAGING DETECTS THERAPEUTIC EFFECTS OF ENDOTHELIAL PROGENITOR CELLS ON TISSUE REPAIR AND MUSCLE REGENERATION INTO ISCHEMIA HINDLIMB

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TARGET AUDIENCE This study aimed to use the diffusion tensor imaging (DTI) and T2 weighted MR imaging to assess the therapeutic effect of endothelia progenitor cell in a mouse model of hind limb ischemia. DTI can be used as a marker *in vivo* and as a diagnostic tool for assessment of ischemia-induced muscle damage and repair. The researchers in the field of DTI and molecule imaging should be interested in this topic.

PURPOSE Endothelia progenitor cells (EPCs) migrating to ischemia tissue and organs do not always participate in the formation of the neovasculature but rather produce a variety of pro-angiogenic cytokines and growth factors to promote proliferation and migration of pre-existing ECs (1-2). The architectural organization of regenerating skeletal muscle or heart left ventricle during remodeling after ischemic injury can be evaluated by analysis of diffusion tensor imaging (DTI) (3-5). Apart from muscle fiber visualization by fiber tractography, a number of quantitative diffusion parameters can be used to characterize the diffusion: λ1, λ2 and λ3 diffusion tensor eigenvectors, which represent the three-dimensional directions and the forces associated with water diffusion in the muscle relative to fiber orientation and cross-sectional area; the fractional anisotropy (FA), which provide the information about diffusion anisotropy; the apparent diffusion coefficient (ADC), an estimate of the diffusing compartment size. The purpose of this study was to study the feasibility and accuracy of *in vivo* MRI to evaluate tissue repair and muscle fiber regeneration in a mouse model of hind limb ischemia.

Methods All animal experiments were approved by the institutional Committee on Animal Research. EPCs were isolated from the tibias and femurs of 4-week-old male C57BLKS/J mice. Bone marrow-derived EPCs were characterized with immunofluorescent staining and flow cytometry. The right femoral artery of 5-week-old male mice was exposed and excised with an electrocoagulator from proximal origin of external iliac artery to the bifurcation into saphenous and popliteal arteries. At 24 hour after surgery, mice were randomly assigned to blinded intracardiac delivery of control saline (CON, 150-200 μl each) and EPCs (1×10^6, 150-200 μl each). *In vivo* T1WI, T2WI, T2 mapping and DTI were performed at 24 h, 2, 7, 14, 21, 28 days post-ischemia to evaluate tissue edema and muscle fiber regeneration. Fiber counts obtained through DTI were compared to reference standard by histological semiquantitative survival fiber in Masson's staining section.

Results T2-weighted images and diffusion-weighted images of ischemic hindlimb indicated the presence of edema in the ischemic muscle. T2 relaxation time was increased throughout the cross section, however, the largest increase was observed at the periphery of the muscle. At 21 days after ischemia, the T2 relaxation time in ischemic muscle treated by EPCs was significantly lower than that of control group. Accordingly, the regions of edema in ischemia muscle after 14 days treated by EPCs were smaller than that of control group and the T2 relaxation time was increased within the center of muscle (Figure 1A, B and C). At 1 and 3 days after treatment, the ADC in ischemia muscle increased and FA in ischemia muscle decreased, but there were no different between EPCs treated and control group. However, at 7 and 14 days after treatment, there were significantl differences between EPCs treated and control group, ADC of ischemic hind limb in EPCs transplantation was lower than that of control ischemic hind limb and FA of muscle in EPCs treated mice were higher than that of control muscle (Figure 1D and Figure 2A-B). Immediately after ligation, λ2 and λ3 increased. Seven to fourteen days after treatment, λ2 and λ3 of ischemia muscle in EPCs group were lower compared to the control group. In addition, the fiber count of ischemia muscle treated by EPCs was higher than those treated with saline at 28 days (Figure 2 C-D). There was a significant correlation between fiber counts calculated by DTI and survival fiber evaluated by histopathology ($r = 0.903$, $P<0.01$).

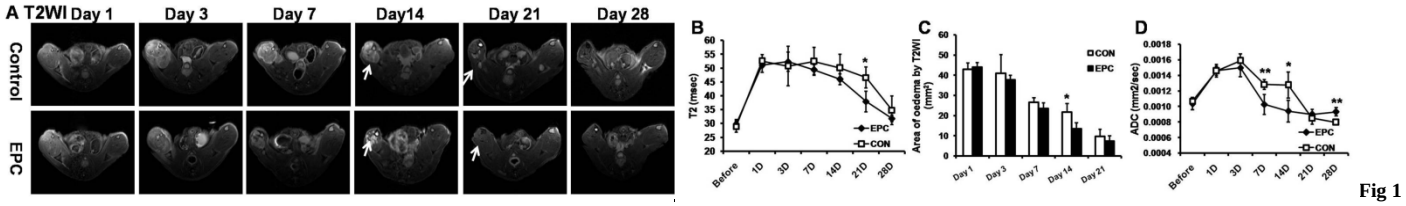

Fig 1

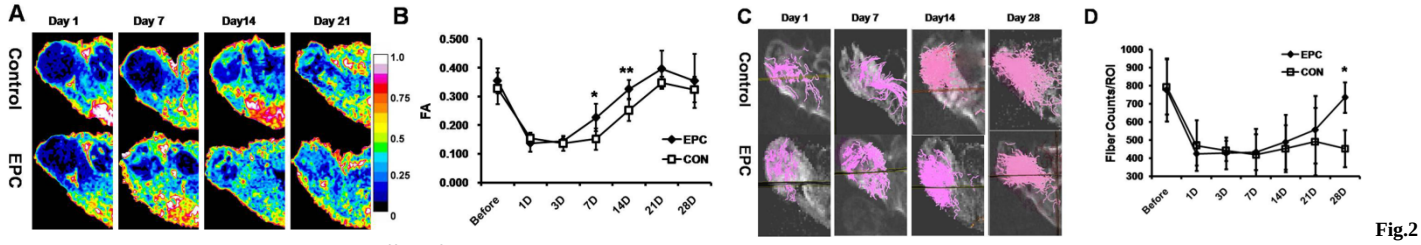

Fig.2

Figure 1. *In vivo* ischemic muscle regeneration effect of EPCs by T2WI. **A:** T2WI showed that transplanted EPCs improved the oedema condition in ischemia muscle. **B:** The line graphes showed that administration of EPCs significantly reduced T2 relaxation time at day 21 after treated. **C:** The line graph showed that administration of EPCs significantly decreased ADC at day 7 and 14 after transplantation. **D:** The area of oedema at 14 days after treated by EPCs was decreased compared with treated by saline.

Figure 2. *In vivo* ischemic muscle regeneration effect of EPCs by DTI. **A:** Representative pixel maps of FA at 1, 3, 7, and 21 days in ischemic gastrocnemius muscles in control and EPCs treated mice. **B:** A line graph showed that FA of EPCs transplantation improved better than that of control group at day 7 and 14 after ischemia. **C:** Representative maps of fiber tracking map at 1, 7, 14, and 28 days in ischemic gastrocnemius muscles in control and EPCs transplanted mice. **D:** A line graph showed that fiber counts of EPCs transplantation improved better than that of control group at day 28 after ischemia.

Discussion Bone marrow-derived endothelia progenitor cells (EPCs) that contributed to neovascularization in ischemic tissues were reported over ten years ago (6). The recent opinion is that the benefits of EPCs therapy are not a result of the structural integration of grafted cells within new vessels, but of the paracrine activation of angiogenesis, arteriogenesis and vasculogenesis pathways by the cytokines, chemokines and growth factors released from such cells (7). The muscle regeneration process followed a centripetal gradient, which is from the outer regions to the inner regions (8). The T2 relaxation time and ADC value in EPCs treated group was lower compared to control group and the area of edema in ischemia muscle was also smaller after 14 days. The results demonstrated that EPCs transplantation promoted the tissue recovery. Furthermore, the results of FA, λ2, λ3 and fiber counts from DTI suggested that the regeneration of ischemia muscle fibers in the transplanted EPCs group was better than that of control group. The histologic findings also indicated that the survival muscle fibers after EPCs treated were more than that of in control mice. Diffusion MR imaging can be used as a marker *in vivo* and as a diagnostic tool for assessment of ischemia-induced muscle damage and repair (3, 9).

Conclusion Out results indicate that T2 weighted imaging and DTI are useful for longitudinally evaluating the effect of EPCs transplanted treatment in a mouse model of hind limb ischemia. DTI is accurate in the regeneration of ischemia muscle fibers. The tissue repair and ischemia muscle fiber regeneration after EPCs treatment was significantly better than that of control group. We propose that MRI can be used for noninvasive evaluation of muscle tissue damage and repair in animal models and patients with ischemia diseases.

Reference (1) Alev C., et al, *Antioxid Redox Signal.* 2011;15:949-65. (2) Sieveking DP., et al, *Vasc Med.* 2009; 14: 153-166. (3) Gianella A., et al, *Cardiovasc Res.* 2010; 85: 503-513. (4) Wu MT., et al, *Circulation.* 2006; 114: 1036-1045. (5) Heemskerk AM., et al. *Radiology.* 2007; 243: 413-421. (6) Asahara T., et al. *Circ Res.* 1999; 85: 221-228. (7) Asahara T., et al, *Stem Cells.* 2011; 29: 1650-1655. (8) Carlson BM., et al. *Anat Rec.* 1975; 183: 47-62. (9) Heemskerk, AM. *Radiology*. 2007; 243: 413-421.